

# Effects of *Cryptococcus laurentii* and chitosan on postharvest decay and storage quality in stored peaches

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## ABSTRACT

The synthetic effects of *Cryptococcus laurentii* and chitosan on postharvest decay and the quality of peach fruit stored at 28°C were evaluated in this study. The results showed that the combined treatment with *C. laurentii* and 1% chitosan was the most effective way to control gray mold in peach fruit, which remarkably reduced the disease incidence and lesion diameter. Addition of chitosan increased the growth of *C. laurentii*, the population of which increased faster in the first 24 hours and reached a higher cell concentration than *C. laurentii* alone after 72 h. The combined treatment with *C. laurentii* and chitosan was the best to storage effect. Spray experiment showed that chitosan and *C. laurentii* effectively reduced the weight loss; maintain the firmness and the contents of total soluble solids of peaches during the storage. Moreover, the activities of polyphenol oxidase and peroxidase were markedly enhanced by the combination of *C. laurentii* and chitosan, which may explain its bacteriostasis mechanism.

**Keywords:** *Cryptococcus laurentii*, Chitosan, Biocontrol, Storage quality, Peach

## INTRODUCTION

Peach (*Prunus persica*), a model species in the Rosaceae, is one of the most popular and economically important tree crops, which is widely cultivated in all parts of the world. Peach fruit is deeply loved by people for its high nutrition and delicious taste over the world. However, due to the high temperature and large amount of temporary harvest in summer season, peaches are susceptible to mechanical damage, water loss and fungal infection, leading to decay and deterioration. The perishable characteristics of peach make the harvest decline in quality, causing great economic losses (Robertson et al., 1990). Several physicochemical treatment methods have been applied to reduce postharvest diseases and preserve fruit quality during the storage such as cold storage, vacuum packing, preservation packaging paper and fungicides (Palou et al., 2002; Gang et al., 2015; Du et al., 2017). These methods are expensive and some of them may face the problem of food safety with a potential risk on human health and environment (Droby, 2006). Thus, much attention has been paid to biological control methods in recent years, especially the antagonistic yeast (Janisiewicz and Korsten, 2002). Antagonist has been

regarded as the most promising alternative to germicide to control the decay and prolong the shelf life of fruits for its high efficiency and security (Sharma et al., 2009).

Strains of *Cryptococcus laurentii* have been widely studied for biological control of postharvest diseases in various fruits, including strawberries (Wei et al., 2014), apples (Roberts, 1991) and pears (Zhang et al., 2006). The yeast is mainly isolated from the surface and wounds of the fruit itself ensuring high security and can survive in the environment of low temperature, low oxygen and high carbon dioxide. The application of *C. laurentii* to certain fruit has shown effective inhabitation on gray mold and blue mold etc. and can maintain the weight, delay the decrease of firmness and avoid losing nutrition (Sharma et al., 2009;). However, antagonistic yeast alone usually cannot attain the expected germicidal efficacy as pesticides (Chand-Goyal and Spotts, 1996), thus numerous researches have focused on improving the antagonistic effect of *C. laurentii*. Antagonistic activity of antagonistic yeast against the pathogens has been found increased in several reports. The combination of physical method and antagonistic yeast can obviously improve the biocontrol effect of the

antagonistic yeast, including heat treatment (Conway et al., 2005), microwave (Zhang et al., 2006) and so on. Adding nutrients is one of the ways to improve the biocontrol effect of the yeast, including the addition of carbohydrates, amino acids and other micronutrients. It is reported that sucrose could enhance the tolerance of *Candida oleophila* to osmotic stress, thus improving its inhibitory effect on *Penicillium* (Rachid L et al., 2009). Antagonistic yeast combined with inorganic compounds that can promote its growth can effectively enhance its biocontrol effect, such as calcium chloride (Lima G et al., 2005), salicylic acid (Yu et al., 2007) etc. The use of yeast mixtures also showed higher effectiveness against *Penicillium expansum* in apples (Calvo et al., 2003).

Chitosan is a kind of film-coating material with antimicrobial properties and biological activity, which can keep fruit fresh and has been applied to the food preservation (Qiu et al., 2014). When chitosan water solution is applied to the surface of vegetables and fruits, a closed environment of low oxygen and high carbon dioxide can be formed, which can inhibit the respiration and bacterial growth, improve the glossiness and sensory quality of fruit and vegetable (Devlieghere et al. 2004). Recent studies have found that fruits treated with chitosan could significantly reduce the postharvest disease incidence and inhibit the growth of pathogens. Sweet cherries coated with chitosan could prevent and control various diseases effectively, such as *Penicillium* and brown rot disease (Romanazzi et al., 2003). Chitosan not only has convincing inhibitory effect on a variety of plant pathogens (bacteria, fungi) and viruses, but also can induce plant disease resistance as a kind of pathogenic elicitor. Reports indicated that chitosan-coating treatment to fruits could exhibit high antimicrobial activity against a wide variety of bacteria, fungi and other microorganisms (Alvarez, 2013). Preharvest spraying treatment of chitosan could improve the activities of PAL, PPO, POD in grapes, increase the total phenolic content of fruits and reduce the occurrence of natural diseases in fruits (Romanazzi et al., 2006; Romanazzi et al., 2010; Meng et al., 2008). These results all demonstrated chitosan exhibit high antimicrobial activity, broad antimicrobial spectrum, and non-toxic, harmless and was applied to fresh preserving of fruit and vegetable.

To our knowledge, there have been few reports on the synthetic effects of *Cryptococcus laurentii* and chitosan on postharvest decay and storage quality of peaches. Therefore, based on the years of experiments, the objective of this study was to investigate the effects of the *Cryptococcus laurentii* treatment alone or in combination with chitosan on the postharvest decay and to evaluate the fruit quality of peaches during the storage at ambient temperature.

## MATERIALS AND METHODS

### Fruit Material

Peach fruit (*Prunus persica*) cultivar "Baihua" were harvested at commercial maturity stage, selected for uniformity of size and ripeness. Any fruit with injuries and pathogen infections were removed. After the peach fruit were selected, the fruit samples were rinsed with fresh water and allowed to air dry at room temperature (28°C).

### Antagonist and Pathogen

The antagonistic yeast, *Cryptococcus laurentii* (2.2257) was purchased from China General Microbiological Culture Collection Center (CGMCC), and maintained on nutrient yeast dextrose agar (NYDA, containing 8 g nutrient broth, 5 g yeast extract, 10 g glucose and 20 g agar in 1 liter of distilled water) at 4°C before used. The yeast was grown in 250 mL flask containing 50 mL nutrient yeast dextrose broth (NYDB) for 24 h at 28°C on an incubator shaker at 160 rpm. Then, cells were centrifuged at 1007 g for 10 min and washed twice with sterile distilled water to remove the growth medium. The cell concentration was adjusted to 108 cells mL<sup>-1</sup> with sterile distilled water counted by a hemocytometer.

The pathogen *Botrytis cinerea* was isolated from the surface of the infected peaches and stored on potato dextrose agar (PDA) medium at 4°C. After being cultured at 28°C for 48h, the cell suspension was suspended with sterile distilled water. The spore concentration was adjusted to 104 cells mL<sup>-1</sup> by sterile distilled water using a hemocytometer.

### Efficacy of *C. laurentii* and Chitosan for Control of Gray Mold in Peach Fruit

Peach fruit were wounded (6-mm-diameter and 3-mm-deep) with a sterile cork borer and treated with 50 µL of one of the following: (1) *C. laurentii* (108 cells mL<sup>-1</sup>); (2) chitosan (w/v, 1%); (3) *C. laurentii* (1 × 108 cells mL<sup>-1</sup>) mixed with chitosan (w/v, 1%); (4) sterile distilled water. 4 hours later, 30 µL of pathogen spore suspension was injected into it. After the treatment, all fruit were sealed with polyethylene plastic film to maintain the relative high humidity and stored at room temperature (28°C). The number of infected peaches and the lesion diameter were recorded daily until the fruit of the control group were all rotted. There were three replicates of seven peaches per replicate per treatment and the experiment was conducted twice.

### Determination of Population Dynamics of *C. laurentii* in the Wounds of Peach Fruit

The peaches were treated as described above. The wounds were treated with 30 µL of *C. laurentii* (1 × 108 cells mL<sup>-1</sup>) cell suspension alone or in combination with chitosan (w/v, 1%). The population of the yeast was assessed at different

times (0, 12, 24, 48 and 72 h at 28°C) after the treatment. The wounded tissue was removed with a sterile cork borer (6-mm-diameter and 3-mm-deep) and ground with a mortar and pestle in 10 mL of sterile water at 4°C. The cells were counted using a hemocytometer. There were three replicates of three fruit per treatment and the experiment was repeated twice.

### Effect of Spraying *C. laurentii* and Chitosan on Postharvest Quality of Peach Fruit

The preservation effect of *C. laurentii* and chitosan on postharvest peaches was determined. Peach fruit were sprayed with the liquid of the following: (1) *C. laurentii* (108 cells mL<sup>-1</sup>); (2) chitosan (w/v, 1%); (3) *C. laurentii* (1×108 cells mL<sup>-1</sup>) mixed with chitosan (w/v, 1%); (4) sterile distilled water. After spraying, the fruits were stored at room temperature (28°C). The quality parameters of peaches were measured daily. There were three replicates of seven fruit per treatment and the experiment was repeated twice.

### Weight Loss and Firmness

The weight of peaches was measured by an MP2000-2 balance (± 0.05 g) (Shanghai Balance Instruments, China). The calculation formula of weight loss rate was as following:

Weight loss = (W1-W2)/W1 × 100%, where W1 represents the original weight, W2 represents the weight after storage.

Fruit firmness was measured by GY-3 firmness tester (Zhejiang Tuopu, China). The hardness was measured at 3 points at the maximum transverse diameter at the middle of each fruit after the skin was removed. The average value was taken.

### Total Soluble Solid

The total soluble solid was measured on a few drops of peach juice using a Refractometer (WZS-I, Shanghai Optical Instruments, China). Results were recorded and expressed as Brix (C. Larrigaudière et al., 2002).

### Polyphenol Oxidase (PPO) and Peroxidase (POD)

The PPO activity was measured using the method of Abbasi et al. (1998) with some modification. 0.5 g tissue from each fruit was homogenized in 10 mL of 0.1 M phosphate buffer (pH 7.0) and the supernatant was collected as enzyme extracts after centrifugation (7, 162 g 5 min at 4°C). The reaction mixture contained a total volume of 3 mL consisted of 2 mL phosphate buffer (pH 7.0), 0.5 mL Catechol and 0.5 mL enzyme extract. The absorbance at 410 nm against blank (prepared in the absence of enzyme) was recorded using a spectrophotometer (756MC). PPO activity was calculated according to the change in OD over a 2 min period and one unit (u) of enzyme activity was defined as the amount that caused a change of 0.001 in absorbance per minute.

The POD activity was measured by the method as described by Abbasi et al. (1998) with some modification. 0.5 g tissue from each fruit was homogenized in 5 mL of 0.1 M phosphate buffer (pH 7.0) and the supernatant was collected as enzyme extracts after centrifugation (7, 162 g 10 min at 4°C). The reaction mixture consisted of 50 mL 0.1 M phosphate buffer (pH 7.0), 28 µl guaiacol and 19 µL H<sub>2</sub>O<sub>2</sub>. The absorbance of 3 mL reaction mixture and 1 mL enzyme extract at 470 nm was recorded using a spectrophotometer (756MC). POD activity was calculated according to the change in OD over a 2 min period and expressed as ΔOD min<sup>-1</sup>g<sup>-1</sup> protein.

### Statistical Analysis

Experiments were performed using a completely randomized design. Origin software (version 9.1) was used for statistical analysis. Data were subjected to one-way analysis of variance (ANOVA). Mean separations were performed by Tukey's multiple range test. Differences at P<0.05 were considered significant.

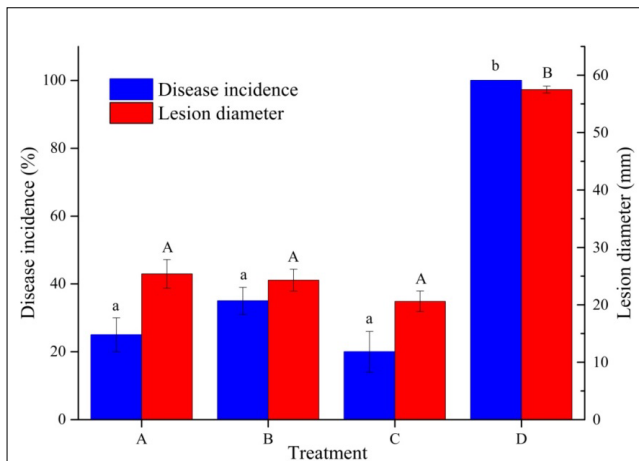
## RESULTS AND DISCUSSION

### Efficacy of *C. laurentii* and Chitosan for Control of Gray Mold in Peach Fruit

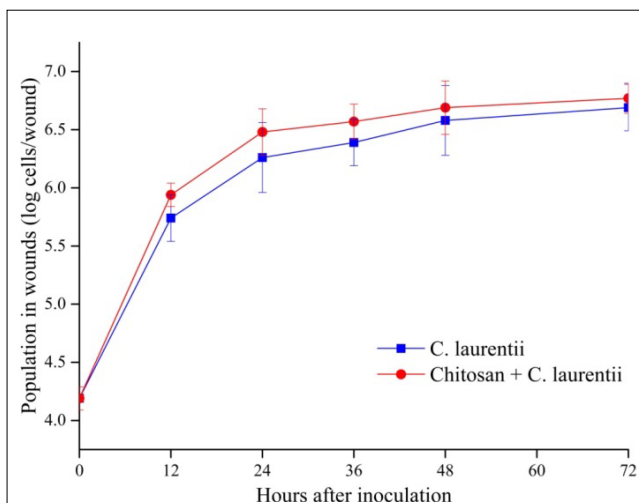
The application of *Cryptococcus laurentii* and chitosan could remarkably reduce the incidence of gray mold caused by *Botrytis cinerea* on peach fruit at ambient temperature. It was shown that the disease incidence and lesion diameter in all treatment groups were significantly lower than the control (P<0.05). The application of single chitosan and *C. laurentii* reduced the incidence of gray mold to 26% and 35% compared with the control 100% 4 days after inoculation, and lesion diameter from 57.5 mm to 25.4 mm and 24.3 mm respectively (Figure 1). The fruit treated with *Cryptococcus laurentii* in combination with 1% chitosan reduced the disease incidence to 20% and the lesion diameter to 20.6 mm, lower than the chitosan and *Cryptococcus laurentii* alone, more effective in controlling gray mold. There was no significant difference among the three treatments.

### Population Dynamics of *C. laurentii* in the Wounds of Peach Fruit

The population dynamics of *C. laurentii* in the peach wounds was investigated during the storage. As shown in Figure 2, the yeast population ranged from 5 × 10<sup>6</sup> CFU wound<sup>-1</sup> (at time 0) to 8.4 × 10<sup>7</sup> CFU wound<sup>-1</sup> (yeast alone) and 8.8 × 10<sup>7</sup> CFU wound<sup>-1</sup> (yeast mixed with chitosan) respectively during the 72 h after inoculation. The results showed that the population density of the antagonist in the wounds grew rapidly in the first 24 h after the inoculation, continued with the gradually stabilizes. It could be observed that the yeast population of yeast alone or combined with chitosan reached a higher cell concentration at the 72 h and the growth of the yeast applied with 1% chitosan was different



**Figure 1.** Efficacy of *Cryptococcus laurentii* and chitosan on disease incidence and lesion diameter of gray mold in peach fruit at 28°C. Wounds were treated with 50 ul of the following: A=*C.laurentii*,  $1 \times 10^8$  CFU/ml; B=1% chitosan; C=1% chitosan + *C.laurentii*; D=control, sterile distilled water, and then were inoculated with 30 ul *Botrytis cinerea* at  $1 \times 10^4$  CFU/ml. Bars represent the standard error.

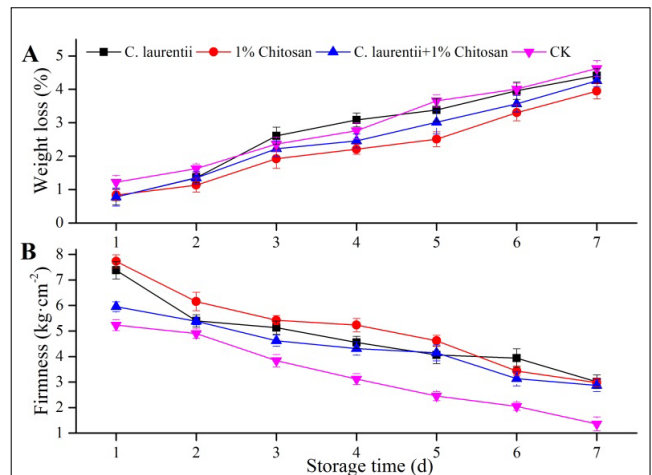


**Figure 2:** Effect of chitosan on growth of *Cryptococcus laurentii* in the wounds of peach fruit at 28°C. Wounds were treated with 30 ul of a cell suspension of *C. laurentii* at  $1 \times 10^8$  cells per ml alone or plus 1% chitosan. Bars represent the standard error.

from the yeast alone. The cell numbers of *C. laurentii* mixed with 1% chitosan were higher than those of isolate *C. laurentii*, 76% and 68% more than the initial number respectively up to 72 h.

#### Effect of Spraying *C. laurentii* and Chitosan on Fruit Weight Loss and Firmness

Effect of chitosan and *C. laurentii* alone or in combination on weight loss and firmness in this experiment was shown



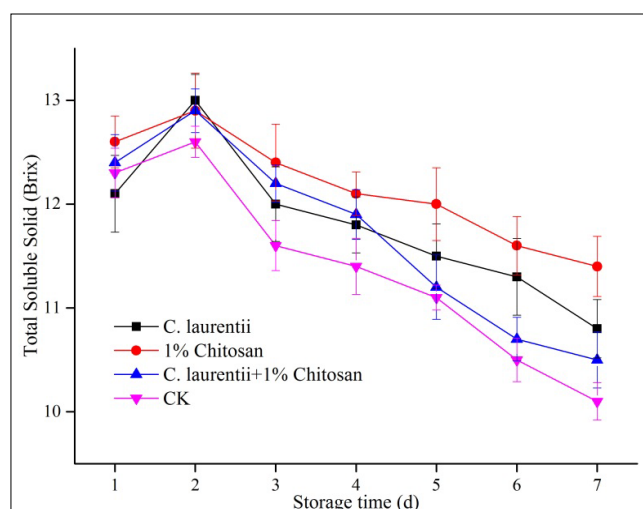
**Figure 3:** Effect of chitosan and *Cryptococcus laurentii* on (A) weight loss and (B) firmness of peach fruit during 7 days of storage at 28°C. Bars represent the standard error.

in Figure 3A. The weight of peach fruit was gradually decreasing in all treatments during the storage. The control recorded the highest weight loss compared with the other treatments. Least weight losses were found in 1% chitosan treated fruit, which reduced mass loss by 3.95%, followed with 4.26% in those of 1% chitosan mixed with *C. laurentii* and 4.41% in those of *C. laurentii* respectively after 7 days of storage at ambient temperature, whereas the untreated fruit only reduced weight loss by 4.61%.

Similar result was also found in firmness. Peach fruit softened rapidly during the storage at ambient temperature, especially in the first 4 d and then the decrease of firmness slowed down (Figure 3B). The reduction of firmness was remarkably higher in control than those in treated fruit. The firmness of peach fruit untreated ranged from  $1.48 \pm 0.06$  N to  $0.38 \pm 0.08$  N, 74% less than the firmness at harvest. Despite the firmness of peach fruit of all treatments and untreated decreased consistently, the fruit treated with 1% chitosan mixed with *C. laurentii* maintained the highest firmness throughout the storage time relatively, 51.9% lower than the firmness at harvest. The firmness of peach fruit applied with single *C. laurentii* and chitosan was  $0.85 \pm 0.08$  N and  $0.84 \pm 0.05$  N by the end of the storage time, which was 59.2% and 61.4% lower than those of peaches freshly harvested. It was shown that the combined treatment was more effective in maintaining firmness than other treatments.

#### Effect of Spraying *C. laurentii* and Chitosan on Total Soluble Solids

The changes in the contents of total soluble solids in peaches were investigated during the storage time at 28°C. As shown in Figure 4, the total soluble solids of four groups



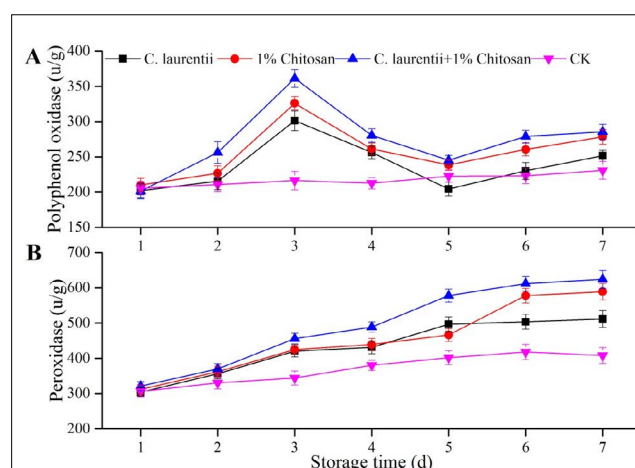
**Figure 4:** Effect of chitosan and *Cryptococcus laurentii* on total soluble solid of peach fruit during 7 days of storage at 28°C. Bars represent the standard error.

all presented the trend that first rised and then declined in turns along with the time. It was shown that at the end of the storage time, the content of total soluble solids of the control fruit was  $10.1 \pm 1.2$ , while in treated fruit they were  $10.8 \pm 0.58$ ,  $11.4 \pm 0.74$  and  $10.5 \pm 0.69$  respectively. Highest total soluble solids was observed in fruit treated with single chitosan that maintained 90.5% of the original, compared with that the *C. laurentii* mixed with 1% chitosan treatment maintaining 84.7%, single *C. laurentii* 89.3% and the control 82.1%. It was indicated that single chitosan treatment was more effective in maintaining the content of total soluble solids than other treatments.

### Effect of Spraying *C. laurentii* and Chitosan on PPO and POD Activity

The effect of *C. laurentii* and chitosan on the activities of polyphenol oxidase and peroxidase in peach fruit was evaluated during the storage. As can be seen in Figure 5A, for all fruit in treatment except the control, the activity of PPO presented the trend that increased first and then decreased with time during the storage. The PPO activity of fruit in the control only slightly increased during the storage. By contrast, treatments with *C. laurentii* and chitosan led to a rapid increase in PPO activity at day 2 and maintained a high level at day 7 of their vase life. The PPO activity of fruit treated with *C. laurentii* mixed with 1% chitosan ranging from 201.6  $\mu\text{g}$  to 285.6  $\mu\text{g}$  recorded the maximum by the end of the storage. Peach fruit treated with single chitosan and *C. laurentii* had a little lower PPO activity than the maximum but still much higher than the control at the same time.

As shown in Figure 5B, the activities of POD presented a steady increment trend in all treatments during the storage



**Figure 5:** Effect of chitosan and *Cryptococcus laurentii* on (A) PPO activity and (B) POD activity of peach fruit during 7 days of storage at 28°C. Bars represent the standard error.

period. Similarly, small increase was found in the POD activity of the control. The POD activity in all treatment groups was higher than that of the control group. Peach fruit treated with *C. laurentii* mixed with 1% chitosan was noted the highest POD activity compared with the other two treatments, followed by the single chitosan treatment and single *C. laurentii* treatment. It was indicated that the combined treatment was more effective in increasing PPO and POD activity.

## DISCUSSION

### Biocontrol Effect of *C. laurentii* and Chitosan on Gray Mold

In this study, the efficacy of chitosan and *Cryptococcus laurentii* on the postharvest decay and quality of peach fruit was evaluated. Our results showed that the incidence of gray mold in peach fruit was significantly reduced by single *C. laurentii* and chitosan, which was in accordance with previous studies. The antagonistic yeast *C. laurentii* had an inhibitory effect on various pathogens such as *Penicillium expansum* and *Glomerella cingulata* (Sugar et al., 1999; Blum et al., 2005). The application of high-molecular and low-molecular weight chitosan coatings on postharvest green asparagus efficiently inhibited the radial growth of *Fusarium concentricum*, which did not show any apparent sign of phytotoxicity and maintained good quality (Qiu et al., 2014). What is worth noting is that peach fruit applied with *C. laurentii* mixed with 1% chitosan recorded the lowest disease incidence and lesion diameter, which may indicate that chitosan can improve the antagonism of *C. laurentii*. This result was in contrary to past studies that no enhancement of plant induced resistance was found when the antagonistic yeast and chitosan were used together. Reports showed that

single yeast (*Candida oleophila* and *Candia saitoana*) and single chitosan could both stimulate resistance responses of fruit but combination of the yeast and chitosan did not reach a good result (Fajardo et al., 1998; De et al., 2002). However, it is remarkable that the concentration of chitosan used in their experiments was relatively high and their research did not show the effect of chitosan on the growth of yeasts. On the other side, related research suggested that glycolchitosan mixed with *C. saitoana* exhibited a positive synergistic effect on diseases of apples and citrus, the premise of which was that chitosan did not affect the growth of *C. saitoana* on fruit. Whereas when *C. saitoana* was applied with the chitosan-chloride which could inhibit the growth of yeast, the biocontrol effect on diseases of fruit decreased significantly (El-Ghaouth et al., 2000). This may reveal the mechanism that 1% chitosan improved the antagonistic effect.

#### Effect of Chitosan on the Growth of *C. laurentii*

Current results in this experiment showed that combined treatment with *C. laurentii* and 1% chitosan did not limit the growth of *C. laurentii* but slightly promoted the growth of yeast, though no significant difference was found between them. This conclusion is consistent with the result of previous study that chitosan at low concentration (0.01 to 1%, w/v) applied with *C. laurentii* did not influence the population growth of the yeast (Yu et al., 2007). It is generally accepted that the main antagonistic mechanism of postharvest antagonistic yeast including *C. laurentii* is nutrition and space competition (Filonow, 1998). Therefore, one of the prerequisites for the integration of antagonistic yeast and other disease control methods is to ensure that yeast growth is not seriously affected for which the number of yeast populations determines the ability to compete in nutrition and space. It has been found that high concentration of chitosan significantly inhibited the growth of *C. laurentii* (Yu et al., 2007). Previous studies also suggested high concentration of other additives such as chitin, salicylic acid and calcium chloride etc. abrogated antagonist growth (Lima et al., 2005; Yu et al., 2006; Yu et al., 2007). The application of antagonistic yeast to postharvest fruit helps to occupy the wound position earlier and quickly consume nutrients from fruit wounds, so that pathogens cannot grow. In that case, fruit and vegetable can be more durable to protect against pathogens during the storage. Therefore, a low concentration of chitosan (1%) was applied with *C. laurentii* on the basis of previous studies and its positive biocontrol effect on gray mold of postharvest peach fruit was played in this study.

#### Effect of *C. laurentii* and Chitosan on Physiological Changes of Peach

The establishment of plant induced resistance is always accompanied by changes in resistance related indicators,

especially the enzymes. Polyphenol oxidase plays a major role in enzymatic browning of fruit and the increase of PPO activity will negatively affect the quality of fruit (Mayer, 1987; Frank et al., 2007). PPO can catalyze the formation of lignin and other phenolic oxidation products, form a protective shield to resist the invasion of pathogens and play a direct role in the disease resistance by forming quinones. Our results showed that the activities of PPO in peach fruit spraying with *C. laurentii* and chitosan decreased gradually after a rapid increase first. Similar results can also be found that fruits treated with *C. laurentii* such as apples, strawberries showed high activity of PPO during the storage period (Wei et al., 2014; Roberts, 1990). Peroxidase is an important antioxidant enzyme that can catalytic decomposition of  $H_2O_2$  in lignin biosynthesis (Vander., 1998; Mohammadi., 2002). High POD activity is considered to be a physiological and biochemical marker of resistance induction of plants. Our results showed that fruit in all treatments exhibited higher POD activity than the control. Previous research works have also reported that fruit such as grapes treated with chitosan and *C. laurentii* induced higher POD activity (Meng et al., 2010). Inducible resistance experiments and determination of physiological indexes related to resistance of fruit indicated that chitosan and *C. laurentii* alone or in combination can not only stimulate fruit resistance to pathogens, but also can stimulate the enzyme activity of fruit resistance such as PPO and POD. These enzymes are closely related to fruit resistance or ripening and senescence, and the rapid activation of these resistance related enzymes may imply the positive effect of fruit tissues on the elicitors.

The fruit were still an organism that is constantly metabolized after packing. Meanwhile, they cannot get the supply of water and other substances through the tree body but use their own moisture and material to maintain their life activities. With the extension of storage time, the weight and firmness of fruit gradually decreased and therefore the resistance to mechanical damage and the invasion of external fungi decreased. Our results showed that *C. laurentii* and chitosan used alone or in combination both decreased the weight loss and maintained the firmness in varying degrees in which the combined treatment performed the best. Chitosan has good spontaneous film formation that can form a selective membrane preventing the entry of  $O_2$ , limiting the discharge of  $CO_2$ . It can effectively reduce the respiration intensity of fruit and allow ethylene to pass through. And it can prevent the transpiration of fruit, thus reducing the weight loss of fruit and vegetable (Pen et al., 2003). Lots of studies have found chitosan coating treatment improved storage quality and prolonged the storage period (Rabea et al., 2003; Coma, 2008). All these above show that *C. laurentii* combined with 1% chitosan has a good effect on maintaining the quality of fruit during storage.



## CONCLUSION

In conclusion, the combined treatment with *C. laurentii* and chitosan showed the best inhibiting efficacy on gray mold in postharvest peach fruit. Antagonistic yeast *Cryptococcus laurentii* and chitosan both have good inhibitory effects on postharvest diseases but the combined treatment performed the best in reducing disease incidence and lesion diameter, indicating that the antibacterial activity of *C. laurentii* was improved when amended with 1% chitosan compared with that without chitosan. The increase in the population growth of *C. laurentii* with the addition of 1% chitosan illustrates this point. Moreover, spraying treatment with 1% chitosan combined with *C. laurentii* effectively alleviated weight loss and decrease of firmness, increased PPO and POD activity. It was suggested the combination of 1% chitosan and *C. laurentii* could control postharvest decay and maintain fruit quality of peach during the storage time. All this work presented the potential use of combined *C. laurentii* and 1% chitosan in anticorrosion and preservation of postharvest fruit and vegetable.

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## CONFLICT OF INTEREST

There are no conflicts of interest to declare.

## REFERENCES

- Abassi NA, Kushad MM, Endress AG (1998). Active oxygen-scavenging enzymes activities in developing apple flowers and fruits [J]. *Scientia Horticulturae*, 74(3):183-194.
- Alvarez MV, Ponce AG, Moreira MDR (2013). Antimicrobial efficiency of chitosan coating enriched with bioactive compounds to improve the safety of fresh cut broccoli. *LWT - Food Science and Technology*. 50(1): 78-87.
- Blum LEB, Amarante CVT, Valdebenitosanhueza RM (2005). Postharvest application of the yeast *Cryptococcus laurentii* reduces apple rots. *Acta Hort.* 682: 2109-2114.
- Calvo J, Calvente V, Orellano MED, Benuzzi D, de Tosetti MIS (2003). Improvement in the biocontrol of postharvest diseases of apples with the use of yeast mixtures. *Biocontrol*. 48(5): 579-593.
- Chand-Goyal T, Spotts RA (1996). Control of postharvest pear diseases using natural saprophytic yeast colonists and their combination with a low dosage of thiabendazole. *Postharvest Biol. Technol.* 7(1-2): 51-64.
- Coma V (2008). Bioactive packaging technologies for extended shelf life of meat-based products. *Meat Sci.* 78(1-2): 90.
- Conway WS, Leverentz B, Janisiewicz WJ, Saftner RA, Camp MJ (2005). Improving biocontrol using antagonist mixtures with heat and/or sodium bicarbonate to control postharvest decay of apple fruit. *Postharvest Biol. Technol.* 36(3): 235-244.
- de Capdeville G, Wilson CL, Beer SV, Aist JR (2002). Alternative disease control agents induce resistance to blue mold in harvested 'red delicious' apple fruit. *Phytopathology*. 92(8): 900-908.
- Devlieghere F, Vermeulen A, Debevere J (2004). Chitosan: Antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiol.* 21(6): 703-714.
- Droby S (2006). Improving quality and safety of fresh fruit and vegetable after harvest by the use of biocontrol agents and natural materials. *Acta Hort.* 709: 45-52.
- Du X L, Li H, Zhou WH, Liu Y, Li JL (2017). Determination of quality changes in peaches wrapped in active paper and stored at ambient temperature in summer. *Sci Rep.* 7(1): 11830.
- El-Ghaouth A, Smilanick JL, Wilson CL (2000). Enhancement of the performance of *Candida saitoana*, by the addition of glycolchitosan for the control of postharvest decay of apple and citrus fruit. *Postharvest Biol. Technol.* 19(1): 103-110.
- Fajardo J E, Mccollum T G, Mcdonald R E, Mayer RT (1998). Differential induction of proteins in orange flavedo by biologically based elicitors and challenged by *Penicillium digitatum* Sacc. *Biological Control*. 13(3): 143-151.
- Filonow AB (1998). Role of competition for sugars by yeasts in the biocontrol of gray mold of apple. *Biocontrol Science & Technology*. 8(2): 243-256.
- Franck C, Lammertyn J, Ho QT, Verboven P, Verlinden B, et al. (2007). Browning disorders in pear fruit. *Postharvest Biol. Technol.* 43(1): 1-13.
- Gang C, Li J, Chen Y, Chen Y, Wang Y, Li H, et al. (2015). Synergistic effect of chemical treatments on storage quality and chilling injury of honey peaches. *J. Food Process. Preserv.* 39(6): 1108-1117.
- Janisiewicz WJ, Korsten L (2002). Biological control of

- postharvest diseases of fruits. *Annu Rev Phytopathol.* 40(1): 411-441.
- Larrigaudière C, Pons J, Torres R, Usall J (2015). Storage performance of clementines treated with hot water, sodium carbonate and sodium bicarbonate dips. *Journal of Pomology & Horticultural Science.* 77(3): 314-319.
- Lima G, Spina AM, Castoria R, De Curtis F, De Cicco V (2005). Integration of biocontrol agents and food-grade additives for enhancing protection of stored apples from *Penicillium expansum*. *J. Food Prot.* 68(10): 2100-2106.
- Mayer AM (1986). Polyphenol oxidases in plants-recent progress. *Phytochemistry.* 26(1): 11-20.
- Meng XH, Li BQ, Liu J, Tian S (2008). Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage. *Food Chem.* 106(2): 501-508.
- Meng XH, Qin GZ, Tian SP (2010). Influences of preharvest spraying *Cryptococcus laurentii* combined with postharvest chitosan coating on postharvest diseases and quality of table grapes in storage. *LWT - Food Science and Technology.* 43(4): 596-601.
- Mohammadi M, Kazemi H (2002). Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. *Plant Sci.* 162(4): 491-498.
- Palou L, Usall J, Muñoz JA, Smilanick JL, Viñas I (2002). Hot water, sodium carbonate, and sodium bicarbonate for the control of postharvest green and blue molds of clementine mandarins. *Postharvest Biol. Technol.* 24(1): 93-96.
- Pen LT, Jiang YM (2003). Effects of chitosan coating on shelf life and quality of fresh-cut Chinese water chestnut. *LWT - Food Science and Technology* 36(3): 359-364.
- Qiu M, Wu C, Ren G, Liang X, Wang X, et al. (2014). Effect of chitosan and its derivatives as antifungal and preservative agents on postharvest green asparagus. *Food Chem.* 155(4): 105.
- Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W (2016). Chitosan as antimicrobial agent: applications and mode of action. *Biomacromolecules.* 4(6): 1457.
- Rachid L, Mhaïssam J (2009). Enhancement of the biocontrol agent *Candida oleophila* (strain O) survival and control efficiency under extreme conditions of water activity and relative humidity. *Biological Control* 51(3): 403-408.
- Roberts RG (1991). Postharvest biological control of gray mold of apple by *Cryptococcus laurentii*. *Phytopathology.* 80(6): 526-530.
- Robertson JA, Meredith FI, Horvat RJ, Senter SD (1990). Effect of cold storage and maturity on the physical and chemical characteristics and volatile constituents of peaches (Cv. Cresthaven). *J. Agric. Food Chem.* 38(3): 620-624.
- Romanazzi G, Gabler FM, Smilanick JL (2006). Preharvest chitosan and postharvest UV irradiation treatments suppress gray mold of table grapes. *Plant Disease.* 90(4): 445-450.
- Romanazzi G, Nigro F, Ippolito A, Divenere D, Salerno M (2010). Effects of pre- and postharvest chitosan treatments to control storage grey mold of table grapes. *Journal of Food Science.* 67(5): 1862-1867.
- Romanazzi G, Nigro F, Ippolito A (2003). Short hypobaric treatments potentiate the effect of chitosan in reducing storage decay of sweet cherries. *Postharvest Biol. Technol.* 29(1): 73-80.
- Sharma RR, Singh D, Singh R (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biological Control.* 50(3): 205-221.
- Sugar D, Spotts RA (1999). Control of postharvest decay in pear by four laboratory-grown yeasts and two registered biocontrol products. *Plant Disease.* 83(2): 155-158.
- Vander P, Km VR, Domard A, Eddine El Gueddari N, Moerschbacher BM (1998) Comparison of the ability of partially N-acetylated chitosans and chitoooligosaccharides to elicit resistance reactions in wheat leaves. *Plant Physiol.* 118(4): 1353-1359.
- Wei Y, Mao S, Tu K (2014). Effect of preharvest spraying *Cryptococcus laurentii*, on postharvest decay and quality of strawberry. *Biological Control* 73(3): 68-74.
- Yu T, Chen J, Chen R, Huang B, Liu D et al. (2007). Biocontrol of blue and gray mold diseases of pear fruit by integration of antagonistic yeast with salicylic acid. *Int. J. Food Microbiol.* 116(3): 339-345.
- Yu T, Li HY, Zheng XD (2007). Synergistic effect of chitosan and *Cryptococcus laurentii* on inhibition of *Penicillium expansum* infections. *Int. J. Food Microbiol.* 114(3): 261-266.
- Yu T, Wu PG, Qi JJ, Zhenga XD, Jiang F, et al. (2006). Improved control of postharvest blue mold



rot in pear fruit by a combination of *Cryptococcus laurentii* and gibberellic acid. *Biological Control*. 39(2): 128-134.

Zhang H, Zheng X, Su D (2006). Postharvest control of blue mold rot of pear by microwave treatment and *Cryptococcus laurentii*. *J. Food Eng.* 77(3): 539-544.