

Chitosan-nanocrystal cellulose composite coating could inhibit fruit decay rate and maintain the texture parameters of fruit in okra (*Abelmoschus esculentus* [L.] Moench)

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Abstract

In order to obtain a better preservation effect on okra fruit, this study examined chitosan (CS)–nanocrystal cellulose (NCC) composite coating as a preservative agent. Four treatments, including CS, NCC, CS–cellulose composite (CS-CC), and ratio of CS:CC: 2:1 (2CS-CC), were implemented to create three different okra fruits (Shuiguo, Naiyou, and *Hibiscus coccineus*). Rate of weight loss, decay rate, and texture profiles (e.g., hardness, springiness, cohesiveness, gumminess, chewiness, and resilience) of the fruits were determined regularly during storage. Our results showed that a CS-NCC composite coating could not reduce rate of weight loss of the fruits. Only 2CS-CC treatment inhibited the fruit decay rate in *Hibiscus coccineus* (C). Nevertheless, NCC treatment did not result in a distinct improvement compared with CS treatment (1%). CS-CC treatment could be advantageous for maintaining the texture parameters of okra fruit during storage. Notably, change in the fruit texture parameters presents a significant cultivar-dependent pattern.

Keywords: okra (*Abelmoschus esculentus* [L.] Moench); fruit; nanocrystal cellulose (NCC); chitosan; texture profile analysis

Introduction

Okra (*Abelmoschus esculentus* [L.] Moench) is a global tropical annual herb belonging to the mallow family. Considered a healthy vegetable with high nutritional values (Shi *et al.*, 2020), okra fruit is canned and used to make *kimchi* because of its tender, mucilaginous, and flavorful features (Chen *et al.*, 2010). In China, the market price of okra fruit in Guizhou province, especially in winter, is much higher than that of staple-growing regions for okra (e.g., Hainan province). Hence, extending the shelf life of the fruits to ensure long-distance transportation is a key to okra preservation. Generally, okra is preserved ecologically in non-chilling low-temperature conditions

using safe and effective preservatives. Polysaccharides are typically used as biological coating materials for preservation of okra. Adding physiologically active substances (e.g., phytic acid) to the polysaccharide coating solution can further improve the preservation effect (He *et al.*, 2021). Recently, other techniques have been used to preserve fresh okra, including low-density polyethylene (Paulus *et al.*, 2021) and nano-ZnO combined with ultraviolet irradiation (Ji and Wang, 2020).

Nanocrystal cellulose (NCC) is a type of rod-shaped cellulose (length: 10–100 nm and diameter: 1–100 nm) that exhibits excellent mechanical properties because of its large surface area, biodegradability, high safety,

nontoxicity, abundant source, biocompatibility, high water solubility, and bioactivity (Ayadi *et al.*, 2016; Debele *et al.*, 2016; Peres *et al.*, 2015; Tran *et al.*, 2017). NCC can be synthesized from animals, algae, microorganisms, and plants, and has been actively used in the chemical industry, and food, medicine (Wang *et al.*, 2017), and other fields (Mohd *et al.*, 2018). However, the effects of NCC on fruit texture have not been explored. Li *et al.* (2021) demonstrated that a chitosan–NCC composite (CS–NCC) coating could enhance the preservation of *shatangju* (*Citrus reticulata* Blanco). This demonstrates that NCC can be used to improve fruit preservation in place of using chemical preservatives. Our previous research (Wang *et al.*, 2020) showed that okra fruit texture is significantly influenced by CS coating. In this study, we investigated the effect of CS–NCC coating on okra fruit during storage in order to improve the preservation effect of CS on the fruit. Particularly, great attention has been paid to changes in okra fruit texture parameters, as very little is known about the preservation mechanism of CS–NCC.

Materials and Methods

Materials

Three okra cultivars, Shuiguo, Naiyou, and *Hibiscus coccineus*, were planted in the experimental plot of the Guiyang University in Qingzhen, Guiyang City, China. The above-mentioned okra cultivars were harvested as experimental materials for further experimentation, and were labeled as A, B, and C, respectively. In all, 10 fruits of each cultivar were used to determine their morphological properties (Table 1). Different okra cultivars have different fruit sizes, ranging from 14 cm to 17 cm. Fruits of cultivars Shuiguo (A) and Naiyou (B) were green in color, while color of *H. coccineus* was red. Chitosan (with a deacetylation of 90% and a molecular weight of 543.51948) was purchased from Weifang Haizhiyuan Biological Products Co. Ltd, (WeiFang, China) and NCC with a final concentration of 1.15% was purchased from Tianjin Mugenie Biotechnology Co. Ltd (TianJin, China). NCC was produced from bleached coniferous wood pulp by the hyper 2,2,6,6-tetramethylpiperidinol oxidation (TEMPO) method.

Fruit coating treatment process

Based on a comprehensive analysis of the previous research (Li *et al.*, 2021; Mohd *et al.*, 2018; Tran *et al.*, 2017; Wang *et al.*, 2017), this study treated okra fruits with CS solution, NCC, and mix I (ratio of CS:NCC: 1:1) (CS–CC) and mix II (ratio of CS:NCC: 2:1) (2CS–CC). N,O-carboxymethyl chitosan (NOCC) solution with a

mass fraction of 1% was prepared before mixing. Four treatments were then used to coat three different okra fruits. Hence, 12 samples were processed immediately after harvesting and cleaning with distilled water. Texture profile analysis (TPA) parameters of the fruit were determined before and after coating. Five biological replicates of each test were performed for comparison.

The coating treatment was implemented according to a previously reported method (Wang *et al.*, 2020). Samples were soaked for 2 min; air-dried; wrapped in a plastic bag; and stored at -10°C in a refrigerator. The controls were also wrapped in plastic and stored at 4°C in a refrigerator. Texture parameters of each sample were measured after 0, 6, and 14 days of storage. The weight loss rate and decay rate of fruits were determined during storage according to the method described by Li *et al.* (2021). Texture profile (e.g., hardness, springiness, cohesiveness, gumminess, chewiness, and resilience) analysis was performed based on past experience (Wang *et al.*, 2020) using TA.XTPlus analyzer (Stable Micro Systems, Surrey, UK).

Results

Change in fruit weight loss rate during storage

Weight loss rate in both treatment samples and control increased significantly with storage time (Figure 1) and was significantly lower in all treatment samples than in the control during storage. Different cultivars showed different results depending on coating treatments. For Shuiguo (A), the minimum weight loss rate was found in the CS treatment at 6 and 14 days of storage. Conversely, no significant difference between coating treatments was observed in Naiyou (B) and *Hibiscus coccineus* (C), despite the CS treatment having the lowest weight loss rate. Particularly, the maximum reduction in weight loss rate in the CS coating treatment was found in *Hibiscus coccineus* (C) compared to other treatments.

Change in fruit decay rate during storage

Fruits in both CS treatment samples and control showed a significant increase in decay rate after 14 days of storage (Figure 2). The decay rate of the fruit after CS treatment was highest at 6 days of storage in case of both Shuiguo (A) and *Hibiscus coccineus* (C). Conversely, no rotten fruits were found in Naiyou (B) for both coating treatments and the control. When stored for 14 days, both Shuiguo (A) and Naiyou (B) had the lowest decay rate of fruit in the CS treatment group, which was dramatically lower than that of the control. However, decay rates of the fruit after NCC and 2CS–CC treatments of Naiyou (B) cultivar were significantly higher than that of the control.

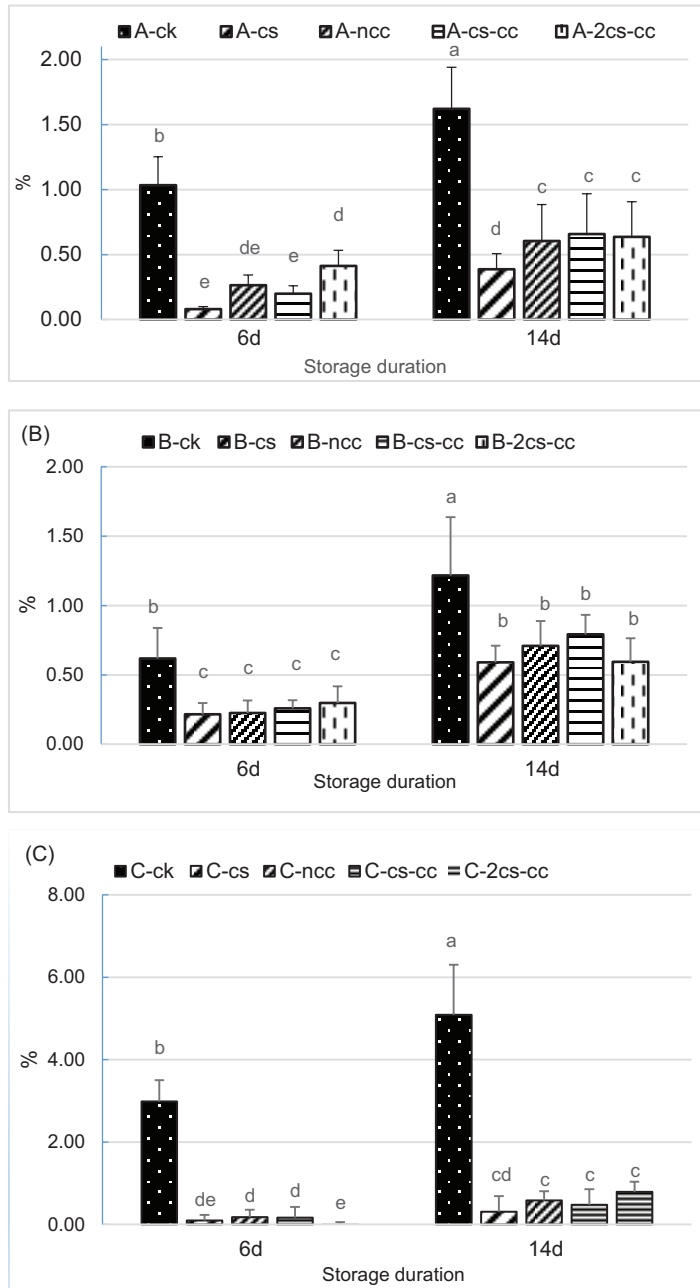


Figure 1. Changes in weight loss rate of okra fruit during storage. (A) Shuiguo, (B) Naiyou, (C) *Hibiscus coccineus*. Different lowercase letters marked on data point indicated significant differences between samples ($p < 0.05$)

Particularly, *Hibiscus coccineus* (C) cultivar showed minimum fruit decay rate in 2CS-CC treatment after 14 days of storage, which was dramatically lower than that of the control and other treated samples.

Change in okra fruit TPA parameters during storage

Hardness of okra fruits exhibited distinct changes between three cultivars during different storage periods (Figure 3). For Shuiguo (A), okra fruit hardness increased

with extension of storage under controlled conditions, but this decreased with both CS and NCC treatments. Particularly, the fruit hardness of Shuiguo (A) treated with CS was significantly higher than that of other treatments after 6 days of storage period. Conversely, the highest fruit hardness was discovered with NCC treatment of Naiyou (B) and *Hibiscus coccineus* (C). When stored for 14 days, fruit hardness with CS-CC treatment was highest in both Naiyou (B) and *Hibiscus coccineus* (C). However, fruit hardness of cultivars was significantly lower than that of the control.

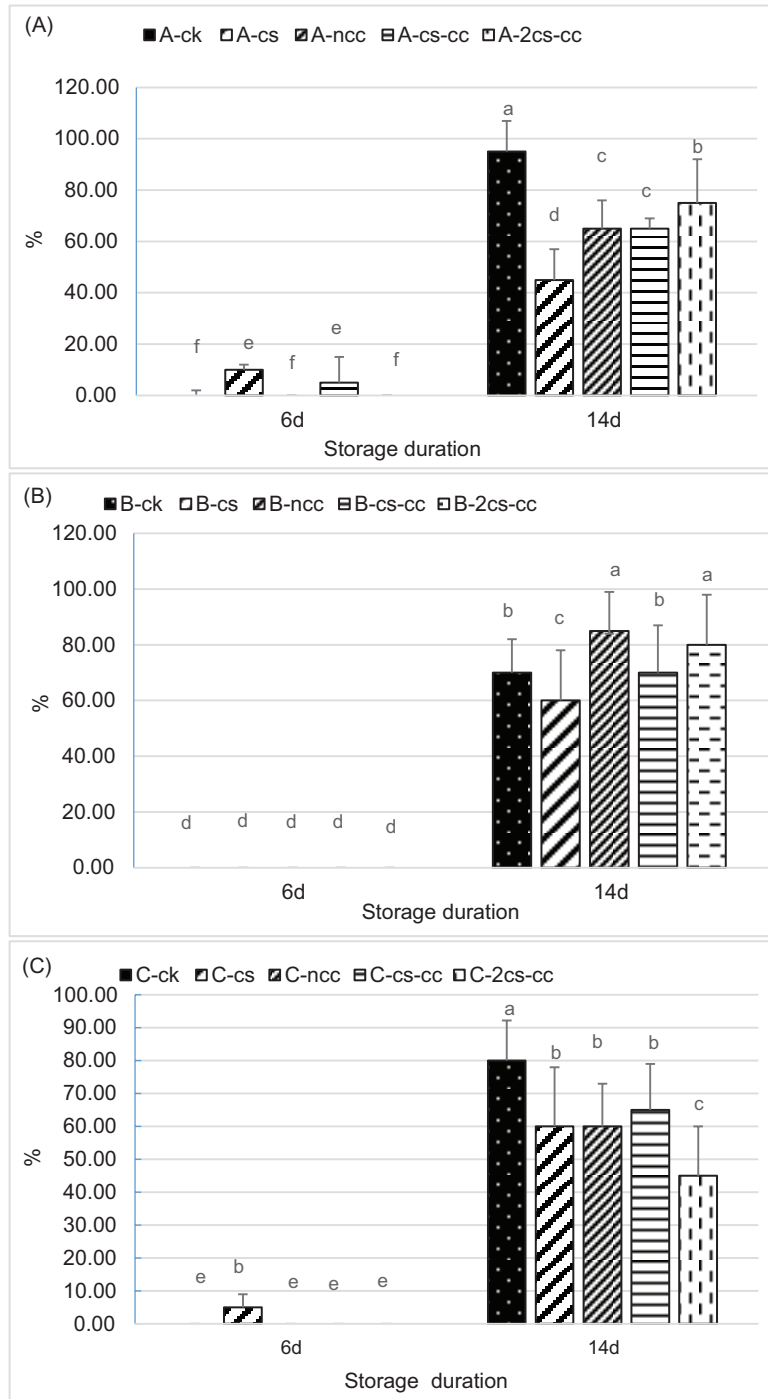


Figure 2. The decay rate of okra fruit during storage. (A) Shuiguoguo, (B) Naiyou, (C) *Hibiscus coccineus*.

Figure 4 shows that the resilience of *Hibiscus coccineus* (C) fruit appeared to decrease over time with the extension of storage period, except for CS-CC treatment. Similar changes were also observed in Shuiguoguo (A) treated with CS and Naiyou (B) with CS and 2CS-CC treatments. Notably, only the resilience of *Hibiscus coccineus* (C) fruit was significantly higher than that of the control after 14 days of storage.

Figure 5 shows the data for okra fruit cohesion. For Shuiguoguo (A) cultivar, fruit cohesion of the control was highest at 6 and 14 days of storage. However, this was not remarkably different from CS-CC and NC treatments at corresponding periods. We also observed such variations in Naiyou (B) cultivar. The fruit cohesion of *Hibiscus coccineus* (C) cultivar revealed the same pattern as that of Shuiguoguo (A) after 6 days of storage. The highest fruit

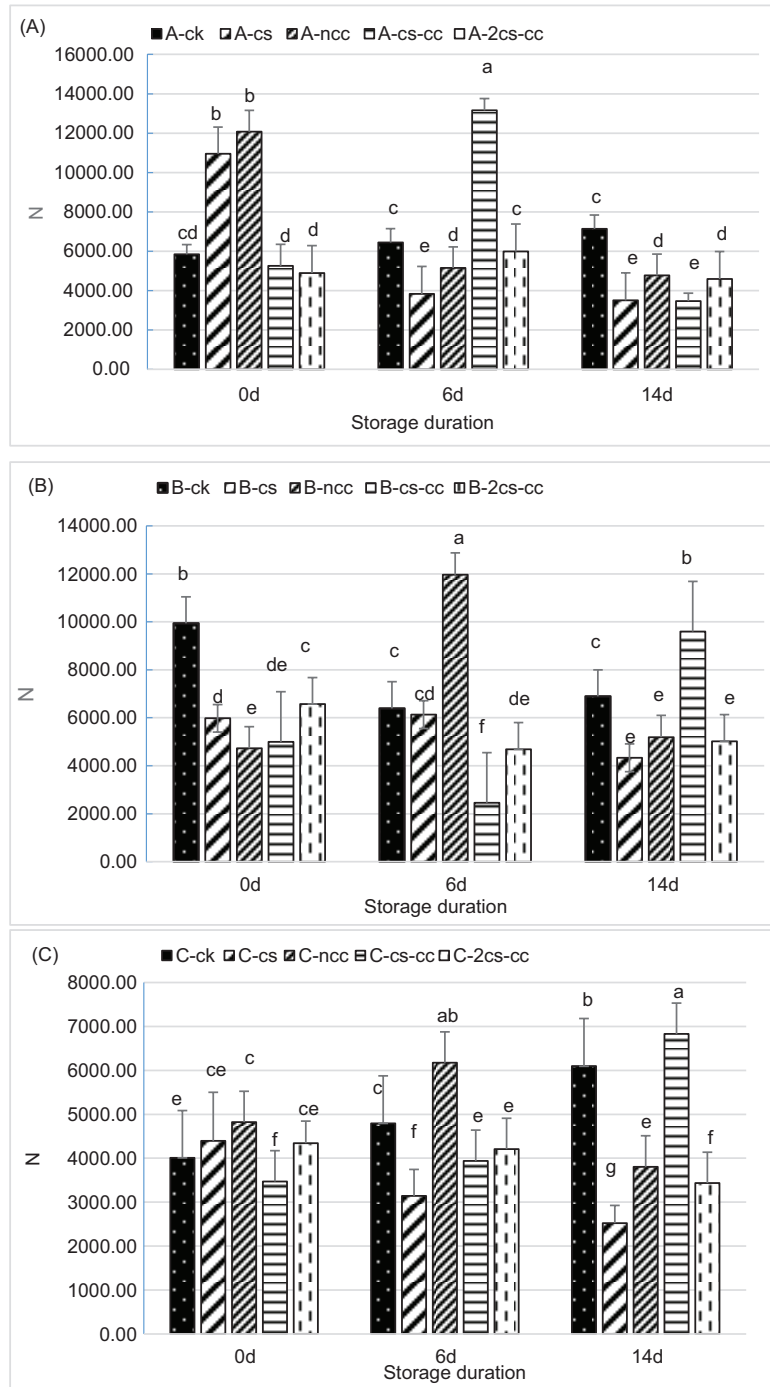


Figure 3. Hardness of okra fruit during storage. (A) Shuiguo, (B) Naiyou, (C) *Hibiscus coccineus*.

cohesion was found in CS-CC treatment when stored for 14 days, which was significantly higher than other treatments and control.

Figure 6 shows that the fruit springiness of three cultivars coated with CS-CC first increased dramatically and then decreased during storage, that is, the highest fruit springiness occurred after 6 days of storage. Naiyou (B) and *Hibiscus coccineus* (C) exhibited similar variability

in springiness with CS treatment. Unique fruit springiness with 2CS-CC treatment in Shuiguo (A) and *Hibiscus coccineus* (C) exhibited a decreasing trend with storage, although there was no significant difference in springiness between 6 and 14 days of storage.

Figure 7 shows that the fruit gumminess of okra appeared to decrease during storage in Shuiguo (A) cultivar with NCC and 2CS-CC treatments, in Naiyou (B) with CS and

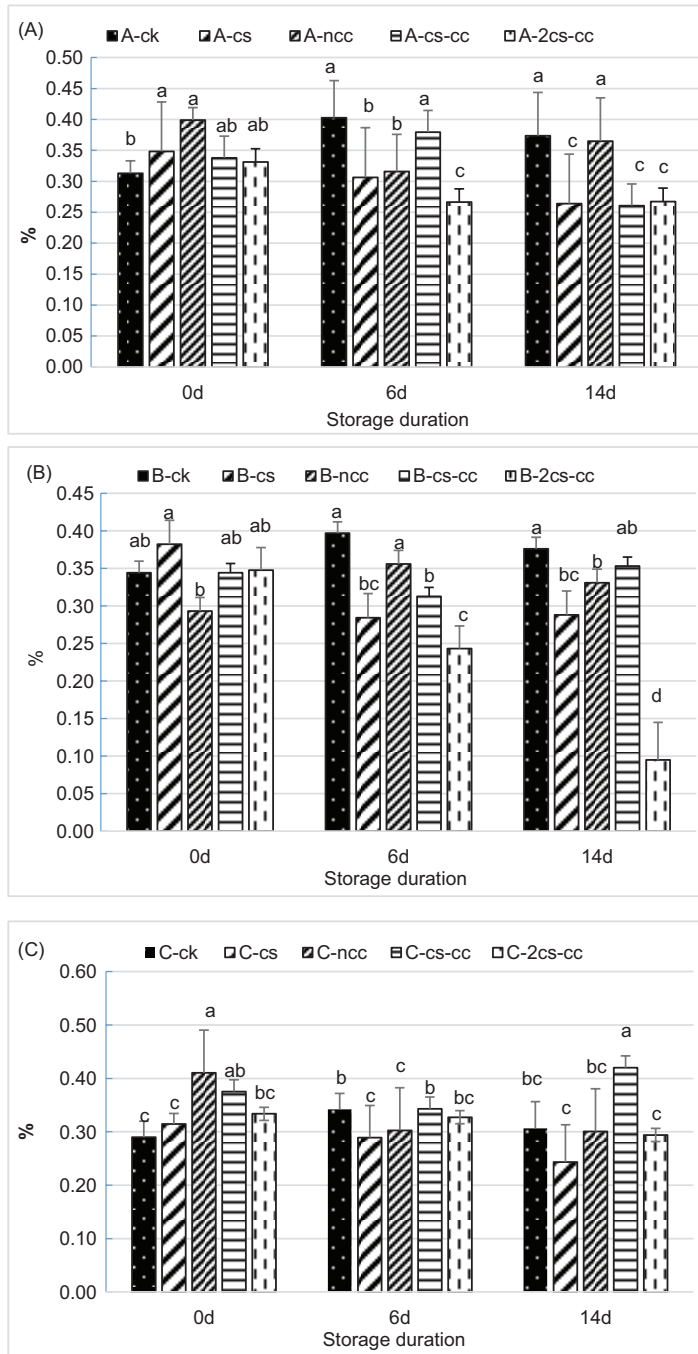


Figure 4. Resilience of okra fruit during storage. (A) Shuigu, (B) Naiyou, (C) *Hibiscus coccineus*.

2CS-CC treatments, and in *Hibiscus coccineus* (C) with CS, NCC, and 2CS-CC treatments. However, fruit gumminess increased during storage in *Hibiscus coccineus* (C) coated with CS-CC. This increase with CS-CC treatment was dramatically higher than that of the control for 6–14 days of storage. Particularly, fruit gumminess in Shuigu (A) with CS-CC treatment and control was significantly increased at 6 days of storage compared to that at 0 day of

storage, despite the increase with CS-CC treatment being dramatically higher than that in the control.

The fruit chewiness of Shuigu (A) cultivar with all treatments showed a decreasing trend during storage, except for with CS-CC treatment (Figure 8). However, only the magnitude of reduction with 2CS-CC treatment was significantly lower than that with control.

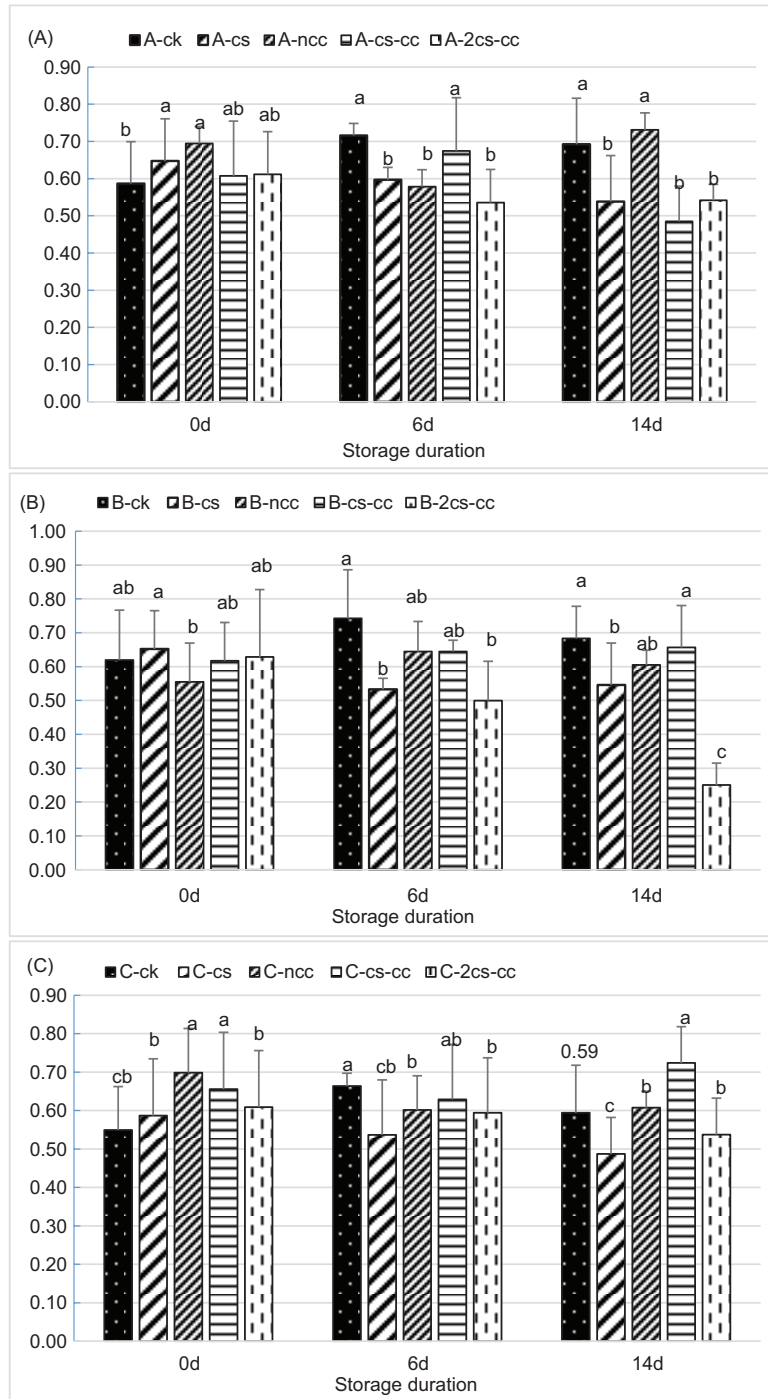


Figure 5. Cohesion of fruit during storage. (A) Shuiguo, (B) Naiyou, (C) *Hibiscus coccineus*.

Particularly, fruit chewiness of Shuiguo (A) coated with CS-CC was dramatically increased at 6 days of storage compared to that at 0 day of storage. Conversely, fruit chewiness decreased significantly at 14 days of storage compared to 6 days of storage. With change in control, the fruit chewiness of Naiyou (B) decreased dramatically during storage with CS and 2CS-CC treatments. The chewiness of Naiyou (B) cultivar coated with NC and

CS-CC increased significantly at 14 days of storage compared to that at 0 day. Surprisingly, the fruit chewiness of *Hibiscus coccineus* (C) coated with CS, NC, and 2CS-CC treatments exhibited a decreasing trend during storage, while that of CS-CC treatment and control increased with the extension of storage period. However, increase in chewiness with CS-CC treatment was less than that in the control.

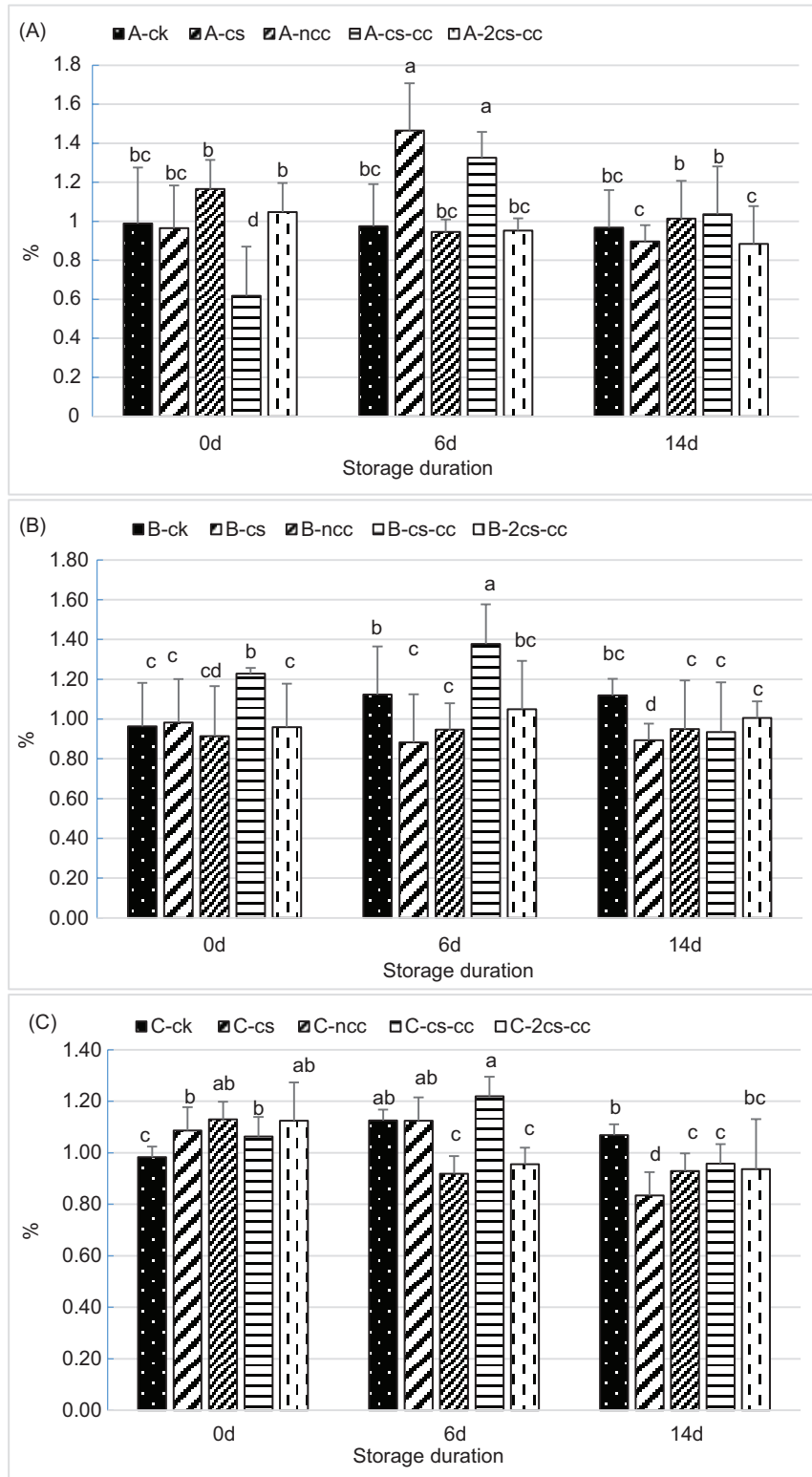


Figure 6. Springiness of okra fruit during storage. (A) Shuiguo, (B) Naiyou, (C) *Hibiscus coccineus*.

Table 2 reports correlations between various parameters of TPA. The hardness of fruit had a significant positive correlation with gumminess and chewiness during storage ($P < 0.01$). This implies that the higher the hardness

of the fruit, the higher its gumminess and chewiness. Resilience of the fruit was significantly positively correlated with cohesiveness ($P < 0.01$) and gumminess ($P < 0.05$). This indicates that the higher the resilience

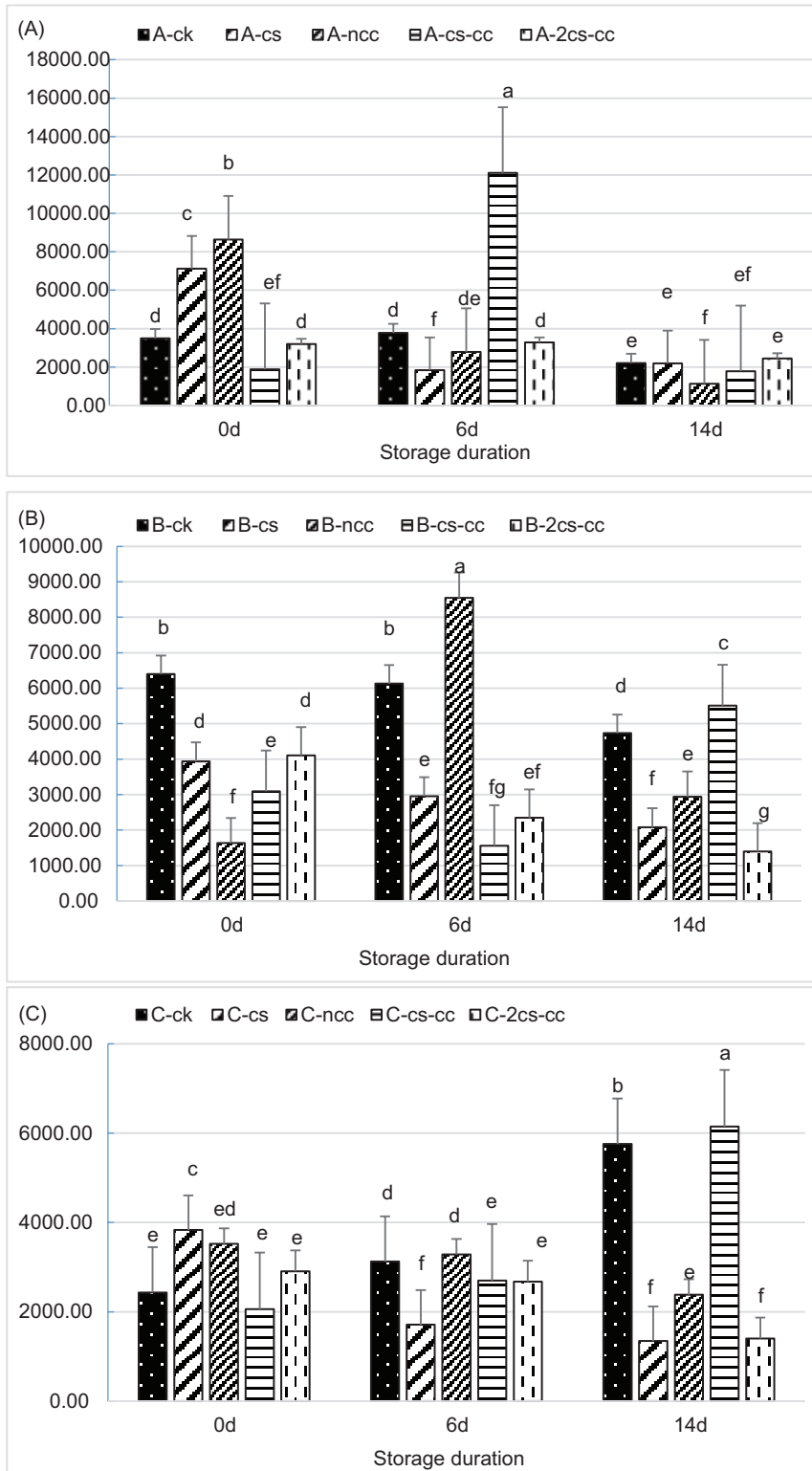


Figure 7. Gumminess of okra fruit during storage. (A) Shuiguo, (B) Naiyou, (C) *Hibiscus coccineus*.

of the fruit, the higher the cohesiveness and gumminess. Furthermore, chewiness was significantly correlated with hardness and gumminess ($P < 0.01$). No correlation was observed between the fruit decay rate and weight loss

rate of any of the three cultivars. The fruit decay proportions of Shuiguo (A) and Naiyou (B) were significantly positively correlated with the weight loss proportion of fruit (0.601^* , 0.741^* , $P < 0.05$).

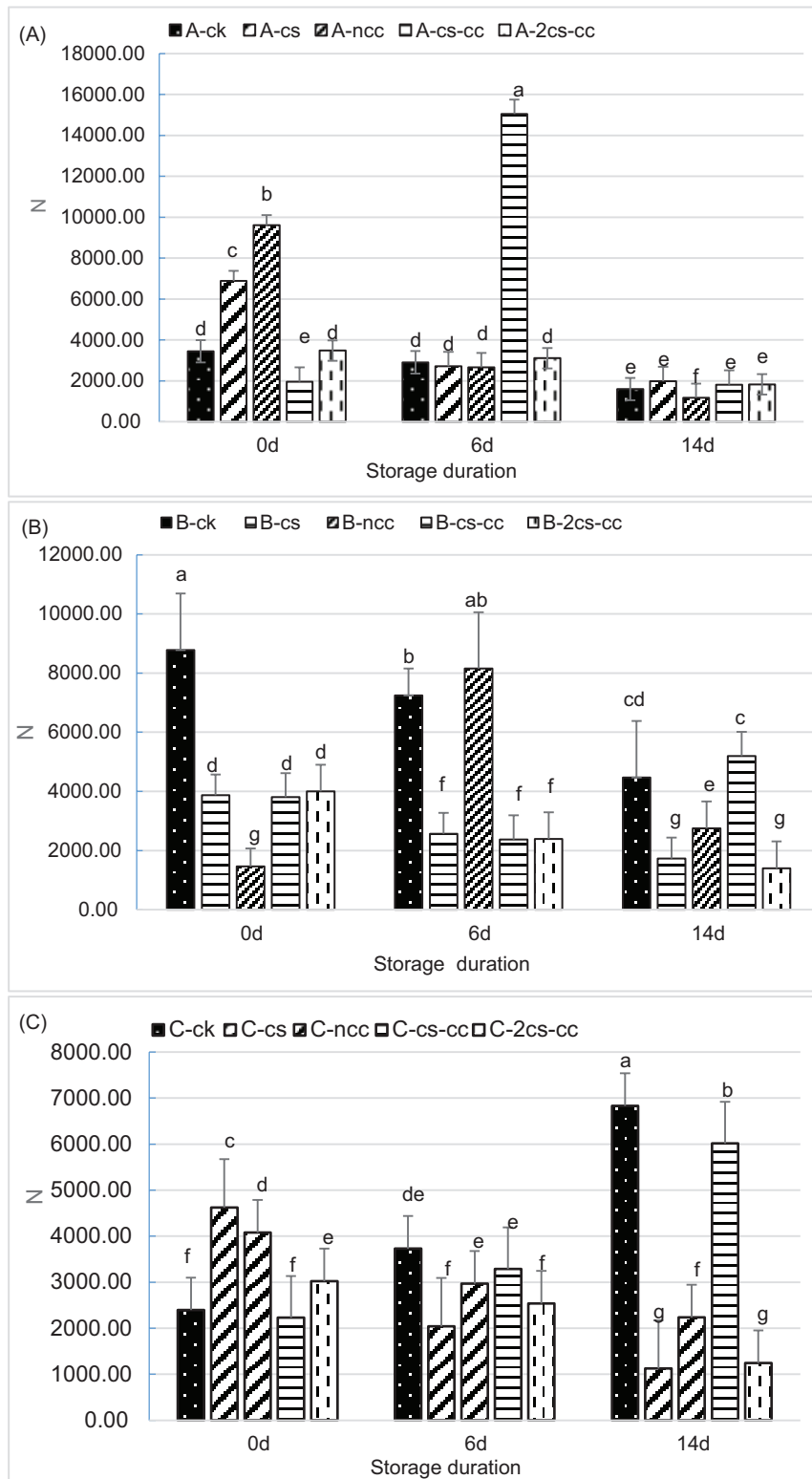


Figure 8. Chewiness of okra fruit during storage. (A) Shuiguo, (B) Naiyou, (C) *Hibiscus coccineus*.

Discussion

Chitosan-NCC composite coatings have assorted effects on different cultivars. The lowest fruit weight loss

rate after CS treatment was observed in all three cultivars, although there was no significant difference in Naiyou (B). This suggests that coating with CS can effectively inhibit weight loss rate of fruit compared to NCC

Table 1. Morphological trait of fruit.

Cultivar	Length (cm)	Diameter (cm)	Weight of each fruit (g)	sides	Fruit color
A	15.62	2.30	30.42	5.64	green
B	14.39	2.43	30.74	5.4	green
C	17.21	2.42	36.16	5.6	red

Table 2. Correlation (R) among texture parameters of okra fruit.

Parameter	Resilience	Hardness	Cohesiveness	Springiness	Gumminess	Chewiness
Resilience	1	–	–	–	–	–
Hardness	0.424	1	–	–	–	–
Cohesiveness	0.965**	0.382	1	–	–	–
Springiness	0.044	0.031	0.086	1	–	–
Gumminess	0.500*	0.907**	0.452	0.220	1	–
Chewiness	0.472	0.845**	0.430	0.296	0.974**	1

Note: *represents $p < 0.05$, **represents $p < 0.01$.

and CS-NCC composite coatings. These results partially agreed with those of Li *et al.* (2021), who studied the influence of CS-NCC composite coating on the preservation of *shatangju*. Chitosan is one of the most abundantly found polysaccharides in nature and has specific biocompatibility, biodegradability, film formation, and barrier properties (Ali *et al.*, 2011). These characteristics contribute to the maintenance of okra fruit weight. Adding NCC into CS solution did not improve the barrier properties of CS in our study, similar to the results observed in *citrus* (Li *et al.*, 2021), which may be related to the proportion of NCC in CS solution. Moreover, in our study, CS had a smaller molecular weight.

We found minimum decay rate of fruits coated with CS in Shuiguoguo (A) and Naiyou (B) cultivars, while coating with 2CS-CC had the lowest decay rate in *Hibiscus coccineus* (C), probably because of difference in breeds. As a polysaccharide with certain antibacterial activity, coating with CS solution forms a protective film on fruit surface to avoid infection of external pathogens, thus exerting an antibacterial effect close to that exerted by a chemical preservative. Adding NCC to CS solution can improve the tensile properties of composite film, thereby enhancing its structural stability and prolonging its shelf life (Khan *et al.*, 2013). Yu *et al.* (2020) reported a similar result in a study conducted on the effects of CS-NCC composite coatings for preservation of *red tangerine*. Unique 2CS-CC treatment demonstrated an advantage in *Hibiscus coccineus* (C), indicating that the effect of CS-NCC composite treatment depends on the breed of cultivar.

Data referring to the texture parameters of okra fruit did not show consistent changes between three cultivars

during storage. The hardness, gumminess, and chewiness of okra fruit in Shuiguoguo (A) were improved or maintained by CS-CC treatment after 6 days of storage, and springiness was improved after 14 days of storage compared to other treatments and control. In case of Naiyou (B) cultivar, CS-CC treatment increased or maintained the hardness, cohesiveness, gumminess, and chewiness of okra fruit after 14 days of storage and springiness after 6 days of storage, compared to other treatments and control. Except for chewiness and springiness, the remaining four parameters (hardness, gumminess, cohesion, and resilience) of okra fruit in *Hibiscus coccineus* (C) treated with CS-CC showed distinct improvement at 14 days of storage. However, springiness also improved at 6 days of storage, compared with other treatments and the control. Hence, CS-CC treatment had a positive role in maintaining okra fruit texture parameters during storage, which is due to the best compatibility of CS-CC treatment. A possible explanation is that the response of texture parameters to CS-NCC composite coatings varies, and the optimal proportion of specific cultivars deserves further study. Owing to the negatively charged sulfate (SO_4^{2-}) groups on cellulosic backbone (Huq *et al.*, 2012), NCC may affect the structure of CS. CS-CC can form a more hydrated but smaller complex (Wang *et al.*, 2017). The underlying mechanism of interaction between two component interactions is particularly complicated and could lead to an uncertain effect on fruit texture.

Concerning correlation analysis, hardness presented a significant positive correlation with gumminess and chewiness during storage ($P < 0.01$). Resilience of fruit was significantly positively correlated with cohesiveness ($P < 0.01$) and gumminess ($P < 0.05$), which agrees with

our previous results (Wang *et al.*, 2020), probably because of the characteristics of fruit texture parameters in okra.

Conclusion

Our data showed that differences in the effects of CS-NCC composite coating on preservation of okra fruit are based on the cultivar. 2CS-CC treatment showed significant positive results in reducing the incidence of fruit decay in *Hibiscus coccineus* (C). Furthermore, CS-CC treatment may be adapted to sustain the textural parameters of okra fruit during storage. As a green and safe preservative, CS-NCC is expected to have good applicability to the extension of okra shelf life.

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Conflict of Interest

The authors stated that they had no conflict of interest to declare.

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