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Effects of chitosan coating on enzymatic browning and decay during postharvest storage of litchi (*Litchi chinensis* Sonn.) fruit

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Abstract

Litchi (*Litchi chinensis* Sonn. cv. Huaizhi) fruit were treated with aqueous solutions of 1.0 or 2.0% chitosan coating 1 h after dipping in 0.1% thiabendazole (TBZ), and then stored at 4°C and 90% relative humidity (RH). Changes in browning, anthocyanins, flavonoids, total phenolic contents, polyphenol oxidase (PPO) and peroxidase (POD) activities, and weight loss were measured. The effects of chitosan coating on decay control were also evaluated. The application of chitosan coating delayed changes in contents of anthocyanin, flavonoid, total phenolics, delayed the increase in PPO activity, reduced weight loss, and partially inhibited the increase in POD activity. All these changes corresponded to changes in browning. The application of chitosan coating partially inhibited decay of fruit during storage. However, increasing the concentration of chitosan coating did not significantly increase the beneficial effects of chitosan on browning and decay of the fruit. © 1997 Elsevier Science B.V.

Keywords: Browning; Chitosan coating; Litchi (*Litchi chinensis* Sonn. cv. Huaizhi); Peroxidase (POD); Polyphenol oxidase (PPO); Storage decay

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a tropical fruit of high commercial value in the international fruit market. However, within 2 or 3 days after harvest its pericarp becomes desiccated and turns brown; it decays and its flavour is lost. Storing the fruit for any longer than 3 or 4 days without treatment is difficult. Pericarp browning reduces its commercial value and has long been considered the main postharvest problem (Akamine, 1960). The browning of litchi fruit pericarp after harvest is the result of polyphenol oxidase activity (Guangdong Postharvest Research Group, 1975; Tan and Zhou, 1987; Lin et al., 1988a,b), desiccation (Scott et al., 1982), changes in anthocyanins (Underhill and Critchley, 1994), attack by pathogens (Chen, 1984), and other unknown factors. At

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present, rapid cooling after harvest and storage at low temperatures with high humidity, treating with fungicides and other preservatives, modifying the atmosphere surrounding the fruit, appropriate packaging, and treating with SO_2 and acids are the most prevalent methods for maintaining the appearance and quality of litchi fruit (Chen et al., 1986; Chen and Zhang, 1988; Nip, 1988; Zauberman et al., 1991). However, a complete solution to storage problems is not available, and the use of fungicides on fruits needs strict control due to potential health risks (Wilson and Wisniewiski, 1989).

Tissue browning of fruits is due to cellular breakdown leading to mixing of browning-related enzymes and substrates, which results in enzymatic oxidation in the presence of oxygen (Ju and Zhu, 1988). Therefore, delaying or reducing enzymatic oxidation should be an important way to extend storage life and preserve the quality of the fruit.

Application of semi-permeable coatings has been shown to improve the storability of perishable crops (Lowings and Cutts, 1982). For example, application of Pro-Long coating to bananas delayed ripening through modifying the internal atmospheres (Banks, 1984), and ripening was delayed in pears and apples coated with Nutri-Save® (Davis et al., 1988). However, little research on semi-permeable coatings for litchi fruit has been done. York (1994) demonstrated that pericarp browning of litchi fruit was delayed significantly by two polysaccharide coatings. Zhang et al. (1997) found that an edible coating based on sucrose esters of fatty acids significantly delayed pericarp browning of litchi fruit.

Chitosan, a high molecular weight cationic polysaccharide, is soluble in dilute organic acids, and could theoretically be used as a preservative coating material for fruits. It can inhibit the growth of several fungi (Allan and Hadwiger, 1979; El Ghaouth et al., 1989, 1991), induce chitinase, a defense enzyme (Mauch et al., 1984), and elicit the production of phytoalexin in pea pods (Kendra and Hadwiger, 1984). Also, due to its ability to form a semi-permeable film (Bai et al., 1988), chitosan coating might be expected to modify the internal atmosphere as well as decrease transpiration losses in fruits. A research study by El Ghaouth et al. (1991) indicates that chitosan coating has the potential to prolong the storage life and control decay of strawberries even at higher storage temperatures, and has the ability to modify internal atmospheres in strawberries. Feeding trials with domestic animals have recently demonstrated that chitosan is non-toxic and biologically safe (Hirano et al., 1990).

The objective of our research was to assess the potential of chitosan coating in maintaining appearance and controlling decay of litchi fruit during postharvest storage.

2. Methods and materials

2.1. Plant material

Litchi (*Litchi chinensis* Sonn. cv. Huaizhi) fruit were harvested in local farms, Guangdong, China and transported to the research laboratory within 2 h. Fruits of uniform size with 80% red colour, free of physical damage, injury caused by insects, and fungal infection were used, and were distributed randomly into groups of 15 fruit. Each group represented one replicate, and for each treatment three replicates were used. Three duplicate experiments were set up. Fruit were dipped in 0.1% TBZ (thiabendazole, Deco Chemicals), dried for 1 h, and then treated with aqueous solutions of 1.0 or 2.0% chitosan. To prepare 100 ml of 1.0 or 2.0% chitosan solutions, 1.0 or 2.0 g of chitosan (Crab-shell chitosan, Sigma Chemicals) was dispersed in 100 ml of distilled water to which 2 g of L-glutamic acid was added, and the mixture was heated to dissolve the chitosan. Tween 80 (0.1 ml) was added to the solution to improve wettability. Fruit were allowed to dry for 1 h after dipping. Fruits dipped in 0.1% TBZ alone were regarded as the control. They were stored at 4°C, 90% RH. Here we used TBZ-treated fruit as the control and chitosan treatments in combination with TBZ as treatments in order to extend storage time for the assessments of browning, enzyme activities, and total phenolics.

2.2. Browning assessment

Browning of fruit was assessed by measuring the extent of the browned area on each fruit pericarp on the following scale: 0 = no browning; 1 = slight browning or a few browning spots; 2 = less than 1/4 browning; 3 = 1/4–1/2 browning; 4 = more than 1/2 browning. The browning grade was calculated using the following formula: Browning grade = Σ (browning scale × proportion of corresponding fruit within each class).

2.3. Assays of contents of anthocyanin, total phenolics, and flavonoids

Contents of anthocyanins, total phenolics, and flavonoids were measured according to Pirie and Mullins (1976). Litchi fruit peel (2 g) were extracted with 1% HCl–methanol (10 ml), the homogenate was filtered and washed, and the filtrate was diluted with 1% HCl–methanol to 50 ml. Absorption of the diluent was measured at 600 and 530 nm for anthocyanins, 325 nm for flavonoids, and 280 nm for total phenolics. Anthocyanin contents were expressed as the change of 0.1 unit of difference between $A_{530\,nm}$ and $A_{600\,nm}$. Flavonoid contents were expressed as the absorbance at 325 nm per g fruit peel. Total phenolics were calculated from a standard curve made with gallic acid.

2.4. Enzyme assays

Fruit peels (2 g) were homogenized in 5 ml of 0.05 M phosphate buffer (pH 6.8) at 4°C. The homogenate was centrifuged at 19 000 × *g* for 20 min and polyphenol oxidase (PPO) activity in the supernatant was determined according to the method of Tan and Li (1984), by measuring the oxidation of 4-methylcatechol. PPO activity was calculated as the increase in 0.001 unit of absorbance per min at 398 nm per mg protein. To measure peroxidase (POD) activity, fruit peels (2 g) were homogenized in 5 ml of 0.1 M phosphate buffer (pH 7.1) at 4°C. The homogenate was centrifuged at 1500 × *g* for 20 min and peroxidase (POD) in the supernatant was measured according to the method of Kochba et al. (1977), and calculated as the increase in absorbance at 470 nm per mg protein per min.

2.5. Control of decay

Fruits were treated with 0.1% TBZ alone or 1.0% chitosan alone or 2.0% chitosan alone, and then dried for 1 h. To assess the effectiveness of chitosan on the control of decay and compare it with the TBZ treatment, we used fruits dipped in distilled water as the control. They were stored at 4°C with 90% RH. Four replicates of 120 fruits were used for each treatment. Fruits were examined for mould regularly and considered infected when a visible lesion was observed. Results were expressed as percentage of fruits infected.

2.6. Determination of weight loss

Four replicates of 60 fruits were used for each treatment. Fruits were weighed regularly for weight loss.

2.7. Protein assays

The protein content of enzyme extracts was measured according to the method of Bradford (1976).

2.8. Data handling

Data were from three duplicate experiments and were analysed using Duncan's multiple range test for least significant difference at the 5% level and the results were subjected to analysis of variance with 5% LSD values calculated to separate significantly different means of the control and treatments.

3. Results

3.1. Effects of chitosan coating on browning

Changes in the browning grades of both TBZ-treated control and chitosan-treated fruits (Table 1) showed that the browning grades of both TBZ-treated control and chitosan-treated fruits signifi-

cantly increased with increased storage time ($P < 0.05$), indicating that the fruit pericarp turned brown gradually. The browning grades of chitosan treatments changed significantly more slowly than that of TBZ-treated control ($P < 0.05$), while there was no significant difference between the chitosan treatments according to Duncan's multiple range test.

3.2. Changes of contents of anthocyanin, flavonoid, and total phenolics

Changes in the anthocyanin and flavonoid contents of the fruit peel during storage at 4°C are shown in Figs. 1 and 2. Anthocyanin and flavonoid contents of both the TBZ-treated control and chitosan treatments decreased slowly during the first 20 days of storage and then decreased more steeply. Anthocyanin and flavonoid contents of chitosan treatments decreased more slowly than in the TBZ-treated control. At days 20, 25 and 30 of storage, there were significant differences in anthocyanin and flavonoid contents between chitosan treatments and the TBZ-treated control ($P < 0.05$). However, there was no significant difference in anthocyanin and flavonoid contents between chitosan treatments.

Changes in total phenolics content of the peel of fruits coated with 1.0 and 2.0% chitosan and in the TBZ-treated control during storage at 4°C are shown in Fig. 3. Total phenolics content decreased continuously. The total phenolics contents of chitosan treatments decreased more slowly than in the TBZ-treated control fruit. At days 20, 25 and 30 of storage, there were significant differences in total phenolic contents between chitosan treatments and the TBZ-treated control ($P < 0.05$). However, there was no significant difference between chitosan treatments.

Table 1
Effects of chitosan coating on browning of pericarp of litchi fruit during storage at 4°C*

Days of storage	Browning grade		
	Control	1.0% Chitosan	2.0% Chitosan
0	0a c	0a c	0a c
10	0.88 ± 0.12a d	0.59 ± 0.10b d	0.52 ± 0.11b d
20	1.64 ± 0.18a e	1.11 ± 0.17b e	1.09 ± 0.15b e
25	2.37 ± 0.20a f	1.82 ± 0.16b f	1.78 ± 0.17b f
30	2.99 ± 0.26a g	2.36 ± 0.23b g	2.30 ± 0.20b g
33	3.67 ± 0.30a h	2.89 ± 0.25b h	2.80 ± 0.26b h

Means within a row followed by the same letter (a and b) and a column followed by the same letter (c–h) are not significantly different at the 0.05 level ($n = 9$). Each data point represents the mean of three duplicate experiments where there were three replicates for each treatment ± S.E. ($n = 9$).

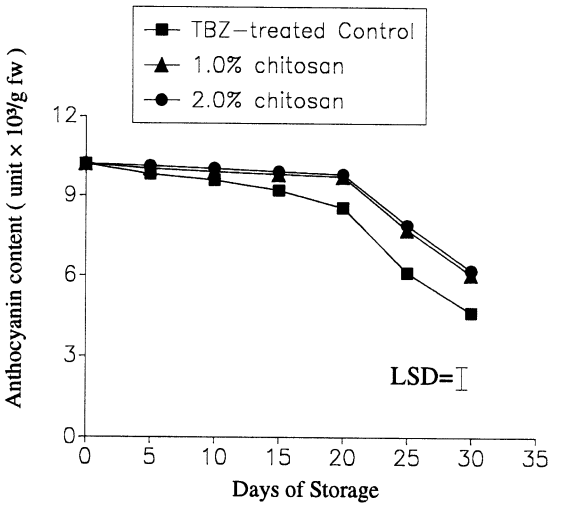

Fig. 1. Changes in anthocyanin contents of the peel of litchi fruits coated with 1.0 and 2.0% chitosan and the TBZ-treated control during storage at 4°C. Each data point represents the mean of three duplicate experiments where there were three replicates for each treatment.

3.3. Effects of chitosan coating on PPO and POD activities

Changes in PPO and POD activities of the fruit peel coated with 1.0 and 2.0% chitosan and of peel from the TBZ-treated control during storage at 4°C are shown in Figs. 4 and 5, respectively. PPO and POD activities of chitosan treatments changed little during the first 14 days of storage, while those in the TBZ-treated control changed significantly. Then, PPO activity of the TBZ-treated control increased and reached a peak at day 26, and then decreased. PPO activity of both chitosan treatments increased slowly and reached

a peak at day 31, and then decreased. At day 26 of storage, there was significant difference in PPO activity between chitosan treatments and the TBZ-treated control ($P < 0.05$) but no significant difference between chitosan treatments.

POD activity of both the TBZ-treated control and chitosan treatments increased continuously after day 14 of storage. POD activity of chitosan treatments increased more slowly than the TBZ-treated control. At days 24, 26, 30 and 33 of storage, there were significant differences in POD activity between chitosan treatments and the TBZ-treated control ($P < 0.05$). However, there was no significant difference between chitosan treatments.

3.4. Effect of chitosan coating on weight loss of fruit

During 4°C storage, weight loss of both the TBZ-treated control and chitosan treatments increased continuously. Weight losses in chitosan treatments were slower than in the TBZ-treated control, and there was no significant difference between chitosan treatments (Table 2).

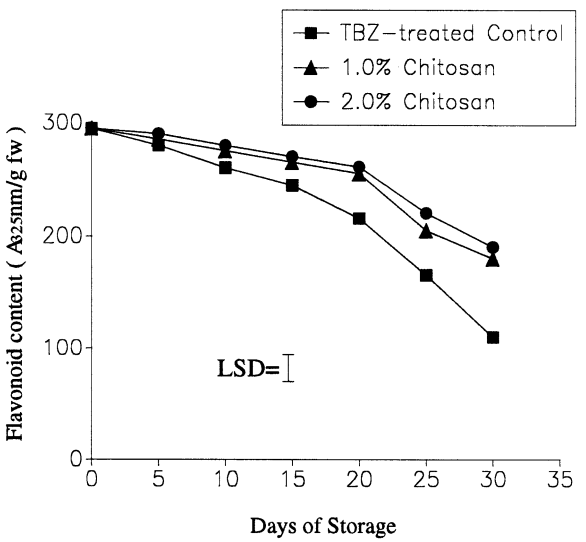

Fig. 2. Changes in flavonoid contents of the peel of litchi fruits coated with 1.0 and 2.0% chitosan and the TBZ-treated control during storage at 4°C. Each data point represents the mean of three duplicate experiments where there were three replicates for each treatment.

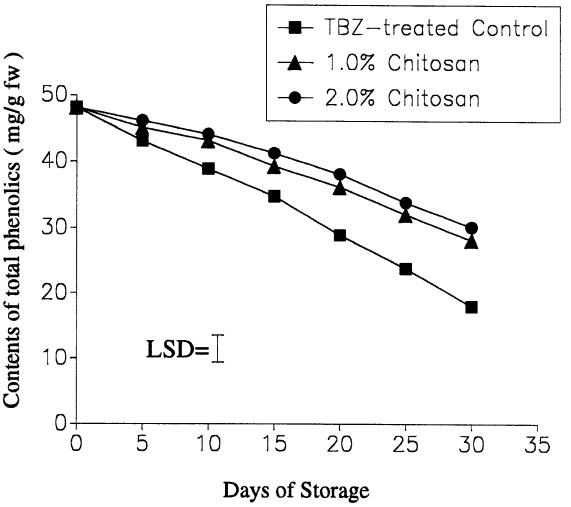

Fig. 3. Changes in contents of total phenolics of the peel of litchi fruits coated with 1.0 and 2.0% chitosan and the TBZ-treated control during storage at 4°C. Each data point represents the mean of three duplicate experiments where there were three replicates for each treatment.

3.5. Effects of chitosan coating on control of decay

Decay in litchi fruit coated with chitosan alone and dipped in TBZ alone was significantly reduced (Table 3). However, the ability of chitosan

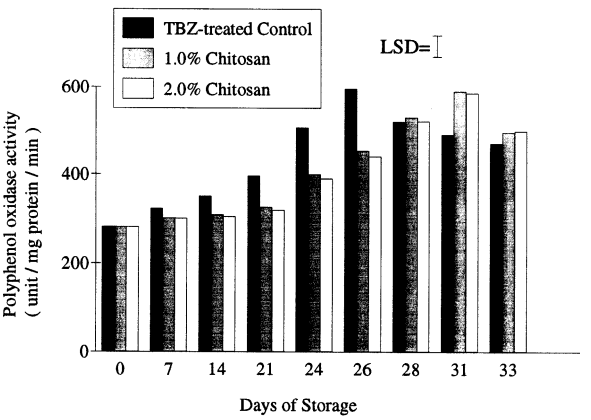

Fig. 4. Changes in polyphenol oxidase activities of the peel of litchi fruits coated with 1.0 and 2.0% chitosan and the TBZ-treated control during storage at 4°C. Each data point represents the mean of three duplicate experiments where there were three replicates for each treatment.

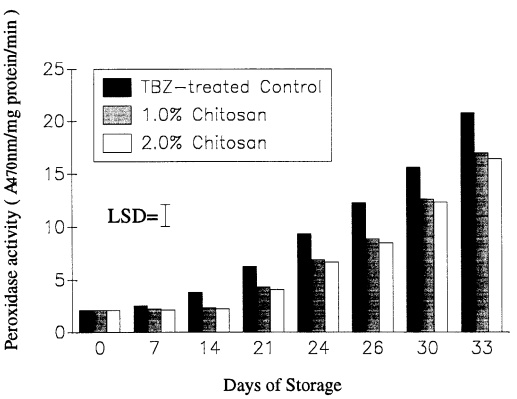

Fig. 5. Changes in peroxidase activities of the peel of litchi fruits coated with 1.0 and 2.0% chitosan and the TBZ-treated control during storage at 4°C. Each data point represents the mean of three duplicate experiments where there were three replicates for each treatment.

to control decay in litchi fruit is limited when compared to TBZ.

4. Discussion

Oxidation of phenolic compounds is the main cause of browning in fruits (Macheix et al., 1990). PPO is a terminal oxidase occurring widely in plants, which catalyzes oxidation of phenolics, resulting in tissue browning in fruits and vegetables. It may be involved in breakdown of anthocyanin, resulting in colour changes of fruits and vegetables (Dong, 1990). Lin et al. (1988b) suggested that peroxidase may be involved in oxidation of phenolics, glutathione and ascorbic acid, also resulting in colour changes of fruit and vegetables.

Coating fruit with semi-permeable films has generally been shown to retard ripening by modifying the endogenous CO_2, O_2 and ethylene levels of fruits (Lowings and Cutts, 1982; Banks, 1984; El Ghaouth et al., 1991). In our studies, the application of chitosan coating delayed changes of contents of anthocyanin, flavonoid, total phenolics, and the increase in PPO activity, and partially inhibited the increase in POD activity which is associated with tissue browning. This implies that a chitosan coating may form a protective barrier on the surface of the fruit and reduce the supply of oxygen for enzymatic oxidation of phenolics.

Scott et al. (1982) pointed out that desiccation is one of main factors causing browning of the fruit. Underhill et al. (1992) indicated that with the development of litchi fruit, cuticle thickness decreases significantly and micro-cracking of the pericarp appears; thus, harvested litchi fruit desiccate quickly. During storage, as desiccation occurred, the pH of the pericarp homogenate increased and the permeability of cell membranes changed so as to influence the micro-structure of pericarp cells (Underhill and Critchley, 1994). Desiccation also prompts the breakdown of vacuoles and leakage of anthocyanin and destroys the compartmentation of browning-related enzymes and their substrates (Chen and Hong, 1992; Underhill and Critchley, 1994). Desiccation can be reduced by the use of plastic films (Chen and Zhang, 1988; Nip, 1988; Chen and Hong, 1992). In our study, the application of chitosan may form a layer of film on the outer pericarp surface, reducing weight loss and desiccation of the fruit (Table 2), and also resulting in less browning.

In some research, chitosan has been shown to be able to inhibit the growth of some fungi (Allan and Hadwiger, 1979; El Ghaouth et al., 1989, 1991). In our study, to assess the effectiveness of

Table 2
Changes in percentage of weight loss of litchi fruits coated with 1.0 and 2.0% chitosan and the control during storage at 4°C (%)*

Days of storage	Control	1.0% Chitosan	2.0% Chitosan
0	0a d	0a d	0a d
7	1.3 ± 0.1a e	1.0 ± 0.1b e	1.0 ± 0.1c e
14	2.4 ± 0.1a f	2.1 ± 0.1b f	2.0 ± 0.1b f
21	3.7 ± 0.2a g	3.1 ± 0.2b g	3.0 ± 0.2b g
26	5.0 ± 0.2a h	4.1 ± 0.2b h	4.1 ± 0.2b h
30	6.1 ± 0.2a i	5.2 ± 0.2b i	5.1 ± 0.2b i
33	7.0 ± 0.2a j	6.1 ± 0.2b j	6.1 ± 0.2b j

Means within a row followed by the same letter (a–c) and a column followed by the same letter (d–j) are not significantly different at 0.05 level ($n = 9$). Each data point represents the mean of three duplicate experiments where there were three replicates for each treatment ± S.E. ($n = 9$).

Table 3
Changes in percentage of decay of litchi fruits coated with 1.0 and 2.0% chitosan, treated with 0.1% TBZ, and the water-dipped control during storage at 4°C (%)*

Days of storage	Control	1.0% Chitosan	2.0% Chitosan	0.1% TBZ
0	0a	0a	0a	0a
7	15.5 ± 1.1b	0a	0a	0a
14	33.8 ± 1.7c	9.7 ± 1.0b	8.5 ± 1.0b	0a
21	65.5 ± 2.5d	28.3 ± 1.5c	26.8 ± 1.4c	0a
26	95.2 ± 3.2e	53.3 ± 2.1d	50.9 ± 2.0d	3.5 ± 0.6b
30		72.1 ± 2.6e	69.9 ± 2.5e	7.8 ± 0.7c
33		89.1 ± 3.1f	87.5 ± 3.1f	20.4 ± 1.3d

* Means within a column followed by the same letter are not significantly different at 0.05 level ($n = 9$). Each data point represents the mean of three duplicate experiments where there were three replicates for each treatment ± S.E. ($n = 9$).

chitosan on the control of decay and compare it with TBZ treatment, we used water-dipped fruit as the control. Results indicate that to some extent the application of chitosan coating delayed the increase in decay of stored litchi fruit, indicating that chitosan coating reduced pathogen growth in some way (Table 3). Since attack by pathogens is also a major factor causing browning of the fruit (Chen, 1984), inhibiting decay partially could be beneficial in delaying browning. The antifungal effects suggested here are in line with those which El Ghaouth et al. (1991) observed in strawberry. However, the increase in concentration of chitosan coating did not control browning or decay of the fruit significantly more, and also the effectiveness of chitosan on decay control is far from reaching that of TBZ and so is limited. Further studies will be needed to fully evaluate the action model of chitosan coating and the possibility of it replacing the use of fungicides.

Taste panels of the stored fruits were done regularly while the other assessments were taken and no off-flavours in chitosan-treated fruits were detected because the low temperature of storage reduced physiological metabolism.

We suggest that the application of chitosan coating could be beneficial in the control of browning and to some extent could be beneficial in decay control of litchi fruit. In using chitosan for decay control, we consider it might be suitable for treatment of fruit stored for shorter periods (e.g. 2 weeks), for short-distance transport and distribution. We recommend the application of chitosan coating to control browning and decay in litchi fruit in combination with other methods such as low temperature and suitable packaging.

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