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Application of a chitosan coating as a carrier for natamycin to maintain the storage quality of ground cherry (*Physalis pubescens* L.)*

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Abstract: Ground cherry (*Physalis pubescens* L.) is a kind of berry fruit favored by consumers in China; however, this fruit is particularly vulnerable to physiological senescence and pathogen attack during the traditional cold storage period. In order to maintain storage quality, a 1.5% (w/w) chitosan (CS) water solution containing 50 mg/L of natamycin (NA) was introduced. After all treatments were completed, the fruit was stored at 0 °C and sampled every 10 d. At each sampling date, the following tests were performed: mold and yeast analyses; enzyme activity and content analyses which included superoxide dismutase (SOD), ascorbate peroxidase (APX), and malondialdehyde (MDA); and color analysis. In addition, a sensory evaluation was carried out for quality assessment at the end of the storage period. The results showed that the application of a chitosan coating combined with natamycin (CSNA) enhanced the activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX), reduced the physiological rate of senescence, and inhibited pathogen attack. Thus, CSNA treatment can maintain ground cherries at an acceptable level of storage quality for 50 d.

Key words: Chitosan coating; Natamycin; Storage quality; *Physalis pubescens* L.
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1 Introduction

The genus *Physalis* L. (Solanaceae) is for the most part wild, but some species are cultivated in countries such as Colombia, Mexico, China, Japan, and recently Brazil (Afsah, 2015). Ground cherry (*Physalis pubescens* L.) is one of the species and it is cultivated mainly in Northeast China. There are many other informal names in Chinese such as "gu niao" and "mao suan jiang". Its fruit is characterized by a spherical berry with a diameter between 1.25 and 2.50 cm, which turns from green to yellow during the ripening period. It is protected by a complete covering of a papery persistent calyx during its development and ripening (Luchese *et al*., 2015). The ripe fruit tastes sweet with a slightly acidic and pleasant flavor, and therefore ground cherry has been accepted as a fashionable, healthy, and nutritional fruit in recent years in China (Ji *et al*., 2013). However, it is difficult to preserve the fruit for any length of time due to the physiological senescence and pathogen attack that commonly occurs during storage.

In order to maintain storage quality, some measures must be taken to protect the fruit from physiological senescence and pathogen attack, and therefore a chitosan (CS) coating with natamycin (NA) was introduced. Chitin is a natural polymer found in the exoskeleton of crustaceans and insects, as well as in the cell walls of certain fungi, particularly zygomycetes (Moussa *et al*., 2013). CS is ordinarily produced by the deacetylation of chitin in a concentrated

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alkali solution (Sabaghi *et al*., 2015). CS has been widely used in the preservation of many fruits, such as grapes (Gao *et al*., 2013), walnuts (Sabaghi *et al*., 2015), and strawberries (Perdones *et al*., 2012), to name a few. A CS coating has been considered the most edible and biologically safe preservative coating for various types of foods because of its lack of toxicity, and its biodegradability, film-forming properties, and antimicrobial action (Pasquariello *et al*., 2015). The CS coating can form a thin film on the fruit surface, which can protect the fruit from pathogen attack and hinder oxygen and carbon oxygen exchanges between the inner fruit and outside atmosphere (Genskowsky *et al*., 2015). Moreover, CS can also induce the activity of various antioxidant enzymes to enhance the defense mechanism of the fruit's cell (Pasquariello *et al*., 2015).

With regard to ground cherry storage, another problem is the issue of pathogen attack on the fruit surface. Based on previous assays, we have found that the main pathogens to affect ground cherry during storage are molds, such as black mold, hairy mold, and blue mold. Ground cherry fruit is a succulent berry with a thin surface, so it is more vulnerable to these kinds of pathogen attacks. Pathogens release mycotoxins and enzymes such as cellulose and pectinase, which will degrade the fruit surface and cell wall and accelerate the process of senescence (Soliman *et al*., 2015; Salem *et al*., 2016). Therefore, NA was introduced to inhibit pathogen attacks by acting as a kind of fungicide from the polyene macrolide group. It is colorless, odorless, tasteless, and has a broad specificity against molds and yeasts. NA can bind to the ergosterol in the fungal cell membrane so as to induce cell death. In addition, NA can also form micelles at relative low solution concentrations, which, however, are very effective in inhibiting fungal cells. Moreover, it has a low water solubility, around 30–50 mg/L (Moatsou *et al*., 2015). Together with some coating materials, NA has been widely applied in the area of cheese preservation (Moatsou *et al*., 2015), and some fruits and vegetables such as Hami melons (Cong *et al*., 2007) and mushrooms (Akata *et al*., 2015).

As mentioned, NA has a low water solubility and its primary site of activity is on the surface of the fruit. Therefore, the application of NA usually requires certain coating materials as a carrier, such as gum arabic (Akata *et al*., 2015), tapioca starch (Olle Resa *et al*., 2014), polymer films (Hanusova *et al*., 2010), or CS (Fajardo *et al*., 2010). In this study, CS was applied as a carrier for NA as well as an edible film coated on the surface of the fruit. We studied the effects of CS combined with NA on the storage quality of ground cherry.

2 Materials and methods

2.1 Fruit samples

Fresh ground cherry fruit were harvested when ripe in a commercial orchard located in Hailun County, Heilongjiang Province, China, and then transported to a laboratory in Tianjin by airplane within 24 h at ambient temperature. After arrival, the fruit in the best condition were selected and the ones whose size or appearance were abnormal due to injury, mold, or infection were eliminated. The selected fruit were then placed in a pre-cooling room at 0 °C for 24 h. Before experimental treatment, the persistent calyx on all samples was removed and then the berries were sanitized by immersion in chlorinated water (200 μl/L) for 2 min.

2.2 Experimental materials

All reagents and solvents were of analytical grade and were purchased from a local commercial reagent company, except for some special reagents. Food-grade CS, with an approximately 95% degree of deacetylation, was purchased from Haidebei Marine Bioengineering Co., Ltd. (Jinan, China). The NA at 95% purity was purchased from Freda Biotechnology Co., Ltd., China. The method for the CS coating containing NA (CSNA) solution was based on Sabaghi *et al*. (2015) with a slight modification. The CSNA solution was prepared by dissolving 1.5 g of CS and 5 mg of NA in 100 ml sterilized distilled water containing 1% (w/w) acetic acid and 2% (v/v) Tween 80, using moderate magnetic stirring at room temperature for 1 h to achieve complete dispersion. The preparation for the CS coating solution was the same as the CSNA solution except that no NA was added.

2.3 Experimental treatments

All experimental treatments were performed in the pre-cooling room (0 °C with 90% relative humidity

(RH)). After the sterilized samples were drained, they were randomly divided into three groups for treatment: CSNA, CS, and the control groups. The samples from the CSNA, CS, and the control group were immersed in the corresponding solutions (control group was immersed in sterilized distilled water) for 2 min, and then removed to drain. After each treatment was completed, the fruit samples were packaged (1 kg/bag) and sealed in a polyethylene (200 mm length×300 mm width×30 μm thickness) plastic bag and then stored in a cold room at 0 °C and 90% RH.

2.4 Mold and yeast analyses

The pathogens that affect ground cherry during storage are mainly molds. Therefore, mold and yeast analyses were performed according to ISO 21527-2-2008 (International Organization for Standardization, 2008). Ten fruit were randomly sampled in each treatment and used for the mold and yeast analyses. All samples were weighed and then mashed with a sterile mortar, after which a series of decimal dilutions were conducted. Then, 1 ml of sample dilution was inoculated on the plate surface. All plates were cultivated at 25 °C for 5 d. The results were expressed in terms of log colony-forming unit (CFU)/g.

2.5 Color analysis

The external color of the fruit was evaluated with a chroma meter (CR 300, Konica Minolta Sensing, Inc., Osaka, Japan) equipped with a CR 300 measuring head and the results were expressed as l^*, a^*, and b^*. Generally, l^* represents luminosity, depending on the reflectivity of the determined surface; a^* and b^* represent chromaticity on a green (−) to red (+) axis and chromaticity on a blue (−) to yellow (+) axis, respectively. Measurement was carried out with four sites in the equatorial of each sample, and 10 fruit were used per treatment. The chroma meter was calibrated with a standard white tile (l^*=97.06, a^*=0.04, and b^*=2.01) before each series of measurements.

2.6 Enzyme extraction and activity assays

2.6.1 Superoxide dismutase activity

As described by Lu *et al*. (2015), the superoxide dismutase (SOD) activity of the samples was measured with a total SOD (T-SOD) detection kit (Nanjing Jiancheng Biological Engineering Inst., China). There were three replicates for each sample. The tissues from the samples were homogenized in a chilled 20 mmol/L Tris-HCl buffer (pH 7.1), and the homogenates were centrifuged at 1000 r/min for 20 min at 4 °C, then the supernatant fraction was used to determine activity following the manufacturer's instructions. Thus, 50 μl of this supernatant fraction was used to determine SOD activity, according to the calculation formula from the manufacturer's instructions based on absorbance values. The result was expressed in terms of U/g.

2.6.2 Ascorbate peroxidase activity

Ascorbate peroxidase (APX) activity was measured according to the method described by Ullah *et al*. (2016) with a slight modification. The sample's tissue was homogenized in a chilled 50 mmol/L potassium phosphate buffer (pH 7.5), and the homogenate was centrifuged at 1000*g* for 10 min at 4 °C; the supernatant fraction containing a crude APX enzyme then began reacting at 25 °C in a 1-ml medium that included 50 mmol/L, pH 7.5, potassium phosphate, 0.5 mmol/L ascorbate, and 0.1 mmol/L H_2O_2. We then monitored the decrease in absorbance at 290 nm for 1.5 min. One APX unit was defined as the amount oxidizing 1 μmol of ascorbate per minute at 25 °C. The result was expressed in terms of U/g.

2.7 Determination of malondialdehyde content

Malondialdehyde (MDA) content was evaluated by high-performance liquid chromatography (HPLC), according to Li *et al*. (2013). In brief, the liquid nitrogen-frozen samples were homogenized with 7.5% (v/v) trichloroacetic acid (TCA), and then centrifuged at 5000 r/min for 5 min. Thus, the extracted MDA reacted with 0.02 mol/L 2-thiobarbituric acid (TBA) and formed a kind of chromogen, after which the chromogen was injected into the HPLC for further analysis.

The chromatographic conditions were as follows: C18 reversed phase HPLC column (5 μm, 250 mm× 4.6 mm, Agilent Technologies, Palo Alto, CA, USA); column temperature, 35 °C; mobile phase, 0.05 mol/L potassium dihydrogen phosphate water solution-acetonitrile (82:18, v/v); flow rate, 1.0 ml/min; determination wavelength, 532 nm; injection volume, 10 μl. The limit of detection (LOD) and limit of quantification (LOQ) for the method were determined

as 4 and 8 μg/kg of the sample, respectively. The result was expressed in terms of μg/kg.

2.8 Sensory evaluation

Sensory evaluation was carried out to investigate the storage quality. After the storage period ended, in a manner described by Pasquariello *et al.* (2015), we stored the samples at 20 °C for 3 d to analyze the quality of the shelf life and the sensory evaluation was conducted using a nine-point category scale, which was based on appearance, color, visible structural integrity, and taste (Hernandez-Munoz *et al*., 2006). The sensory score ranging from 1 (dislike very much) to 9 (like very much) was used to mark the overall storage quality. For each treatment, 10 fruit were randomly selected for sensory evaluation, which was performed by 10 trained members.

2.9 Statistical analysis

All evaluations excluding the sensory evaluation were performed in triplicate every 10 d, and the first day of storage was defined as Day 0. All values were expressed as mean±standard deviation (SD). The difference between two treatments was analyzed by SPSS Version 19.0 software (SPSS Inc., Chicago, IL, USA). The result was considered significantly different if the P value was <0.05.

3 Results and discussion

3.1 Effects of different treatments on log CFU/g

Previous assays have demonstrated that the main pathogens affecting ground cherry fruit storage are molds, some of which can release enzymes such as cellulose and pectase, as well as some mycotoxins, which may accelerate the senescence process in the fruit (Matan *et al*., 2015). Therefore, it is important to inhibit these pathogens to maintain the fruit storage quality.

In the mold and yeast analyses, Day 0 was defined as the day when the samples had completed the pre-cooling process and before each treatment was conducted. As shown in Fig. 1, the log CFU/g values for all treatments varied at different rates. For the control group, the log CFU/g value was both constantly and significantly increased on each sampling date; especially from Day 30, the increased rate was higher and some very small pathogen colonies were visible on the surface of the sampling fruit according to the sensory evaluation, which showed that simplex cold storage cannot inhibit some low-temperature-resistant pathogens. For the CS and CSNA treatments, however, compared with Day 0, the log CFU/g values were both significantly decreased on Day 10, and the value of the CSNA treatment was decreased more than that of the CS treatment, which showed that both CS and NA can inhibit pathogens (mold and yeast); the differences between the CS and CSNA treatments were still significant on the subsequent sampling dates, especially during the later sampling dates. The log CFU/g value for the CS treatment was 3.26 on Day 50, while the CSNA was 2.04. The results showed that although CS can inhibit pathogens (Ramezani *et al*., 2015), simplex CS cannot maintain this inhibitory effect for a greater length of time, and CS combined with NA performs better than the single CS treatment.

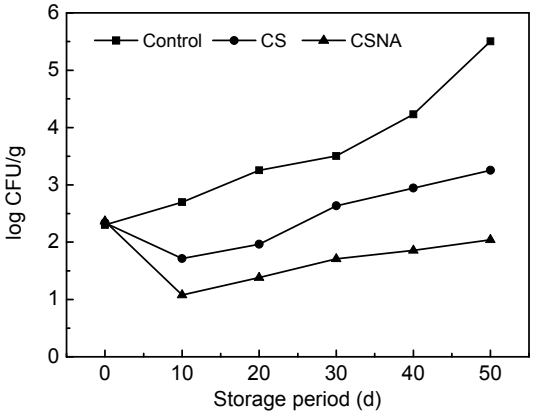

Fig. 1 log CFU/g values for the samples during storage

3.2 Effects of different treatments on external color

Color is an important nutritional and commercial indicator of proper fruit storage. External fruit color depends on the pigment compounds it contains. These compounds can be degraded depending upon many variables such as temperature, light, time, and oxygen during storage (Aral and Bese, 2016). In addition, physiological senescence and pathogen attack can also cause color degradation (Roidoung *et al*., 2016). As mentioned, this color analysis was based on l^*, a^*, and b^* analyses, in which l^*, a^*, and b^* represent

brightness, redness (+) or greenness (−), and yellowness (+) or blueness (−), respectively (Ayour *et al*., 2016). As shown in Fig. 2a, the l^* values for all treatments were decreased during storage, and all of them decreased faster in the latter half of the storage. However, there were significant differences in the l^* values between each two treatments from Day 20 of the storage, and the reduction in l^* values for CSNA were the smallest with the lowest decrease rate. On the other hand, the reduction in l^* values for the control group were the greatest with the highest decrease rate compared with the other two treatments. However, for the a^* values in Fig. 2b, we can see that the a^* values for all treatments increased with a faster rate during storage, while the a^* value for the control group increased to the highest among all the treatments at the end of storage. However, regarding the CSNA treatment, its a^* value was significantly lower than those of the CS and control groups during storage. Moreover, the trends in the b^* values were similar to the l^* values (Fig. 2c). From these three graphs, we concluded that compared with the control group and the single CS treatment, the CSNA treatment can slow the decrease in l^* and b^* values and the increase in a^* values during storage, thus maintaining the luminosity, greenness, and yellowness of the fruit surface. On the other hand, the color of fresh ground cherry is golden, which means the more luminosity, greenness, and yellowness there is, the better the fruit's appearance is going to be. Moreover, anthocyanins give color to fruits and vegetables and they also possess various biological activity including reactive oxygen species (ROS) scavenging activity. Thus, their contents are related to pathogen attack and some enzyme (such as SOD, APX) activity. We concluded that the CSNA treatment can maintain a better color quality for ground cherry than the other two treatments.

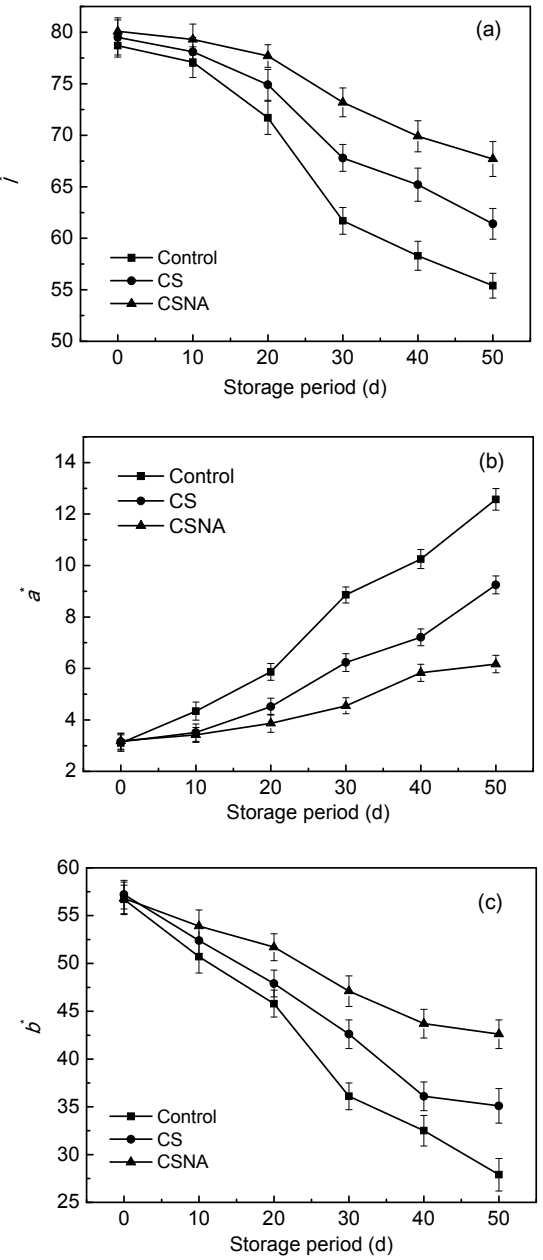

Fig. 2 Effects of different treatments on the l^* (a), a^* (b), and b^* (c) values during storage
Error bars indicate standard deviations (n=3)

3.3 Effects of different treatments on SOD and APX activity

ROS are a series of substances in fruit cells that include $O_2^{\bullet-}$, H_2O_2, $HO_2^{\bullet}$, and $OH^{\bullet}$ (Berto *et al*., 2015). ROS are related to some biochemical reactions in fruit, and the ROS levels will increase to defend the fruit cells when the fruit is stressed by negative circumstances or factors such as pathogen attacks, mechanical injury, or chilling injury, but high levels of ROS will, in turn, hurt the normal cells (Gundala *et al*., 2014; Ptackova *et al*., 2015). Therefore, some enzymes are involved in protecting the normal cells by scavenging ROS so as to maintain the ROS in cells at relatively reasonable levels. SOD and APX are two kinds of ROS scavenging enzymes (Kim *et al*., 2015).

By catalyzing the dismutation of toxic oxygen (radical superoxide), SOD can reduce the toxic oxygen to H_2O_2 and molecular oxygen (O_2). Although

H_2O_2 is still a kind of ROS, APX is involved in the detoxification of H_2O_2, using ascorbate as an electron donor to reduce H_2O_2 to H_2O (Sarowar *et al*., 2005). As shown in Figs. 3a and 3b, in the process of physiological metabolism, accumulation of substrates can incite the activity of responding enzymes according to Pasquariello *et al*. (2015). For the control group, the SOD activity and APX activity were both significantly increased to reach a peak on Day 20. However, for the CS and CSNA treatments, their SOD and APX activity increased to a peak on Day 30, which was later than the control. Moreover, compared with the control group, the SOD and APX activity for the CS and CSNA treatments were both significantly higher on Day 20, which shows that CS induces an effect on both SOD and APX activity. There were insignificant differences between the CS and CSNA treatments in both SOD activity and APX activity from Day 0 through Day 30; however, compared with the CSNA treatment, both SOD activity and APX activity for the CS treatment were significantly reduced after Day 30. According to Fig. 1, the pathogen attacks in the CS treatment were significantly increased after Day 30, which may cause the increase in ROS as well as the decrease in SOD and APX activity.

3.4 Effects of different treatments on MDA content

MDA is one of the most common reactive aldehydes, most of the activity of which results from the oxidative degradation of polyunsaturated fatty acids (PUFAs) of a cell membrane; its content is an indicator of membrane integrity (Luengwilai *et al*., 2014). MDA is also thought to be involved in the deterioration of various biological functions through its attachment to biomolecules such as proteins and nucleic acid (Yamauchi *et al*., 2008). As shown in Fig. 4, there were insignificant differences (P>0.05) in the MDA content between each two treatments on both Day 0 and Day 10, but a significant difference emerged between the CSNA and the control group from Day 20. Comparing the CS with the CSNA and control groups, respectively, there were insignificant differences on Day 20. The MDA content of the control group increased rapidly on the following sampling dates and reached a peak at (90.93±1.98) μg/kg on Day 40 and then was reduced to (77.40±2.90) μg/kg on Day 50, while for the CS and CSNA treatments, compared with Day 0, their MDA contents were constantly increased to about three-fold and two-fold, respectively, on Day 50. The results show that CS can decrease the accumulation of the MDA content, and CS combined with NA has a better decreasing effect on the accumulation of MDA.

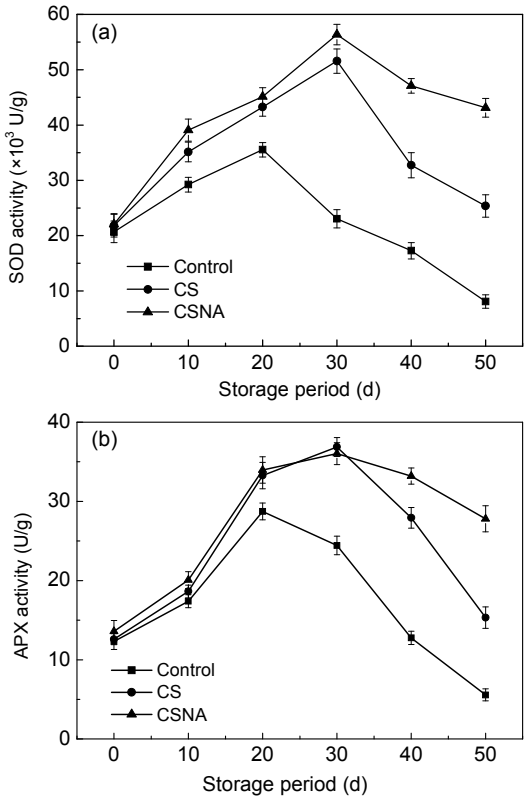

Fig. 3 Superoxide dismutase (SOD) (a) and ascorbate peroxidase (APX) (b) activity of the samples during storage
Error bars indicate standard deviations (n=3)

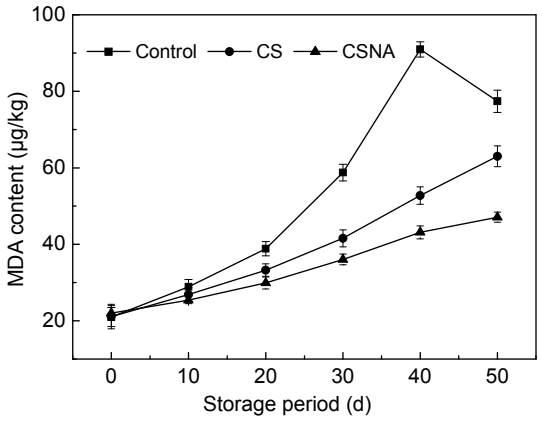

Fig. 4 Malondialdehyde (MDA) content of the samples during storage
Error bars indicate standard deviations (n=3)

3.5 Effects of different treatments on the sensory score

Sensory evaluation is a direct indicator of storage quality and commercial value (Phat *et al*., 2016). Thus, we stored the samples at 20 °C for 3 d after the storage ended to assay the storage quality during shelf life. As shown in Fig. 5, there were significant differences between any two sensory scores for the treatments based on storage quality. For the control group, the sensory score was the lowest at 1.2±0.3, which suggests the worst flavor or taste, appearance, and aroma. Compared with the samples on Day 0, the CSNA samples show little deterioration in quality, and their sensory score was the highest at 7.6±0.4 on Day 53. For the CS treatment, the sensory score for these samples was between the CSNA treatment and the control group at 5.4±0.5. The results show that the CSNA treatment can maintain a better storage quality for a longer period of time than the CS treatment.

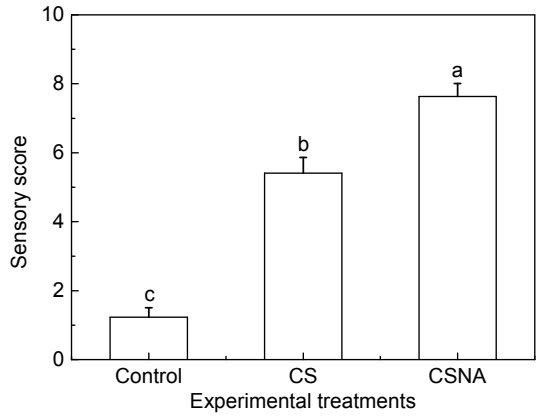

Fig. 5 Sensory score of the samples on Day 53 during room temperature storage

Error bars indicate standard deviations (n=3). a, b, and c represent statistically significant differences between different treatments

4 Conclusions

This study showed that the storage quality of ground cherry is associated with physiological senescence and pathogen attack. In order to maintain storage quality, a CS coating with NA was applied, because CS can induce several defensive genes and enzymes in plants (Pichyangkura and Chadchawan, 2015), which can slow the deterioration in quality by reducing oxidative stress. Moreover, when a CS film is formed on the surface of the fruit, it can protect the fruit from pathogen attack. Moreover, NA, acting as a fungicide, was introduced in combination with CS for a longer pathogen-resistant effect. Thus, a CSNA was proven to work as a valid tool in maintaining the storage quality of ground cherry.

Compliance with ethics guidelines

Xiao-lei HAO, Jiao-jiao ZHANG, Xi-hong LI, and Wei WANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Afsah, A.F.E., 2015. Survey of insects & mite associated cape gooseberry plants (*Physalis peruviana* L.) and impact of some selected safe materials against the main pests. *Ann. Agric. Sci.*, **60**(1):183-191. https://doi.org/10.1016/j.aoas.2015.04.005

Akata, I., Torlak, E., Erci, F., 2015. Efficacy of gaseous ozone for reducing microflora and foodborne pathogens on button mushroom. *Postharv. Biol. Technol.*, **109**:40-44. http://dx.doi.org/10.1016/j.postharvbio.2015.06.008

Aral, S., Bese, A.V., 2016. Convective drying of hawthorn fruit (*Crataegus* spp.): effect of experimental parameters on drying kinetics, color, shrinkage, and rehydration capacity. *Food Chem.*, **210**:577-584. http://dx.doi.org/10.1016/j.foodchem.2016.04.128

Ayour, J., Sagar, M., Alfeddy, M.N., *et al.*, 2016. Evolution of pigments and their relationship with skin color based on ripening in fruits of different Moroccan genotypes of apricots (*Prunus armeniaca* L.). *Sci. Hortic.-Amsterdam*, **207**:168-175. http://dx.doi.org/10.1016/j.scienta.2016.05.027

Berto, A., Ribeiro, A.B., de Souza, N.E., *et al.*, 2015. Bioactive compounds and scavenging capacity of pulp, peel and seed extracts of the Amazonian fruit *Quararibea cordata* against ROS and RNS. *Food Res. Int.*, **77**:236-243. http://dx.doi.org/10.1016/j.foodres.2015.06.018

Cong, F.S., Zhang, Y.G., Dong, W.Y., 2007. Use of surface coatings with natamycin to improve the storability of Hami melon at ambient temperature. *Postharv. Biol. Technol.*, **46**(1):71-75. http://dx.doi.org/10.1016/j.postharvbio.2007.04.005

Fajardo, P., Martins, J.T., Fucinos, C., *et al.*, 2010. Evaluation of a chitosan-based edible film as carrier of natamycin to improve the storability of Saloio cheese. *J. Food Eng.*, **101**(4):349-356. http://dx.doi.org/10.1016/j.jfoodeng.2010.06.029

Gao, P.S., Zhu, Z.Q., Zhang, P., 2013. Effects of chitosan-glucose complex coating on postharvest quality and shelf life of table grapes. *Carbohyd. Polym.*, **95**(1):371-378. http://dx.doi.org/10.1016/j.carbpol.2013.03.029

Genskowsky, E., Puente, L.A., Pérez-Álvarez, J.A., *et al.*,

2015. Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*). *LWT-Food Sci. Technol.*, **64**(2):1057-1062. http://dx.doi.org/10.1016/j.lwt.2015.07.026

Gundala, S.R., Yang, C.H., Mukkavilli, R., *et al.*, 2014. Hydroxychavicol, a betel leaf component, inhibits prostate cancer through ROS-driven DNA damage and apoptosis. *Toxicol. Appl. Pharm.*, **280**(1):86-96. http://dx.doi.org/10.1016/j.taap.2014.07.012

Hanusova, K., Stastna, M., Votavova, L., *et al.*, 2010. Polymer films releasing nisin and/or natamycin from polyvinyldichloride lacquer coating: nisin and natamycin migration, efficiency in cheese packaging. *J. Food Eng.*, **99**(4):491-496. http://dx.doi.org/10.1016/j.jfoodeng.2010.01.034

Hernandez-Munoz, P., Almenar, E., Ocio, M.J., *et al.*, 2006. Effect of calcium dips and chitosan coatings on postharvest life of strawberries (*Fragaria* x *ananassa*). *Postharv. Biol. Technol.*, **39**(3):247-253. http://dx.doi.org/10.1016/j.postharvbio.2005.11.006

International Organization for Standardization, 2008. Microbiology of Food and Animal Feeding Stuffs-horizontal Method for the Enumeration of Yeasts and Moulds-Part 2: Colony Count Technique in Products with Water Activity Less than or Equal to 0.95, ISO 21527-2-2008. International Organization for Standardization, Geneva.

Ji, L., Yuan, Y.L., Ma, Z.J., *et al*, 2013. Induction of quinone reductase (QR) by withanolides isolated from *Physalis pubescens* L. (Solanaceae). *Steroids*, **78**(9):860-865. http://dx.doi.org/10.1016/j.steroids.2013.05.008

Kim, Y.H., Lim, S., Han, S.H., *et al.*, 2015. Expression of both CuZnSOD and *APX* in chloroplasts enhances tolerance to sulfur dioxide in transgenic sweet potato plants. *Compt. Rend. Biol.*, **338**(5):307-313. http://dx.doi.org/10.1016/j.crvi.2015.03.012

Li, P.L., Ding, G.L., Deng, Y.F., *et al.*, 2013. Determination of malondialdehyde in biological fluids by high-performance liquid chromatography using rhodamine B hydrazide as the derivatization reagent. *Free Radic. Biol. Med.*, **65**: 224-231. http://dx.doi.org/10.1016/j.freeradbiomed.2013.06.032

Lu, X., Wang, C., Liu, B.Z., 2015. The role of Cu/Zn-SOD and Mn-SOD in the immune response to oxidative stress and pathogen challenge in the clam *Meretrix meretrix*. *Fish Shellfish Immun.*, **42**(1):58-65. http://dx.doi.org/10.1016/j.fsi.2014.10.027

Luchese, C.L., Gurak, P.D., Marczak, L.D.F., 2015. Osmotic dehydration of physalis (*Physalis peruviana* L.): evaluation of water loss and sucrose incorporation and the quantification of carotenoids. *LWT-Food Sci. Technol.*, **63**(2):1128-1136. http://dx.doi.org/10.1016/j.lwt.2015.04.060

Luengwilai, K., Beckles, D.M., Pluemjit, O., *et al.*, 2014. Postharvest quality and storage life of 'Makapuno' coconut (*Cocos nucifera* L.). *Sci. Hortic.-Amsterdam*, **175**: 105-110. http://dx.doi.org/10.1016/j.scienta.2014.06.005

Matan, N., Puangjinda, K., Phothisuwan, S., *et al.*, 2015. Combined antibacterial activity of green tea extract with atmospheric radio-frequency plasma against pathogens on fresh-cut dragon fruit. *Food Control*, **50**:291-296. http://dx.doi.org/10.1016/j.foodcont.2014.09.005

Moatsou, G., Moschopoulou, E., Beka, A., *et al.*, 2015. Effect of natamycin-containing coating on the evolution of biochemical and microbiological parameters during the ripening and storage of ovine hard-Gruyère-type cheese. *Int. Dairy J.*, **50**:1-8. http://dx.doi.org/10.1016/j.idairyj.2015.05.010

Moussa, S.H., Tayel, A.A., Al-Turki, A.I., 2013. Evaluation of fungal chitosan as a biocontrol and antibacterial agent using fluorescence-labeling. *Int. J. Biol. Macromol.*, **54**: 204-208. http://dx.doi.org/10.1016/j.ijbiomac.2012.12.029

Olle Resa, C.P., Jagus, R.J., Gerschenson, L.N., 2014. Effect of natamycin, nisin and glycerol on the physicochemical properties, roughness and hydrophobicity of tapioca starch edible films. *Mater. Sci. Eng. C Mater. Biol. Appl.*, **40**:281-287. http://dx.doi.org/10.1016/j.msec.2014.04.005

Pasquariello, M.S., Di Patre, D., Mastrobuoni, F., *et al.*, 2015. Influence of postharvest chitosan treatment on enzymatic browning and antioxidant enzyme activity in sweet cherry fruit. *Postharv. Biol. Technol.*, **109**:45-56. http://dx.doi.org/10.1016/j.postharvbio.2015.06.007

Perdones, A., Sanchez-Gonzalez, L., Chiralt, A., *et al.*, 2012. Effect of chitosan-lemon essential oil coatings on storage-keeping quality of strawberry. *Postharv. Biol. Technol.*, **70**:32-41. http://dx.doi.org/10.1016/j.postharvbio.2012.04.002

Phat, C., Moon, B., Lee, C., 2016. Evaluation of umami taste in mushroom extracts by chemical analysis, sensory evaluation, and an electronic tongue system. *Food Chem.*, **192**:1068-1077. http://dx.doi.org/10.1016/j.foodchem.2015.07.113

Pichyangkura, R., Chadchawan, S., 2015. Biostimulant activity of chitosan in horticulture. *Sci. Hortic.-Amsterdam*, **196**:49-65. http://dx.doi.org/10.1016/j.scienta.2015.09.031

Ptackova, N., Klempova, J., Oboril, M., *et al.*, 2015. The effect of cryptogein with changed abilities to transfer sterols and altered charge distribution on extracellular alkalinization, ROS and NO generation, lipid peroxidation and LOX gene transcription in *Nicotiana tabacum*. *Plant Physiol. Biochem.*, **97**:82-95. http://dx.doi.org/10.1016/j.plaphy.2015.09.009

Ramezani, Z., Zarei, M., Raminnejad, N., 2015. Comparing the effectiveness of chitosan and nanochitosan coatings

on the quality of refrigerated silver carp fillets. *Food Control*, **51**:43-48. http://dx.doi.org/10.1016/j.foodcont.2014.11.015

Roidoung, S., Dolan, K.D., Siddiq, M., 2016. Gallic acid as a protective antioxidant against anthocyanin degradation and color loss in vitamin-C fortified cranberry juice. *Food Chem*., **210**:422-427. http://dx.doi.org/10.1016/j.foodchem.2016.04.133

Sabaghi, M., Maghsoudlou, Y., Khomeiri, M., *et al.*, 2015. Active edible coating from chitosan incorporating green tea extract as an antioxidant and antifungal on fresh walnut kernel. *Postharv. Biol. Technol.*, **110**:224-228. http://dx.doi.org/10.1016/j.postharvbio.2015.08.025

Salem, M.Z.M., Zidan, Y.E., Mansour, M.M.A., *et al.*, 2016. Antifungal activities of two essential oils used in the treatment of three commercial woods deteriorated by five common mold fungi. *Int. Biodeter. Biodegr.*, **106**:88-96. http://dx.doi.org/10.1016/j.ibiod.2015.10.010

Sarowar, S., Eui, N.K., Young, J.K., *et al.*, 2005. Overexpression of a pepper ascorbate peroxidase-like 1 gene in tobacco plants enhances tolerance to oxidative stress and pathogens. *Plant Sci.*, **169**(1):55-63. http://dx.doi.org/10.1016/j.plantsci.2005.02.025

Soliman, S., Li, X.Z., Shao, S., *et al.*, 2015. Potential mycotoxin contamination risks of apple products associated with fungal flora of apple core. *Food Control*, **47**:585-591. http://dx.doi.org/10.1016/j.foodcont.2014.07.060

Ullah, S., Kolo, Z., Egbichi, I., *et al.*, 2016. Nitric oxide influences glycine betaine content and ascorbate peroxidase activity in maize. *S. Afr. J. Bot.*, **105**:218-225. http://dx.doi.org/10.1016/j.sajb.2016.04.003

Yamauchi, Y., Furutera, A., Seki, K., *et al.*, 2008. Malondialdehyde generated from peroxidized linolenic acid causes protein modification in heat-stressed plants. *Plant Physiol. Biochem.*, **46**(8-9):786-793. http://dx.doi.org/10.1016/j.plaphy.2008.04.018

中文概要

题　目：壳聚糖和那他霉素联合应用对毛酸浆贮藏品质的影响

目　的：通过壳聚糖和那他霉素在果蔬生理代谢和致病微生物抑制等方面的特点，达到减缓毛酸浆果实在贮藏期间的生理衰老和抑制致病菌发展，进而提高毛酸浆贮藏品质。

创新点：壳聚糖作为一种涂被剂，可以均匀地分布在果蔬表面。许多学者在研究中发现壳聚糖可以延缓果蔬的生理代谢。那他霉素作为一种真菌抑制剂，通常和涂被剂联合用于奶酪的贮藏防霉。本文的创新在于壳聚糖和那他霉素联合在毛酸浆贮藏中的应用。

方　法：按比例制备出壳聚糖水溶液，随后定量添加那他霉素并搅拌均匀。毛酸浆果实在浸泡一定时间后，捞出沥干。随后按照实验设计进行分组处理。在贮藏期内，定期测定菌落总数对数值、果实外部色差、超氧化物歧化酶（SOD）和抗坏血酸过氧化物酶（APX）酶活性、丙二醛（MAD）含量及感官评价等指标。最后进行总结分析。

结　论：单独使用壳聚糖时，可以延缓毛酸浆果实的生理衰老，但是难以抑制贮藏期间的致病微生物（主要是真菌类）；作为一种真菌抑制剂，那他霉素具有水溶性低的特点，难以单独使用。那他霉素与壳聚糖联合使用时，壳聚糖即可对毛酸浆果实起到生理作用，还可以作为那他霉素的载体，使其均匀分布在果实表面。二者联合使用既能延缓毛酸浆果实的生理衰老，又能抑制贮藏期间的致病菌，从而达到提高毛酸浆贮藏品质的目的。

关键词：壳聚糖；那他霉素；贮藏品质；毛酸浆