

EFFECTS OF BIOLOGICAL EDIBLE FILM ON POST HARVEST PRESERVATION AND QUALITY OF APPLES (*MALUS DOMESTICA* BORKH.)

RUIMIN FU^{1*}, WEI TANG¹, HONG ZHANG¹, TIEQI XIA¹, XUE YANG¹,
DING WANG¹ AND WULING CHEN²

*College of Health Management, Henan Finance University, Zhengzhou,
Henan 450046, China*

Keywords: Apple, Fresh-keeping, *Bacillus amyloliquefaciens*, Chitosan, Biological edible film

Abstract

Chitosan, as a carrier of *Bacillus amyloliquefaciens*, is a biological edible film used to control the post harvest disease of Fuji apples. After coating the apples with chitosan, the preservation of Fuji apples in post harvest storage was measured by testing the weightless and decay rates; hardness; malondialdehyde (MDA), soluble solids (SS), titratable acid (TA), and Vitamine C (VC) contents; and all kinds of changes in oxidase activity (e.g., peroxidase, superoxide dismutase, and catalase). Results showed that the biological edible film can reduce the weightless and decay rates of the apples effectively, control the increase in MDA, and decrease TA, VC, and SS contents. These results indicated a good fresh-keeping effect.

Introduction

As one of the main fruits, apples are widely distributed in China, such as Shaanxi, Henan, Gansu, and Shandong. Because of the high water content and the rich nutrition of apples, they are susceptible to various pathogens, such as *Alternaria sp.* and *Penicillium sp.* Mechanical damage, such as vibration and squeezing, during the picking and transportation processes, provides opportunities for spore infection that leads to *Penicillium* infection (Chalupowicz *et al.* 2020). Post harvest diseases are likely to occur in the late storage period, leading to serious economic losses. Therefore, the anticorrosion and the fresh-keeping treatments of post harvest apples are of great practical importance.

Studies showed that the main pathogen causing infection is *Penicillium expansum* (Gong *et al.* 2020). Presently, the main way to control the expansion of *Penicillium* is still based on physical means, such as chemical fungicides and refrigeration (Larbi-Koranteng *et al.* 2020, Zhang *et al.* 2020). Although chemical fungicides can effectively control the growth of *Penicillium sp.* Chemical control methods have gradually become unacceptable with the continuous improvement of the pathogenic fungi's drug resistance and the people's awareness of health and environmental protection. Physical method, such as cold storage, can prevent the growth of pathogenic fungi to a certain extent but can easily affect the flavor and nutritional value of fruits, and improper handling causes frost damage. In addition, the cost of processing is relatively high. The biological control method that uses antagonistic organisms to inhibit the growth of pathogens for fruit preservation is a novel method. This new method of disease prevention after fruit picking will change the nutrition and flavor of the fruit and eliminate the hidden dangers caused by chemical agents to human health. Therefore, this method has become a popular research topic in fruit preservation recently (Timmermans *et al.* 2019, Xu *et al.* 2019, Zhang *et al.* 2019a, Obiekezie *et al.* 2020).

Bacillus amyloliquefaciens (BA-16-8) is a multi effect broad-spectrum antagonistic bacterium that is isolated and screened from the surface of apples in the laboratory (Fu *et al.* 2020a). In the early stage, *B. amyloliquefaciens* is safe and has a certain inhibitory effect on the various diseases

*Author for correspondence: <angelaminmin@163.com>. ¹Henan Finance University, Zhengzhou, 450046, China; ²Shaanxi Normal University, Xi an, 710000, China.

of apples after harvest (Fu *et al.* 2020b). Studies showed that chitosan, as deacetylation product, can effectively delays fruit maturation and senescence at a concentration of 1.5-2% and can be used as a fruit and vegetable film-forming agent for fruit preservation. However, chitosan alone has a limited preservative effect. Previous studies have reported that the combined treatment of chitosan and antagonistic yeast can remarkably increase the antagonistic efficacy of tomato fruit pathogens (Shi *et al.* 2019, Shoaib *et al.* 2019).

In the present study nchitosan with good film-forming properties and the fermentation broth of the *B. amyloliquefaciens* BA-16-8 strain with good antiseptic and bactericidal properties were mixed to prepare a bioactive fresh-keeping film, which was applied on the surfaces of apples. Effects of the film on preservation and the various indicators of apple quality, physiology, and biochemistry were studied to provide a theoretical basis for apple storage and preservation.

Materials and Methods

A fermentation broth of the *Bacillus amyloliquefaciens* BA-16-8 strain was cultured in *Penicillium. sp* spore suspension (10^6 CFU/ml) at 37°C for 24 hrs. The reagents used in this study were: Chitosan, vanillin, absolute ethanol, 2,6-dichlorophenol indophenol, ascorbic acid, oxalic acid, thiobarbituric acid, riboflavin, nitrogen blue tetrazole, methionine, sodium chloride, sodium hydroxide, and oxalic acid.

The instrument used were: Cold storage, electronic balance (1 in 10 000), HHW-21CU600 constant temperature water bath, GY-4 digital-display fruit hardness tester, ATAG digital-display hand held sugar meter, UV1800 UV spectrophotometer, Shimadzu GC-14A Gas chromatograph, DC1212 high-speed refrigerated centrifuge, Telaire7001 infrared carbon dioxide analyzer color meter, centrifuge tube, test tube, micropipette (5, 2 and 1 ml)

The test apples were red Fuji apples purchased from a well-managed orchard in Luochuan County, Shaanxi Province. The fruits were uniform in size, matured in consistency, and free of mechanical damage and disease. The fruits were transported to the laboratory immediately after harvest and refrigerated at 0°C and their surfaces were washed with tap water before testing. The apples were sterilized with sodium hypochlorite at a concentration of 0.1% for 1 min. Then, the residual sodium hypochlorite was rinsed with tap water. After drying, the apples were randomly divided into groups. The experiment was conducted in four treatments (Table 1). Groups of 30 apples were processed. After immersing the apples in liquid for 5 min, they were air dried and placed in a spore suspension of pathogenic bacteria. Subsequently, the apples were placed in a plastic basket at 30°C for 30 days. The fruit decay and weight loss rates of the apples were calculated, and quality analysis was performed by equation (1) and (2). The experiments were repeated three times.

The fruit decay and weight loss rates of the apples were calculated, and quality analysis was performed by equation (1) and (2). The experiments were repeated thrice.

Rotting rate (%) = (number of rotten fruits / total number of fruits inoculated with pathogenic bacteria) \times 100% (1)

Weight loss rate (%) = [(Fruit mass before storage–Fruit mass at test) / Fruit mass before storage] \times 100% (2)

B. amyloliquefaciens BA-16-8 was inoculated in Nutrient broth (NB) medium and cultured in a shaker at 30°C and 200 rpm for 24 hrs, and the cell concentration was adjusted to 10^5 , 10^6 , 10^7 , 10^8 , and 10^9 CFU/ml by using sterile water as the control. Then, 0.1 ml of each of the above solutions was added to 10 ml Potato Dextrose Broth (PDB) medium, and 0.1 ml 10^6 /ml of *Penicillium* spore suspension was added and cultured in a shaker at 28°C and 180 rpm for 18 hrs. The spore germination and the length of the germ tube extension were observed under a

microscope. At least 100 spores per treatment were observed, and the experiment was repeated thrice.

The *Penicillium* spore suspension (0.1 ml, 10^6 CFU/ml) was spread on a Potato dextrose agar (PDA) plate, and a hole (with the diameter of 8 mm) was punched at the center of the Petri dish. Subsequently, 0.1 ml *B. amyloliquefaciens* fermentation broth samples at concentrations of 10^5 , 10^6 , 10^7 , 10^8 and 10^9 CFU/ml in bacteria were added, with sterile water as the control and incubated at 25°C for 48 hrs to determine the diameter of the zone of inhibition. The experiment was repeated thrice.

In accordance with the above experiment, the optimal antagonistic fermentation broth concentration was 10^9 CFU/ml, and a chitosan solution was added to the fermentation liquid of *B. amyloliquefaciens* after fermentation for 32 hrs (to make the concentration of chitosan 2%) to obtain the bioactive film.

Table 1. Different treatments on coating apple.

Group number	Treatment
Control	Distilled water
A treatment 1	2% chitosan solution
B treatment 2	10^9 CFU/ml of <i>B.amyloliquefaciens</i> fermentation broth
C treatment 3	10^9 CFU/ml of <i>B.amyloliquefaciens</i> fermentation broth and 2% chitosan solution

Hardness determination was performed using the TA-XT2i texture analyzer (EI-Gendy *et al.* 2020). Total soluble solids (SS) content was determined using the Atago digital handheld sugar content meter (Makroo *et al.* 2019). Titratable acid (TA) was determined via acid–base titration (Simatende *et al.* 2019). The Vitamine C (VC) content was determined via molybdenum blue spectrophotometry (Najafi *et al.* 2019). Malondialdehyde (MDA) content was determined using the thiobarbituric acid method (Zhang *et al.* 2019b). The peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) were determined by the methods used by Ullah *et al.* (2019).

Results and Discussion

Effects of different concentrations of *B. amyloliquefaciens* fermentation broth on the germination of *P. expansum* are shown in Fig. 1. The fermentation broth of the strain can inhibit the spore germination and hypha extension of *Penicillium sp.* Effects on spore germination and mycelial growth inhibition were more evident. When the fermentation broth concentration was 10^8 and 10^9 CFU/ml, the germination rates of the pathogenic spores decreased to 10 and 2%, respectively, and the lengths of the spore filaments were 30 and 10 μ m, respectively.

Results of the bacteriostatic experiments by using the *B. amyloliquefaciens* fermentation broth with different concentrations on *Penicillium sp.* at 10^8 and 10^9 CFU/ml indicated that the diameters of the inhibition zone formed were not remarkably different. On the basis of the above experiments, the 10^9 CFU/ml concentration of *B. amyloliquefaciens* fermentation broth was selected for subsequent experiments (Fig. 2).

Rotten rate is an important indicator in evaluating the effectiveness of biological control. The fruit rotten rates under different treatments are shown in Fig. 3. The inhibition effect of the four different treatments on the apples' rotten rate were shown respectively: C (bioactive film treatment) > B (*B. amyloliquefaciens* fermentation broth) > A (chitosan film treatment) > Control (distilled water treatment). The treatment C can significantly ($P < 0.05$) reduce the apple fruit's

decay rate. The inhibition rate of decay in Group C was higher than that in Group B. This result might be due to the addition of the antagonistic fermentation broth of Group C. Moreover, chitosan can inhibit the entry of oxygen, playing an inhibitory effect in the growth of pathogenic bacteria.

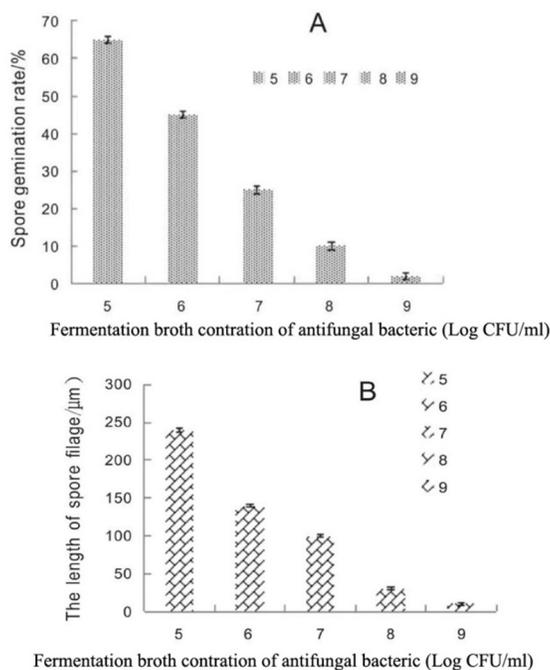


Fig. 1. Effect of different concentrations of *B. amyloliquefaciens* BA-16-8 on the spore germination of *P. expansum*. A shows Germination rate of *Penicillium expansum* spores, B shows length of germinated mycelial.

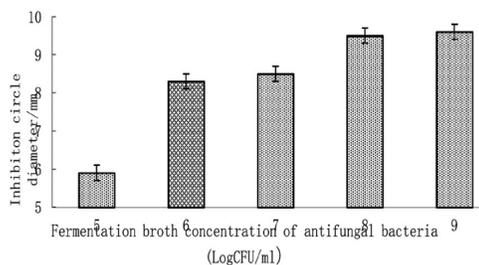


Fig. 2. Inhibition of different concentrations of *B. amyloliquefaciens* BA-16-8 on the spore germination of *P. expansum* on PDA.

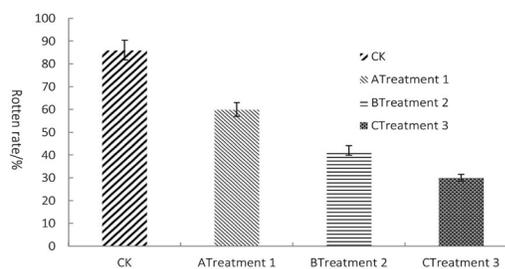


Fig. 3. Effects of different treatments on apple's rotten rate.

Hardness is an important index for evaluating the ripeness of fruit. Results of different treatments presented in Fig. 4, showed that the fruit hardness of each treatment decreased continuously, and the decrease trend followed the order: Control (CK) (distilled water treatment) > A (*B. amyloliquefaciens* fermentation broth) > B (chitosan film treatment) > C (bioactive film

treatment). The decrease in the control group's hardness was the most evident, and its difference from those of the other treatment groups was highly significant ($P < 0.01$). The decrease in the hardness of Group C was the lowest and can still reach 8.6 kg/cm^2 at 30 days. The chitosan film (Group B) maintained the hardness of the fruit better than the antibacterial fermentation broth (Group A). The film blocked the skin pores of the fruit to a certain extent, thereby inhibiting the respiration and metabolism of the fruit and slowing down the decline in hardness.

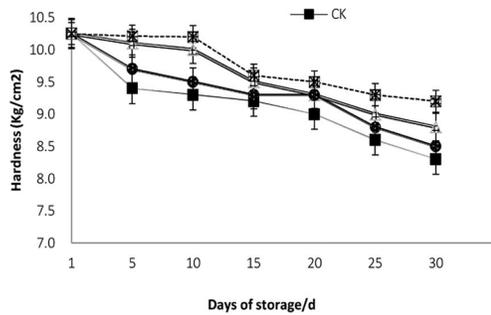


Fig. 4. Effects of different treatments on hardness of apples.

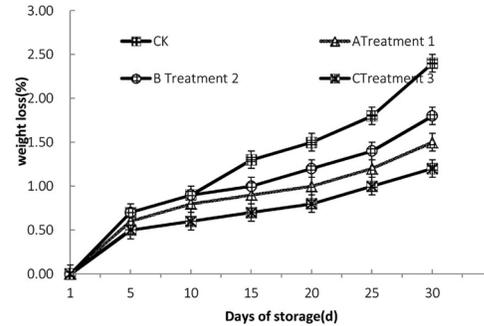


Fig. 5. Effects of different treatments on weight loss of apples.

Water is a necessary condition to ensure the normal physiological metabolism and maintain the quality of fruit cells. The main reason for freshness is water retention, and the weight loss of fruits during storage is mainly due to water evaporation. Therefore, the weight loss rate is an important indicator in measuring the freshness of fruits. The effects of each treatment on the weight loss rate of apples are shown in Fig. 5, which reveals that the weight loss rate of each treatment showed an increasing trend with increased storage time. It is evident that the weight loss rate increased in the control group. In group A, the C group added with the antagonistic fermentation broth showed a lower weight loss rate, indicating that the addition of the antagonistic fermentation broth was beneficial to the fruit's water retention.

Soluble solids (SS) content is an important indicator of apple quality and can reflect the nutrient content retained in the apple fruit. Results presented in Fig. 6 showed that the SS content of each treatment decreased with time especially in the control group. Throughout the process, the SS content of Group C (chitosan and antagonistic antibiotics) ranked third and had the lowest decline in materials, showing proper storage and freshness advantages.

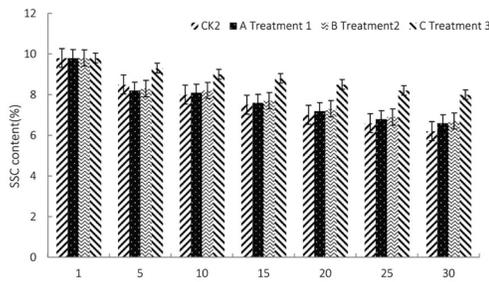


Fig. 6. Effects of different treatments on the soluble solids content of apples.

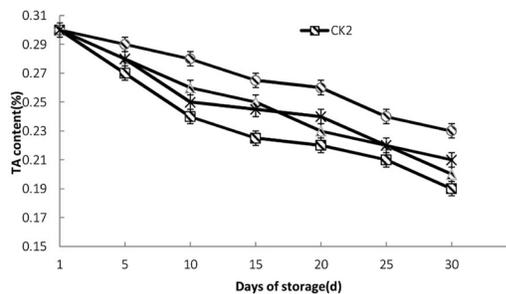


Fig. 7. Effects of different treatments on the titratable acid content of apples.

The titratable acid (TA) content of fruit has an important effect on its flavor, and the TA content is an important indicator of fruit quality. Effects of each treatment on the TA content of apples are shown in Fig. 7. During storage, the acid contents of fruits in all treatments decreased. The control group showed the most evident decline, whereas the group C showed the most gentle decline (i.e., speed of metabolism).

The Vitamine C (VC) in apple is an important component of its nutritional content and an important antioxidant that removes active oxygen and delays aging. The VC content under each treatment group decreased especially in the late storage period (Fig. 8). The VC content in the coating film treatment group was higher than that in the control group. The treatment effect of Group C was the best, and the VC content was the highest in the later storage period indicating a good fresh-keeping performance. This result can be attributed to the ability of the chitosan coating to prevent oxygen from entering and that of the antibacterial fermentation liquid to induce the synthesis of antioxidant enzymes in the fruit, which reduce the oxidation efficiency.

Malondialdehyde (MDA) is a toxic cell film lipid peroxide, and its accumulation can directly reflect the senescence of a fruit, that is, a high MDA content results in serious damage to the plant cell film. Figure 9 showed that the MDA in each group increased with time. The MDA in the control group rose the fastest, and the other groups all had different degrees of retardation especially in Group C, which was treated with bioactive films. The aldehyde rise was the most gradual, and the MDA content of the apples in this group was also the lowest in the later storage period. The MDA content of the control group was always higher than those of the other groups, indicating that the coating film treatment can effectively reduce the MDA content in fruits, thereby delaying fruit senescence. This phenomenon may be attributed to the ability of the chitosan film to reduce the contact efficiency of the fruit surface with oxygen and reduce the peroxidation of film lipids by reducing the respiration rate of the fruit, thereby reducing the degree of damage to the cell film.

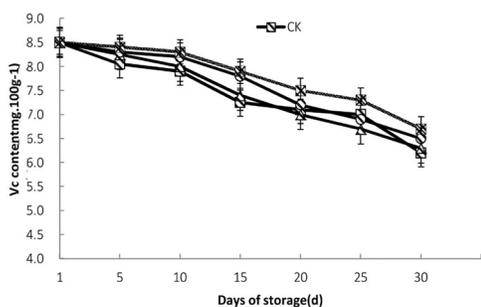


Fig. 8. Effects of different treatments on the VC content of apples.

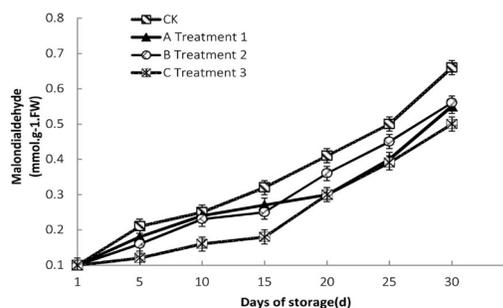


Fig. 9. Effects of different treatments on the MDA content of apples.

Peroxisome (POD), Catalase (CAT), and Superoxide dismutase (SOD) are important oxidases in living organisms. These enzymes scavenge free radicals and reactive oxygen species. The POD, CAT, and SOD contents directly affect how cells mature and are damaged. In particular, SOD has the effect of converting superoxide radicals. The SOD enzyme content of apples under each coating film treatment is shown in Fig. 10. After the coating film treatment, the SOD change in each treated apple was the first to manifest during the entire storage process. The trend showed rising and falling, and the treatments with antibacterial broth and chitosan composite film can maintain the SOD enzyme activity in the fruit to the highest extent, showing a good antiaging effect.

After SOD converts the superoxide anions in the crop into hydrogen peroxide, POD or CAT is needed to continue to break it into water and eliminate the harmful effects of superoxide anions on the body. Studies showed that POD is closely related to plant respiration, photosynthesis, and auxin oxidation, and its content is also positively related to the degree of senescence of crops. Therefore, POD is also an indicator of tissue aging. The apples' POD enzyme contents under each coating treatment, which had a similar trend to that of SOD (Fig. 11). The POD content in each treated apple increased first and then decreased, and the antibacterial and chitosan compound in the entire process. The film treatment can keep the fruit's POD at a low level, which again confirms that the film treatment can delay fruit senescence.

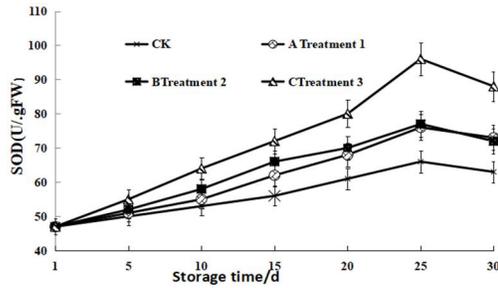


Fig. 10. Effects of different treatments on the SOD enzyme content of apples.

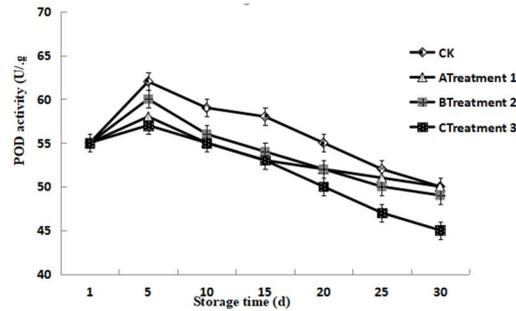


Fig. 11. Effects of different treatments on POD enzyme content of apples.

The CAT activities of apples during storage are shown in Fig. 12. The CAT activities of the three coating treatment groups were maintained for a longer time than that of the control group especially with the addition of antagonistic fermentation broth in Group B and the addition of the antagonistic fermentation broth and chitosan in Group C (i.e. the peak of the CAT activity was much higher than that of the control). The vitality decreased rapidly and was even lower than that of the chitosan-treated group at the 30-day test (Fig.12), indicating that the CAT expression induced by the pure antibacterial fermentation broth treatment on the apples was not stable. Compared with the results for Group C, the antibacterial fermentation The activity of CAT expressed by the fruit mixed with chitosan and fermentation liquid was higher and longer-lasting, which showed better anti aging and antiseptic effects.

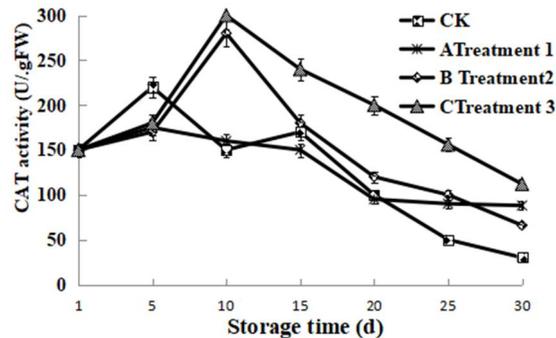


Fig. 12. Effects of different treatments on the activity of CAT of apples.

During the post harvest storage of apples, the weight loss and rotten rates are important indicators for measuring the freshness and preservation of fruits because apples are highly breathable during storage after harvest. Chitosan as a coating agent can block pores to a certain extent, reduce respiration intensity, and slow down water loss. Chitosan can effectively reduce the weight loss rate. In addition, the encapsulation of chitosan prevents apples and the external pathogenic fungus from expanding the contact degree of *Penicillium*, thereby exhibiting a certain antiseptic effect.

In the previous study, some researchers have reported the preparation of anti fungal biological edible films. Yang *et al.* (2021) used high pressure microfluidizer to encapsulate eugenol (EUG), carvacrol (CAR) and cinnamaldehyde (CA) in oil-in water nanoemulsion to produce the biological film. After exploring the antifungal effect of the biofilm against *Penicillium digitatum*, *P. digitatum* spore germination was significantly inhibited, and mycelial morphology was changed. Meanwhile, the soluble solids, vitamin C, and titratable acid contents degradation was delayed, while antioxidant enzyme activities were also significantly increased and maintained during post harvest storage. All these results were in accordance with the result obtained from the present study, which revealed the biological film had better antifungal effect and great potential in prolonging the storage period of fruits such as citrus fruits and apples.

As a commonly used biological control bacterium, *B. amyloliquefaciens* mainly acts as a preservative by secreting antibacterial lipopeptides for space and nutrition competition and can also induce apples to develop resistance and produce more SOD enzymes. Consequently, apples maintain good quality.

B. amyloliquefaciens BA-16-8, which is effectively antagonized by *Penicillium sp.*, was selected in this experiment and added to 2% chitosan. The immersion in the suspension of *Penicillium sp.* composite film, the antagonistic antibacterial effect, and chitosan are beneficial to fruit preservation, and the effects of antagonistic antibacterial and chitosan are the best. They can significantly reduce the weight loss rate, rot rate, and MDA of apples. Furthermore, by effectively inducing the production of SOD enzymes and reducing the content of POD enzymes, the rate of fruit oxidation is retarded. The composite film can maintain high VC, SS, and TA contents, further confirming the fresh-keeping effect of the composite film. In short, cross-linking the antibacterial broth and chitosan on the surface of the apple fruit can effectively maintain the freshness of the fruit and presents a new direction for the preservation of apples after harvest.

Acknowledgements

This work was financially supported by the state scholarship fund (China Scholarship Council Program No.10006), Key scientific research of Institution of higher learning in Henan Province(21B180002), and Henan Provincial Science and Technology Project (182102310995, and 182102110002).

References

- Chalupowicz D, Veltman B, Droby S and Eltzov E 2020. Evaluating the use of biosensors for monitoring of *Penicillium digitatum* infection in citrus fruit. *Sensors and Actuators B: Chemical* **311**: 127896.
- El-Gendy IR, Draz KA, El-Aw MA and Darwish HF 2020. Interaction among peel hardness, fruit-age and gibberellic acid on infestation susceptibility of citrus fruits by peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *African Entomology* **28**(1): 125-132.
- Fu RM, Yu F, Zhang YL and Chen WL 2020a. Fengycin controls the apple blue disease caused by *Penicillium expansum*. *Inter. J. Agricul. Biol.* **24**(4): 655-662.
- Fu RM, Zhang H, Chang HP and Chen WL 2020b. Evaluation of antifungal mechanism of *Bacillus amyloliquefaciens* BA-16-8. *Inter. J. Agricul. Biol.* **23**(2): 405-408.

- Gong L, Liu YF, Xiong YH, Li TT, Yin CX, Zhao JN, Yu JL, Yin Q, Gupta VK, Jiang YM and Duan XW 2020. New insights into the evolution of host specificity of three *Penicillium* species and the pathogenicity of *P. italicum* involving the infection of Valencia orange (*Citrus sinensis*). *Virulence* **11**(1): 748-768.
- Larbi-Koranteng S, Awuah RT and Kankam F 2020. Biological control of black pod disease of cocoa (*Theobroma cacao* L.) with *Bacillus amyloliquefaciens*, *Aspergillus sp.* and *Penicillium sp.* in vitro and in the field. *J. Microbiol. Antimicrobials* **12**(2): 52-63.
- Makroo HA, Prabhakar PK, Rastogi NK and Srivastava B 2019. Characterization of mango puree based on total soluble solids and acid content: Effect on physico-chemical, rheological, thermal and ohmic heating behavior. *LWT-Food Sci. Tech.* **103**: 316-324.
- Najafi A and Hashemi M 2019. Vortex-assisted natural deep eutectic solvent microextraction using response surface methodology optimization for determination of orthophosphate in water samples by molybdenum blue method. *J. Separation Sci.* **42**(19): 3102-3109.
- Obiekezie OP, Efiuvwevwere BJO and Eruteya OC 2020. Production, preservation and shelf-life evaluation of wine from banana fruit (*Musa acuminata Colla*). *J. Adv. Microbiol.* 47-61.
- Shi ZJ, Yang HY, Jiao JY, Wang F, Lu YY and Deng J 2019. Effects of graft copolymer of chitosan and salicylic acid on reducing rot of post harvest fruit and retarding cell wall degradation in grapefruit during storage. *Food Chemist.* **283**: 92-100.
- Shoab A, Awan ZA and Khan KA 2019. Intervention of antagonistic bacteria as a potential inducer of disease resistance in tomato to mitigate early blight. *Scientia Horticult.* **252**: 20-28.
- Simatende P, Siwela M and Gadaga TH 2019. Identification of lactic acid bacteria and determination of selected biochemical properties in emasi and emahewu. *South African J. Sci.* **115**(11-12): 1-7.
- Timmermans RAH, Mastwijk HC, Berendsen LBJM, Nederhoff AL, Matser AM, Van Boekel MAJS and Nierop Groot MN 2019. Moderate intensity Pulsed Electric Fields (PEF) as alternative mild preservation technology for fruit juice. *Inter. J. Food Microbiol.* **298**: 63-73.
- Ullah I, Al-Johny BO, AL-Ghamdi KMS, Al-Zahrani HAA, Anwar Y, Firoz A, AL-Kenani N and Almatry MAA 2019. Endophytic bacteria isolated from *Solanum nigrum* L., alleviate cadmium (Cd) stress response by their antioxidant potentials, including SOD synthesis by sodA gene. *Ecotoxicol. Environ. Safety* **174**: 197-207.
- Xu T, Chen Y and Kang H 2019. Melatonin is a potential target for improving post-harvest preservation of fruits and vegetables. *Frontiers in Plant Sci.* **10**: 388.
- Yang RP, Miao JY, Shen YT, Cai N, Wan CP, Zou LQ, Chen CY and Chen JY 2021. Antifungal effect of cinnamaldehyde, eugenol and carvacrol nanoemulsion against *Penicillium digitatum* and application in postharvest preservation of citrus fruit. *LWT-Food Sci. Tech.* **141**(1): 110924.
- Zhang XK, Li BQ, Zhang ZQ, Chen Y and Tian SP 2020. Antagonistic yeasts: a promising alternative to chemical fungicides for controlling postharvest decay of fruit. *J. Fungi* **6**(3): 158.
- Zhang YM, Holman BWB, Ponnampalam EN, Kerr MG, Bailes KL, Kilgannon AK, Collins D and Hopkins DL 2019a. Understanding beef flavour and overall liking traits using two different methods for determination of thiobarbituric acid reactive substance (TBARS). *Meat Sci.* **149**: 114-119.
- Zhang YB, Zhang Y, Zhu Z, Jiao XY, Shang YL and Wen YQ 2019b. Encapsulation of thymol in biodegradable nanofiber via coaxial eletrospinning and applications in fruit preservation. *J. Agricul. Food Chemist.* **67**(6): 1736-1741.