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ORIGINAL ARTICLE

Control of post-harvest pericarp browning of litchi (*Litchi chinensis* Sonn)

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Abstract Pericarp browning is the major post-harvest problem of litchi (*Litchi chinensis* Sonn) fruit, resulting in reduced commercial value of the fruit. Control of post-harvest pericarp browning of fruit using 9 different post-harvest treatments were studied. The treated fruits were packed in transparent perforated (0.2% ventilation) low density polythene bags (100 gauge). On storage, pericarp browning increased irrespective of treatments with the decrease in pericarp specific activity, total pericarp phenol and total anthocyanin. Anthocyanin degradation index and polymeric colour increased during storage. Pre-cooled (10^0C) fruits treated with 0.6% sodium metabisulphite solution for 10 min, air dried followed by dipping in 2% HCl for 5 min and packing in perforated LDPE bags recorded the lowest polyphenol oxidase specific activity (2.2 units/mg protein) with maximum retention of total anthocyanin (47.3 mg/100g) leading to the lowest pericarp browning after 9 days of storage with attractive red colour, freshness and enhanced shelf life of 9 days at ambient conditions (27.7 ± 1.2°C, RH 78 ± 4%).

Keywords Litchi · *Litchi chinensis* · Pericarp browning · Polyphenol oxidase activity · Anthocyanin · Shelf-life

Introduction

The perishable nature of litchi (*Litchi chinensis* Sonn) poses a serious problem in its transportation and marketing. Pericarp browning is the most important post-harvest problem associated with storage which drastically reduces the commercial value of the fruit (Snowdon 1990). The unharvested ripe fruit has an attractive red colour. Once harvested, the brilliant red colour gets lost within 48 h and turns brown. This browning reaction is believed to be induced by desiccation of the pericarp causing anthocyanin degradation and the subsequent production of brown by-products (Akamine 1960). Several approaches like heat treatment, wax coating, vinyl resin plastic coating, fumigation with ethylene bromide, acid dipping, and use of fungicides have been tried to solve the problem of post-harvest browning of litchi. However, these approaches could not provide satisfactory means for retaining the red colour of the pericarp for a longer period. Huang and Scott (1985) reduced browning and rotting in litchi by dipping fruits in hot benomyl at 52°C and enhanced shelf life of 6 days. Kudachikar et al. (2007) reported the effect of SO_2 -fumigation on litchi pericarp colour retention. Inhibition of browning in litchi by acid dipping was reported by Underhill et al. (1994) and Yueming et al. (2008). Detailed studies on control of polyphenol oxidase (PPO) mediated browning and anthocyanin degradation in litchi pericarp are still lacking. Control of enzyme activity and anthocyanin degradation in relation to post-harvest pericarp browning during storage were studied and results reported here.

Materials and methods

Litchi fruits of uniform maturity cv. 'Muzaffarpur' were harvested at 100 days after anthesis from the Experimental Orchard, Department of Horticulture, AAU, Jorhat. Fruits were subjected to following post-harvest treatments. T_1: Pre-heating in boiling water (98°C for 1 min) + dipping in 2% HCl for 5 min, air dried and packed, T_2: Pre-cooling (10°C for 10 min) + dipping in 0.6% sodium metabisulphite solution for 10 min, air dried + dipping in 2% HCl solution for 5 min, air dried and packed, T_3: Pre-cooling (10°C for 10 min) + dipping in 500 ppm ascorbic acid solution for 5 min, air dried and packed, T_4: Dipping in 2% sodium hypochlorite solution for 5 min, air dried and packed,

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T_5: Dipping in 125 ppm bavistin solution for 2 min, air dried and packed, T_6: Fruits packed with ethylene absorbent, T_7: Dipping in 3% wax emulsion for 1 min, air dried and packed, T_8: Fruits packed without any chemical treatment and T_9: Fruits without packaging and chemical treatment.

In all the treatments except T_9, fruits were packed in perforated (0.2% ventilation) LDPE (100 gauge) bags and stored at ambient conditions (27.7 ± 1.2°C, 78 ± 0.4% RH). The experiment was laid out in a factorial randomized block design with 3 replications. Fruits were analysed for biochemical parameters at 3 days interval up to 9 days.

PPO specific activity: PPO activity was determined by measuring the oxidation of catechol solution, prepared fresh as substrate (Zauberman et al. 1991). The reaction mixture (3 ml) contained 1.9 ml of 0.05 M sodium phosphate buffer, pH 6.8 and 1 ml of 0.1 M catechol as substrate. The reaction was started by adding 0.1 ml of PPO extract and the change in absorbance at 410 nm was recorded at 30 sec interval for 5 min at room temperature (27°C) in a UV-VIS spectrophotometer. The initial linear part of the progressive curve was considered for calculating the change in absorbance per min. One unit of enzyme activity was defined as that which caused a change in absorbance at 410 nm of 0.001 /min / ml enzyme extract. The PPO specific activity was calculated as the units of enzyme activity /mg of protein (Jayaraman et al. 1982).

Total phenols: Absorbance of 1% phenol suspension was recorded at 650 nm against blank in a UV-VIS Spectrophotometer (Sadasivam and Manikam 1992). A standard curve was prepared using different concentrations of catechol. From the standard curve, the concentration of total phenol in the test sample was determined and expressed as mg/100 g.

Total anthocyanin: Total pericarp anthocyanin was determined by pH differential method (Wrolstad 1993). Absorbance of 1% anthocyanin solution was determined at 530 nm in a UV-VIS Spectrphotometer. Turbidity (haze) was corrected for by measuring the absorbance at 700 nm. A check was run to see if the assay deviated from Lambert –Beer Law. Concentration was then calculated (Fuleki and Francis 1968) incorporating the molar absorbance (Jurd and Asen 1966). Finally, the concentratiuon of total anthocyanin was expressed as mg/100g.

Pericarp browning: Pericarp browning was determined by recording the absorbance of 1% anthocyanin solution at 420 nm. Browning was expressed in terms of absorbance at 420 nm (A_{420nm}) as described by (Wrolstad 1993).

Anthocyanin degradation index (ADI)*:* ADI was determined from the same sample and procedure utilized for determination of total anthocyanin and it was estimated (Fuleki and Francis 1968) as

$$\text{ADI} = \frac{\text{Anthocyanin content by single pH method}}{\text{Anthocyanin content by pH differential method}}$$

Colour density, polymeric colour and per cent contribution of tannin to total colour: These parameters were determined by utilizing the same sample extracted for determination of total anthocyanin. Two ml of 1% anthocyanin solution was diluted with distilled water to 10 ml volume, giving a dilution factor of 5. Two hundred microlitres of 20% potassium metabisulphite were added to a 3 ml sample and 200 ml of water was added to a second 3 ml control sample. The absorbance at 420 nm, 530 nm and 700 nm were recorded (Fuleki and Francis 1968).

The colour density was determined by summing the absorbance of the control sample at 420 and 530 nm. Turbidity was corrected for by substracting any absorbance at 700 nm.

$$\text{Colour density} = (A_{530\ nm} - A_{700\ nm}) + (A_{420\ nm} - A_{700\ nm}) \times \text{Dilution factor}$$

Polymeric colour was calculated from the bisulphate treated samples as:

$$\text{Polymeric colour} = (A_{530\ nm} - A_{700\ nm}) + (A420_{\ nm} - A_{700\ nm}) \text{ x Dilution factor}$$

Per cent contribution of tannin (non-monomeric anthocyanin colour) to total colour was determined as follows:

$$\text{Contribution of tannin, \%} = \frac{\text{Polymeric colour}}{\text{Colour density}} \times 100$$

Shelf-life of fruits: Shelf-life of fruits was assessed on the basis of pericarp colour and taste (Sharma and Ray 1992) and rotting of fruits (Chandramonti et al. 1991). Organoleptic score value of 6 for colour and 10% rotting of the fruits were considered as the critical limit of shelf life acceptance.

Results and discussion

PPO activity and pericarp browning: Pericarp browning was maximum (0.577 as $A_{420\ nm}$) in non treated fruits (Table 1) which might be due to higher PPO specific activity (38.2 units/mg protein). PPO activity had positive correlation (r = +0.259) with pericarp browning. During storage, PPO activity showed a decreasing trend, recording the lowest activity of 7.3 units/mg protein on 9 days of storage. Maximum PPO activity was on first day after harvest which subsequently declined during storage in litchi (Zauberman et al. 1991). The decrease in PPO activity might be ascribed to enzymatic copper chelating activity (Mayer 1987). Pericarp browning was minimum in pre-cooled fruits of T_2 (0.218 as A_{420nm}) which could be attributed to the lowest PPO specific activity (2.2 units/mg protein). Sulphite could act by reducing oxygen and making it unavailable for oxidizing polyphenols or they might combine with quinines and prevent their participation in further oxidation, cyclization and condensation reactions and ultimately promoting the reaction of quinines back to the original phenols. Inhibition of PPO activity by SO_2 was also reported by Zauberman et al. (1991). Dipping the fruits in low pH solution (T_2) resulted in lower pericarp pH which might exert inhibitory effect on PPO activity and retard the development of enzymatic browning (Ketsa and Leelawatana 1992). Kudachikar et al.

Table 1 Effect of treatment and storage (27.7 ± 1.20C, 78 ± 0.4% RH) on biochemical quality characteristics of litchi fruits

Storage period (S), days	Treatments (T)								
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
	Polyphenol oxidase specific activity,units /mg protein (IV=88.1)								
3	3.7	2.6	44.1	47.0	26.1	24.1	39.2	53.5	81.0
6	3.6	2.5	30.8	39.8	14.1	14.6	39.2	46.8	26.4
9	3.5	1.6	16.4	19.2	12.9	12.3	22.0	13.6	7.3
	CD(5%), n=3, T=0.030, S=0.017, TXS=0.052								
	Pericarp browning,A_{420nm} (IV=0.215)								
3	0.250	0.217	0.330	0.333	0.220	0.220	0.356	0.349	0.537
6	0.283	0.217	0.371	0.400	0.274	0.277	0.407	0.500	0.593
9	0.411	0.220	0.503	0.520	0.362	0.368	0.543	0.593	0.600
	CD(5%), n=3, T=0.004, S=0.002, TXS=0.006								
	Total phenol, mg/100 gm (IV=195.3)								
3	172.0	170.1	177.8	177.6	174.9	176.1	178.1	180.9	185.7
6	170.0	170.0	177.0	177.0	174.2	176.0	177.1	178.3	180.0
9	169.0	169.2	176.0	177.0	172.0	173.0	177.0	177.3	178.2
	CD(5%), n=3, T=0.032, S=0.019, TXS=0.056								
	Total anthocyanin, mg/100 gm (IV=57.9)								
3	39.0	47.2	40.8	43.1	45.1	44.3	40.9	39.8	34.0
6	38.1	50.3	38.2	40.8	43.5	42.2	37.1	36.0	32.7
9	32.7	44.2	37.0	38.1	40.6	39.1	35.0	33.1	31.6
	CD(5%), n=3, T=0.046, S=0.027, TXS=0.080								
	Anthocyanin degradation index (IV=3.4)								
3	4.0	3.6	3.6	3.6	3.6	3.6	3.7	3.8	4.0
6	4.1	3.6	3.7	3.6	3.6	3.7	3.8	4.0	4.2
9	4.2	3.6	4.0	3.8	3.8	3.8	4.0	4.1	4.2
	CD(5%), n=3, T=0.033, S=0.019, TXS=0.057								
	Colour density (IV=4.2)								
3	4.0	4.1	4.0	4.1	4.1	4.1	4.0	3.9	3.7
6	3.8	4.0	3.8	4.0	4.0	4.0	3.9	3.8	3.7
9	3.6	4.0	3.7	3.8	3.8	3.8	3.7	3.6	3.6
	CD(5%), n=3, T=0.037, S=0.021, TXS=0.064								
	Polymeric colour (IV=0.49)								
3	0.556	0.500	0.561	0.544	0.540	0.550	0.563	0.604	0.712
6	0.603	0.530	0.619	0.617	0.603	0.613	0.620	0.706	0.773
9	0.690	0.578	0.694	0.691	0.653	0.675	0.701	0.792	0.809
	CD(5%), n=3, T=0.003, S=0.002, TXS=0.006								
	Contribution of tannin (%) to total colour (IV=11.8)								
3	13.9	12.2	13.9	13.2	13.2	13.4	13.9	15.5	19.2
6	15.8	13.2	16.1	15.5	15.1	15.5	16.0	18.6	21.0
9	19.0	14.6	18.8	18.4	17.0	18.0	19.0	22.0	22.6
	CD(5%), n=3, T=0.037, S=0.021, TXS=0.063								

T_1–T_9 = see text, IV= initial value

(2007) also reported the effect of SO_2-fumigation on litchi pericarp colour retention. Combined treatments of chitosan and organic acids showed better control of post harvest pericarp browning in litchi (Caro and Joas 2005). Reduced

pericarp browning under this treatment might also be due to packaging of fruits with perforated low density polyethylene bags (Akamine 1960). Pre-cooling, which generally removes the field heat from the harvested produce has been shown to restrict enzymatic and respiratory activities during the post-harvest holding period. Menon and Goswami (2007) explained the effect of pre-cooling in enhancing the efficiency of preservation techniques in mango.

Phenol: Phenols may contribute to browning when enzymatically oxidized to quinines, and the quinine polymerize to relatively stable polymers. Total phenol content was lowest (169.8 mg/100g) in T_2 where bisulphate reacts with the quinines or other intermediates in polyphenol oxidation and thus prevents further polymerization of the phenolic compound (Table 1). Total pericarp phenol had significant and positive correlation (r = +0.565) with pericarp browning and pericarp PPO activity (r = +0.840).

Total anthocyanin and anthocyanin degradation index: Anthocyanins are the principal pigments in mature litchi which are responsible for the bright red colour of fruit. Total anthocyanin concentration decreased during storage which might be due to degradation of anthocyanin (Table 1). Anthocyanin responsible for redness of strawberry was maximum on 6th day of storage in fruits packed in HDPE crates, treated with 2% $CaNO_3$ (Akath Singh et al. 2008). After harvest of litchi fruit, due to physiological stress condition, membrane disintegration in the pericarp occurs due to moisture loss which leads to release of PPO in active form. When PPO comes in contact with anthocyanin in presence of oxygen, the anthocyanin gets irreversibly broken down into melanin by-products creating pericarp browning (Lin et al. 1988). Significant positive correlation of ADI with pericarp browning (r = +0.709) and pericarp PPO activity (r = +0.068) was observed. Anthocyanin degradation has a close correlation with phenolic substrate showing positive correlation (r = +0.236). It was reported that anthocyanin are readily discolourized by PPO in presence of a better substrate such as catechol (Schumaker and Bastin 1965). The product from the anthocyanin degradation has structure similar to catechol, which in turn, could accelerate enzymatic browning reactions by PPO (Zhang et al. 2001). Anthocyanase, which catalyses the hydrolysis of sugar moieties from anthocyanin to anthocyanidin, was identified in litchi pericarp and this might contribute to the browning of litchi pericarp involved in anthocyanase-anthocyanin-PPO reaction (Jiang et al. 2004, Ducamp Collin et al. 2007). ADI was minimum (3.5) in T_2 fruits, resulting in maximum retention of total anthocyanin content of 47.3 mg/100g (Table 1). Sulphite bleaching is an ionic reaction involving a nucleophilic attack by one of the negative ions of sulphurous acid on the flavylium cation to form what is probably chromenol-4-sulphonic acid. These anthocyanin chromenols will instantly regenerate the anthocyanin cation upon acidification (Jurd 1964).

Colour density: Colour density decreased progressively during storage (Table 1). Anthocyanin degradation products decreased colour density in blackcurrant (Skrede et al. 1992). Pre-cooled fruits (T_2) registered highest colour density (4.0), which may be due to the lowest ADI.

Polymeric colour and per cent contribution of tannin to total colour: Polymeric colour increased significantly during storage recording the highest value of 0.698 on 9 days of storage (Table 1). These results suggest that towards the end of storage, polymeric pigments are the predominant pigments in litchi pericarp. During storage, the anthocyanin initially responsible for bright red colour of fruit is displaced progressively and irreversibly by more polymeric pigments (Lee and Wicker 1991). Significant positive correlation (r = +0.923) of polymeric colour with pericarp browning was noticed. Polymeric colour influenced per cent contribution of tannin (Somers 1971). With the increase of polymeric colour, there was corresponding increase in contribution of tannin to the total colour. As the storage period advanced, anthocyanin and other phenolic compounds underwent oxidation and polymerization which ultimately contributed increase in tannin. These degraded products showed higher absorbance (0.765) at 420 nm. Polymeric colour was the lowest (0.536) in T_2 which might be due to lower degree of polymerization, resulting in the lowest per cent contribution of tannin (13.4%).

Shelf-life: Pre-cooled fruits treated with sodium metabisulphite and HCl (T_2) appeared to be the best treatment which extended the shelf-life of the fruits up to 9 days as against 3 days in non treated fruits (Table 2). This treatment effectively controlled pericarp browning and also maintained attractive red colour up to 9 days of storage. The effectiveness of polyethylene packaging in extending shelf-life and maintaining quality of fruits were reported in passion fruit (Yadav et al. 2007, Patel et al. 2009), tomato (Yadav et al. 2009) and citrus (Sonkar et al. 2008). Acidification did not affect the quality of the pulp (Underhill et al. 1994). SO_2 content decreased during storage (Shilpa et al. 2008). The SO_2 –residues of 0.16 ppm was detected on 9 days after storage which was much lower than the tolerated

Table 2 Shelf-life of litchi fruit as influenced by treatments (storage at ambient conditions, 27.7 ± 1.2°C, 78 ± 0.4% RH)

Treatments	Shelf-life, days
T_1	5.0
T_2	9.0
T_3	6.0
T_4	6.0
T_5	8.3
T_6	7.3
T_7	5.0
T_8	3.0
T_9	2.0
S.Ed.	0.222
CD at 5%	0.467

level of 10 ppm in litchi pulp (Normand and Bouffin 1995). This might be a strong support for increasing the comsumer acceptance of the sodium metabisulphite treated fruits.

Conclusion

The study indicated that pre-cooled fruits treated with 0.6% sodium metabisulphite solution for 10 min followed by dipping in 2% HCl solution for 5 min, air dried and packed in transparent perforated (0.2% ventilation) low density polyethylene (100 gauge) bags appeared to be the best treatment for controlling pericarp browning and browning attributing factors which extended the shelf life up to 9 days at ambient conditions (27.7 ± 1.2°C, RH 78 ± 0.4%).

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