Effect of composite biological preservative containing *Lactobacillus plantarum* on postharvest litchi quality

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Received May 13, 2016
Revised September 8, 2016
Accepted September 16, 2016
Published online December 31, 2016

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pISSN 1226-7708
eISSN 2092-6456

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Abstract Biological preservatives containing live microorganisms are environmentally friendly and non-toxic substances used to preserve the quality of fresh fruits. This study investigated whether a composite biological preservative containing live *Lactobacillus plantarum* (designated as DN003) could preserve the quality of postharvest litchi fruits at high temperature and in humid environment. Postharvest litchi fruits were briefly soaked in DN003, then dried and stored at 29–33°C with 95–98% relative humidity; prochloraz treatment was included as positive control and non-treatment as negative control. In comparison with negative control group, litchi fruits in both DN003-treated and positive control groups better retained their appearance with lower polyphenol oxidase and peroxidase activities and showed higher concentrations of vitamin C, titratable acids, and total sugar content. These data demonstrated that the new composite biological preservative containing *L. plantarum* is promising to be used in the preservation of postharvest litchi fruit, particularly in high-temperature and humid environment.

Keywords: litchi, biological preservative, *Lactobacillus plantarum*

Introduction

Litchi (*Litchi chinensis* Sonn.) belongs to the family *Sapindaceae* and is native to China (1). As a tropical and subtropical fruit with high nutrition and commercial value, litchi is being widely cultivated in China, India, and Thailand (2). However, postharvest fresh litchi fruit is liable to quick deterioration, including pericarp browning as the major cause of downgrading their market value (3).

Pericarp browning is attributed to various factors such as degradation of anthocyanidin by polyphenol oxidase (PPO) and peroxidase (POD), dehydration, microbial contamination, and decreased antioxidant levels (4–6). For decades, research has been concentrated on postharvest preservation technology. Preservation methods comprise modified atmosphere, low temperature storage, chemical preservatives, coating preservation, and biological preservation (7–13). In practice, multiple methods have been combined according to their conditions and needs. Nonetheless, there are many disadvantages on employing current preservative methods, for example, low-temperature storage reduces the shelf-life of litchis and chemical preservatives cause residual problem. These problems are gaining more attention owing to people's increasing health concerns. Therefore, high efficiency, safety, stability, and low cost are the key factors in developing new preservation methods.

Biological preservatives containing live microorganisms take the advantages of the existing antagonism among microorganisms and have become a prospective alternative technology that can avoid residual chemicals. Biological preservatives are environmentally friendly and non-toxic. Jiang *et al.* (14) have reported that *Bacillus subtilis* and its crude extract could control wound-inoculated litchi pathogens. More recently, Gustavo *et al.* (13) showed that spraying of live *L. plantarum* onto postharvest litchi fruits could improve their quality in low-temperature and humid environment.

Southern China is a major producer of litchi; owing to its high temperature and humidity, the preservation of the quality of postharvest fresh litchi fruits has been a challenge. *L. plantarum* is a kind of beneficial bacteria, and it is widely used in food fermentation and commonly found in fermented food products such as pickles, cheeses, and fermented sausages (15). Moreover, *L. plantarum* is used in silage inoculants, therapeutics (16,17), antimicrobial property (18), biological antiseptic (19), and against AIDS-defining illnesses (20). This study investigated whether a composite biological preservative containing live *L. plantarum* (designated as DN003) could preserve the quality of postharvest litchi fruits in high-temperature and humid environment.

Materials and Methods

Microorganism *L. plantarum* (named DGNKZX003) was isolated from orchard soil, and 10 g orchard soil was placed into 100 mL sterile water with glass beads and vibrated for 20 min. The soil suspension was diluted 10^2 , 10^4 , and 10^6 times, and a 200- μ L dilution was spread on NA and PDA plates and cultured at 28–30°C for 48–72 h. Single colonies with distinct differences were further purified and identified. DGNKZX003 was cultivated in an MRS medium at 30°C after purification and identification.

Plant material and treatments Litchi fruit (*Litchi chinensis* Sonn. cv. qingpitian) at about 90% maturity was harvested from an orchard in Maoming city, Guangdong province, China. The fruits were sorted and randomly divided into three large groups. Group A was not treated and served as the negative control, group B was treated by prochloraz and served as the positive control. The group C was soaked in preservative DN003 (10^8 – 10^9 cfu/mL DGNKZX003 and 2% citric acid, 1% chitosan, and 1% sodium lactate) for 2 min. Each group included three parallel groups. Furthermore, three batches of litchi were subjected to the same treatment. After air-drying, all groups were put into foam boxes and stored at 29–33°C with 95–98% relative humidity.

Physiologic and biochemical index

Browning index and good fruit rate assessment: Browning index was assessed by the following scale: 0=no browning (excellent quality); 1=slight browning; 2=<1/5 browning; 3=1/5–1/2 browning; 4=1/2–3/4 browning; 5=>3/4 browning.

Browning index

$$= \frac{\sum(\text{browning scale} \times \text{corresponding fruit within each class})}{\text{total fruit}}$$

According to the browning index grading standard, the good fruit rate was calculated using the following formula.

$$\text{Good fruit rate (\%)} = \frac{\text{scale 0} + \text{scale 1} + \text{scale 2}}{\text{total fruit}} \times 100\%$$

Polyphenol oxidase (PPO) and peroxidase (POD) activity assay:

According to Jiang (21), frozen litchi pericarp (2 g) was homogenized with 10 mL of 0.2 M citrate-phosphate buffer and 0.4 g polyvinyl pyrrolidone (PVP) at 4°C. The homogenate was centrifuged at 15,000 $\times g$ for 15 min and the supernatant was collected as crude enzyme. PPO activity was assayed by the reaction of 3 mL reaction mixture containing 2.21 mL phosphate buffer (pH 6.8), 0.75 mL 0.2 M pyrocatechol, and 0.04 mL crude enzyme. The absorbance at 398 nm was recorded every 10 s. One unit of enzyme activity was defined as the amount of the enzyme that caused an increase of 0.001 in absorbance per minute.

POD activity was assayed by the following procedure referred to

Pang *et al.* (22): in a reaction of 3 mL reaction mixture containing 2.07 mL phosphate buffer (pH 6.8), 0.3 mL 0.08% H₂O₂, 0.6 mL guaiacol, and 0.03 mL crude enzyme. The absorbance at 470 nm was recorded every 10 s. One unit of enzyme activity was defined as the amount of the enzyme that caused an increase of 0.001 in absorbance per minute.

Measurement of anthocyanins in pericarp: This method was described by Chen and Wang (23). Pericarp (5 g) was extracted several times with 1% HCl until no red color remained. The extracts were merged and filtered till a constant filtrate volume of 100 mL was obtained. Further, 1 mL filtrate was diluted to 5 mL with 0.4 M KCl-HCl (pH 1.0) and 0.4 M sodium citric acid-sodium hydrogen phosphate buffer (pH 5.0). The absorbance at 510 nm was recorded.

$$\begin{aligned} &\text{Anthocyanins in pericarp (mg/g flesh weight (FW))} \\ &= \Delta A \times 5 \times 0.1 \times 1000 \times 445.2 / 29,600 \times 5 \end{aligned}$$

ΔA : A (pH 1.0)–A (pH 5.0)

5: dilution multiple

0.1: filtrate volume (L)

445.2: the molar mass of cyanidin 3-glucoside

29,600: the molar ratio absorption coefficient of cyanidin 3-glucoside

5: Pericarp weight (g)

Measurement of vitamin C, the total soluble solids, titratable acid, and total sugar content: The total soluble solids were determined by hand refractometer. Titratable acid, vitamin C, and the total sugar content were measured as described by Ning (24).

Statistical analysis Data were analyzed by OriginPro 8 and DPS7.05. Three replications and three sets of duplication data were used for analysis. $p < 0.05$ denotes significant difference.

Results and Discussion

Obvious distinctions between the negative control, prochloraz, and DN003 groups after six days stored at 29–33°C with 95–98% relative humidity were observed (Fig. 1). The appearance of litchi fruits in both prochloraz and DN003 groups was more attractive than that of the negative control group.

The browning indexes of the three groups were measured during the storage period (Fig. 2A). All three groups showed an increase of their browning indexes during the storage period; however, DN003 or prochloraz significantly inhibited the increase of the browning indexes. The browning index of both DN003- and prochloraz-treated groups was 2.6, whereas that of the negative control group was 4 ($p < 0.05$) after six days of storage. Moreover, both DN003- and prochloraz-treated groups exhibited significantly higher values of their good fruit rates than the negative control group (Fig. 2B). It is interesting to note that the treatment of litchi fruits by spraying of live *L. plantarum* in Gustavo *et al.* (13) did not significantly reduce the browning index; however, the effectiveness of DN003 in preserving

Fig. 1. The appearance of negative control (A), prochloraz group (B), and DN003 group (C) after six days stored at 29–33°C with 95–98% relative humidity.

the quality of litchi fruits in this study seemed to be more effective in high-temperature and humid environment than in low-temperature and humid environment. It can be concluded that both DN003- and prochloraz-treated groups maintained better appearance and significantly reduced the browning indexes of litchi fruits than the negative control group in high-temperature and humid environment. Moreover, DN003 was as effective as prochloraz in the maintenance of appearance and reduction of browning indexes.

PPO and POD are recognized as the main factors that cause the pericarp browning of postharvest litchi fruits (5,25). In this study, PPO and POD activities were assayed during the storage period (Fig. 3). In all groups, PPO activity showed a reduction trend in accordance with Underhill's work (26), and POD activity showed an increasing trend. Compared to the negative control group, both DN003- and prochloraz-treated groups showed lower PPO and POD activities during the entire storage period. The POD activities of the negative control group were significantly ($p < 0.05$) higher than that of the other groups. More interestingly, the PPO activity was initially increased in the negative control group, but both DN003 and prochloraz treatment inhibited the initial increase of PPO activity; whether this initial increase is critical to the appearance and browning index

Fig. 2. Browning indexes stored at 29–33°C with 95–98% relative humidity (A) and good fruit rate stored at 29–33°C with 95–98% relative humidity (B). ■, negative control; ○, prochloraz group; ▲, DN003 group. Different letters mean significant difference ($p < 0.05$).

needs further investigation. It could be summarized that DN003 as well as prochloraz significantly inhibited the PPO and POD activities.

Anthocyanins degradation has been believed to play a key role in pericarp browning (8,27,28). The content of anthocyanins was measured during the storage period (Fig. 4). DN003-treated group showed significantly higher concentration of anthocyanins than the negative control group (0.09185 mg/g·FW vs 0.06173 mg/g·FW) ($p < 0.05$). It is interesting to know that DN003-treated group also showed significantly higher concentration of anthocyanins than the prochloraz group (0.09185 mg/g·FW vs 0.07676 mg/g·FW) ($p < 0.05$). It could be summarized that DN003 was the most effective substance in inhibiting the degradation of anthocyanins.

In this study, four kinds of nutritional parameters (vitamin C, total soluble solids, titratable acids, and total sugar content) were determined during the storage period (Fig. 5). During this period, the change in vitamin C and titratable acids contents was significant for all groups; the DN003-treated group as well as prochloraz-treated group retained significantly higher vitamin C and titratable acids contents than the negative control group ($p < 0.05$) (Fig. 5); the change of total soluble solids and total sugar contents was not significant in all three groups (Fig. 6), but DN003- and prochloraz-treated groups retained significantly higher total sugar contents than the negative control

Fig. 3. Polyphenol oxidase (PPO) activity in litchi pericarp stored at 29–33°C with 95–98% relative humidity (A) and peroxidase (POD) activity in litchi pericarp stored at 29–33°C with 95–98% relative humidity (B). ■, negative control; ○, prochloraz group; ▲, DN003 group. Different letters mean significant difference ($p < 0.05$).

Fig. 4. Content of anthocyanins in litchi pericarp stored at 29–33°C with 95–98% relative humidity. ■, negative control; ○, prochloraz group; ▲, DN003 group. Different letters mean significant difference ($p < 0.05$).

group ($p < 0.05$) (Fig. 6B). In short, DN003-treated litchi fruits retained significantly higher nutrition than the negative control fruits, and DN003 was no less than effective than prochloraz.

In this study, an aqueous composition containing live *L. plantarum*, citric acid, chitosan, and sodium lactate (designated as DN003) worked as a composite preservative. All these substances play synergistic roles in litchi preservation. Citric acid helps to maintain the color of litchi pericarp, the main effect of chitosan is to form a

Fig. 5. Content of vitamin C in litchi flesh stored at 29–33°C with 95–98% relative humidity (A), content of titratable acid in litchi flesh stored at 29–33°C with 95–98% relative humidity (B); ■, negative control; ○, prochloraz group; ▲, DN003 group. Different letters mean significant difference ($p < 0.05$).

membrane on the litchi surface, sodium lactate has a bacteriostatic effect, and *L. plantarum* DN003 can inhibit *Penorophythora litchi*, *Colletotrichum gloeosporioides*, polyphenol oxidase, and peroxidase according to our published paper (29).

Live *L. plantarum*, citric acid, chitosan, and sodium lactate were used as single or composite preservatives in our pre-experiments, all preservatives resulted in unattractive appearance of the fruit after 4–5 days of storage in high-temperature and humid environment. The formulation of DN003 resulted in best preservation effects in this research after several experiments. DN003 was investigated for its effects on the preservation of the quality of postharvest fresh litchi fruits in high-temperature and humid environment. The data showed that DN003 significantly improved fruit appearance and good fruit rates and significantly inhibited the degradation of anthocyanins and PPO and POD activities. In addition, DN003 significantly reduced the loss of nutrients, including vitamin C, titratable acids, and total sugar. More importantly, DN003 was no less effective in the preservation of the quality of postharvest fresh litchi fruits than prochloraz, a known chemical preservative for litchi fruits. In conclusion, live *L. plantarum* is an effective preservative for postharvest litchi fruits in high-temperature and humid environment (29), and DN003 could be used to substitute chemical preservatives in the future for the preservation of postharvest litchi fruits.

Fig. 6. Contents of total soluble solids in litchi flesh stored at 29–33°C with 95–98% relative humidity (A), contents of total sugars in litchi flesh stored at 29–33°C with 95–98% relative humidity (B). ■, negative control; ○, prochloraz group; ▲, DN003 group. Different letters mean significant difference ($p < 0.05$).

Acknowledgments Financial support by the Science and Technology Project of the research institutions and colleges of Dongguan city, Guangdong province, China (No. 2011108101008).

Disclosure The authors declare no conflict of interest.

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