

QUALITATIVE ASSESSMENTS OF LITCHI FRUITS BY USING PACKAGING MATERIALS, GLUTATHIONE AND CITRIC ACID AT DIFFERENT STORAGE CONDITIONS

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Abstract

Oxidation of anthocyanin by polyphenol oxidase (PPO) or peroxidase (POD) causes enzymatic browning. It leads to undesirable characteristics of fruits and ultimately decreasing of fruit quality and value. Desiccation, post-harvest decay, loss of quality and micro cracking are the other most important problems restricting the growth of the litchi industry. Based on the fact, glutathione and citric acid inhibits the browning mechanism, the novel strategies of combining glutathione + citric acid with different packaging materials were used to improve litchi storability at refrigerated and room temperature. From the experiment, it was observed that, the application of glutathione (10 mmol/litre) and citric acid (100 mmol/litre) + polypropylene packet + corrugated fiber box gave good control on browning, weight loss, disease incidence and higher amount of anthocyanin in the litchi pericarp. High amount of TSS, total sugar, titratable acidity and ascorbic acid were also achieved in same treatments. Litchi fruits treated with combined application of glutathione and citric acid with packaging materials showed promising method to controlling browning and all the other quality parameters.

Keywords: *Litchi chinensis* Sonn., glutathione, citric acid, packaging materials, qualitative parameters

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1. INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is a non-climacteric, tropical and highly profitable fruit having highly popular in the international market and is known to have originated from South-East Asia (Huang *et al.*, 2005). Litchi fruit have huge demand even in temperate regions. Production has gradually increased over recent decades, particularly with the increase in exports (Huang *et al.*, 2005).

Maintenance of quality (particularly post-harvest) of litchi fruit is particularly a problem when stored at ambient temperature. Wu *et al.*, (2011) estimated the postharvest losses of litchi in the range of 20-30% of the harvested fruit and can be as more as 50% prior to utilization. The postharvest shelf life of litchi fruit is very short and it is highly perishable and can be lasts only for 2-3 days at normal temperature ($26 \pm 2^{\circ}\text{C}$). Once harvested, the pericarp browning also occurs within 48hr which extensively affects the eye appeal and results in

reduced commercial value of the fruit (Kumar *et al.*, 2011).

Pericarp browning and fruit decay are the major quality issues affecting litchi (Jiang *et al.*, 2006; Sivakumar *et al.*, 2010). Polyphenols oxidase (PPO) (Jiang, 2000) and peroxidase (POD) (Zhang *et al.*, 2005) are the two major compounds causes enzymatic browning by oxidation of anthocyanin and it is a common problem in the litchi and other fruit industries, as it leads to detrimental characteristics of fruits, thereby decreasing the quality and value fruit. Desiccation, post-harvest decay, loss of quality and micro-cracking are the other major problems also restricting development of the litchi industry affecting commercial quality during storage, transportation or during shelf-life (Tian *et al.*, 2005; Sivakumar *et al.*, 2007). A variety of physico-chemical techniques have been used to administer browning and/or inactivate the activities of polyphenols oxidase

and peroxidase in litchi fruit (Lichter *et al.*, 2000; Jiang *et al.*, 2004).

2. MATERIALS AND METHODS

Sample collection and preparation

The present study was conducted in the Laboratory of Post Harvest Technology of Horticultural Crops, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, India during the period from 2009 to 2012. In order to study the storage life of litchi cv. Bombai, fruit was harvested manually at commercial maturity by the workers in the early morning from the Horticultural Research Station, Mondouri, BCKV, Mohanpur, India and brought to the laboratory in bags with litchi leaf used as cushioning material for protection from the bruising. Immediately on receipt, the fruits were destalked; sorting is done for uniformity of shape, colour, size and absence of mechanical damage or disease and kept in low temperature (pre-cooling at 4°C temperature) for protection from the heat for 2-3 hrs.

Fruits were grouped in six groups; each group had four replications containing 20 fruits in each replication. These six groups were treated as; polypropylene packet (T₁), corrugated fiber board box (CFB box) (T₂), corrugated fiber board box + polypropylene packet (T₃), glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet (T₄), glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet + corrugated fiber board box (T₅) and control (T₆).

Reagent (reduced glutathione) was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai.

For treatment, fruits of respective treatments were dipped in water containing 10 mmol/litre glutathione and 100 mmol/litre citric acid for 5 min within 3 hour after harvest before being air dried and packed in different packaging materials. The size of ventilated polypropylene bags and CFB boxes were 32 × 22.5 cm and 22.5 × 15.5 × 15.0 cm, respectively.

The samples in polypropylene bags were sealed with rubber band and fruits without any chemical treatments/ packaging materials were kept loose as control. These treated and control samples were again divided in two groups. One group was stored at refrigerated temperature (10°C) while another group was stored at ambient temperature 34.3°C±5°C for comparative assessment. Two fruits from each treatment were randomly sampled every 3 days at refrigerated and every 2 days at room temperature to determine physico-chemical changes during storage periods.

Qualitative analysis

Physiological loss in weight (PLW)

Fruits under each experimental lot were weighed on the day of observation and expressed as percentage of the original fresh weight of the fruit.

Browning assessment

Pericarp appearance was assessed by measuring the extent of the total browned area on each fruit pericarp using following scale; 1 = no browning (excellent quality); 2 = slight browning; 3 = <1/4 browning; 4 = 1/4 -1/2 browning; 5 = >1/2 browning (poor quality). The browning index was calculated using the following formula:

$$\text{Browning index} = \sum (\text{browning scale} \times \text{percentage of corresponding fruit within each class}).$$

Fruit evaluated at an index >3.0 were considered unacceptable for marketing.

Disease index

Severity of post-harvest disease was assessed on a 1-5 scale, describing the severity of post-harvest fungal or bacterial decay as; 1 = none; 2 = slight (up to 5% surface affected); 3 = moderate (5-20% surface affected); 4 = moderately severe (20-50% surface affected) and, 5 = extreme (>50% surface affected) (Liu *et al.*, 2011).

A decay incidence was calculated using following formula:

$$\text{Disease index} = \frac{100 \times \sum (\text{decay score} \times \text{fruit within each class})}{\text{Total fruit} \times \text{the highest score}}$$

Bio-chemical analysis

Total soluble solids (°Brix)

In order to estimate the total soluble solid contents, a hand refractometer (Erma, Japan) ranging from 0 to 32° Brix and 45-82° Brix at 20°C was used. Two or three drops of sample were taken on prism of refractometer and direct reading was taken by reading the scale in meter as described in AOAC (2006) and reading was expressed as °Brix.

Titrateable acidity (%)

Percentage titrateable acidity was determined by titration of 10 ml of fruit juice with 0.1N NaOH using phenolphthalein as an indicator (1-2 drops) till light pink colour was achieved and calculated as citric acid as per standard procedure given in AOAC(2006).

Total Sugar (%)

Total sugar was determined as standard procedure described in AOAC (2006) using Lane and Eynon method.

Ascorbic acid (mg/100 g of fruit pulp)

Ascorbic acid contents of fruit were estimated by titration against 2, 6-dichlorophenol-indophenol dye as method described in AOAC (2006) and was expressed as mg/100 g of fruit pulp.

Anthocyanin (mg/100g of peel)

Anthocyanin of peel was estimated by spectrophotometric method using Mecasys Optizen Pop Spectrophotometer following the procedure expressed by AOAC (2006). Pericarp (1 g) from 2 fruits was quickly sliced and extracted with 15ml HCl-methanol (0.15% HCl: 95%; methanol= 15:85) for 24 hrs. The extract was filtered and its absorbance was determined at 535 nm and presented as mg/100 g peel.

Data analysis

The experiments were arranged as completely randomized design (CRD) and carried out in duplicate. All experiments were performed with four replications having 20 fruits in each replication and average value was used. The data were analyzed by analysis of variance (ANOVA) and means were compared by Duncan's Multiple Range Test (DMRT) by using SAS Version 9.1 for windows, 2002-2003, SAS Institute Inc., Cary, NC, USA. Differences between the means at the 5% level were considered significant.

3. RESULTS AND DISCUSSION

Physiological loss in weight

During refrigerated and room temperature storage, weight loss of treated and untreated fruits significantly increased continuously (Table 1). However, litchi fruits treated with combined application of glutathione (10 mmol/litre) with citric acid (100 mmol/litre) and packed in polypropylene bag and kept in corrugated fiber board box i.e. T₅ recorded minimum weight loss followed by T₄ after 11th day in storage, while control (T₆) exhibited maximum weight loss after 9th day in refrigerated storage.

The same trend was also recorded in room storage samples, where, T₅ showed minimum weight loss followed by T₄ while control (T₆) fruits exhibited maximum weight loss after 5th day of storage.

Browning

During storage at refrigerated and room temperature, pericarp browning index exhibited increasing trends in treated and untreated fruits (Table 2) indicating that the fruit pericarp brown gradually. Packed and treated fruits samples retained its colour longer than unpacked fruits (up to 11 days at refrigerated

temperature). The browning grade of combined treatment of glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene + corrugated fiber board box (T₅) changed more slowly than that of other treatments at both the storage conditions. The rate of browning of fruits was rapid in room temperature as compared to refrigerated temperature. Untreated fruits (control) exhibited higher browning index as compared to treated fruits at both the storage conditions.

The results indicated that postharvest browning of litchi fruit were severe on the 5th day of storage at room temperature.

Disease incidence

The effect of different treatments and packaging on diseases incidence of litchi was presented in Table 3. Litchi fruits treated with treatments T₅ and T₄ had significantly good effect in lowering the disease incidence after 11th day at refrigerated temperature and after 5th day in room temperature, while control fruits showed maximum disease incidence at refrigerated and room temperature after 9th and 5th day of storage, respectively.

Table 1. Effect of different treatments on physiological loss in weight (%) of litchi fruit

Treatments	Days in storage					
	Refrigerated Temperature (10°C)				Room Temperature	
	3 DAS	6 DAS	9 DAS	11 DAS	3 DAS	5 DAS
A. T ₁	2.74 ^b	5.63 ^{abc}	8.02 ^b	--	3.56 ^{ab}	7.10 ^b
B. T ₂	2.97 ^b	6.45 ^{ab}	10.12 ^a	--	4.14 ^{ab}	8.25 ^{ab}
C. T ₃	2.20 ^{bc}	4.03 ^{bc}	6.61 ^{bc}	--	3.26 ^{ab}	6.64 ^b
D. T ₄	1.80 ^{bc}	3.89 ^{bc}	6.48 ^{bc}	8.87 ^a	2.05 ^b	5.34 ^b
E. T ₅	1.01 ^c	2.96 ^c	5.03 ^c	6.45 ^b	1.22 ^b	4.66 ^b
F. T ₆	4.94 ^a	8.60 ^a	11.10 ^a	--	6.94 ^a	12.26 ^a

T₁ = Polypropylene packet, T₂ = Corrugated fiber board box, T₃ = Corrugated fiber board box + polypropylene packet, T₄ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet, T₅ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet + corrugated fiber board box, T₆ = Control. Values followed by different superscript letters are significantly ($P < 0.05$) different from each other. DAS: Days in Storage

Table 2. Effect of different treatments of browning (%) on litchi fruit

Treatments	Days in storage					
	Refrigerated Temperature (10°C)				Room Temperature	
	3 DAS	6 DAS	9 DAS	11 DAS	3 DAS	5 DAS
G. T ₁	10.83 ^c	23.14 ^c	49.99 ^{bc}	--	21.66 ^b	39.58 ^c
H. T ₂	12.49 ^b	29.62 ^b	54.76 ^b	--	24.99 ^b	45.83 ^b
I. T ₃	7.49 ^d	18.51 ^d	44.04 ^{cd}	--	18.33 ^c	36.45 ^c
J. T ₄	5.83 ^e	13.88 ^e	36.90 ^d	48.61 ^a	12.49 ^d	25.00 ^d
K. T ₅	4.16 ^f	9.25 ^f	24.99 ^e	37.49 ^b	9.16 ^d	20.83 ^d
L. T ₆	18.33 ^a	36.10 ^a	58.72 ^b	--	33.33 ^a	64.58 ^a

T₁ = Polypropylene packet, T₂ = Corrugated fiber board box, T₃ = Corrugated fiber board box + polypropylene packet, T₄ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet, T₅ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet + corrugated fiber board box, T₆ = Control. Values followed by different superscript letters are significantly ($P < 0.05$) different from each other. DAS: Days in Storage

Table 3. Disease incidence (percent) as influenced by different treatments during storage

Treatments	Days in storage					
	Refrigerated Temperature (10°C)				Room Temperature	
	3 DAS	6 DAS	9 DAS	11 DAS	3 DAS	5 DAS
M. T ₁	19.16 ^c	52.77 ^c	83.33 ^b	--	24.99 ^c	71.87 ^c
N. T ₂	22.49 ^b	62.03 ^b	88.09 ^b	--	29.99 ^b	78.12 ^b
O. T ₃	17.49 ^d	41.66 ^d	73.80 ^c	--	23.33 ^d	64.58 ^d
P. T ₄	12.49 ^e	34.25 ^e	65.47 ^d	87.49 ^a	16.66 ^e	58.33 ^e
Q. T ₅	9.16 ^f	21.29 ^f	47.61 ^e	73.61 ^a	12.50 ^f	39.58 ^f
R. T ₆	24.99 ^a	82.40 ^a	100.00 ^a	--	33.33 ^a	91.67 ^a

T₁= Polypropylene packet, T₂ = Corrugated fiber board box, T₃ = Corrugated fiber board box + polypropylene packet, T₄ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet, T₅ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet + corrugated fiber board box, T₆ = Control. Values followed by different superscript letters are significantly ($P < 0.05$) different from each other. DAS: Days in Storage

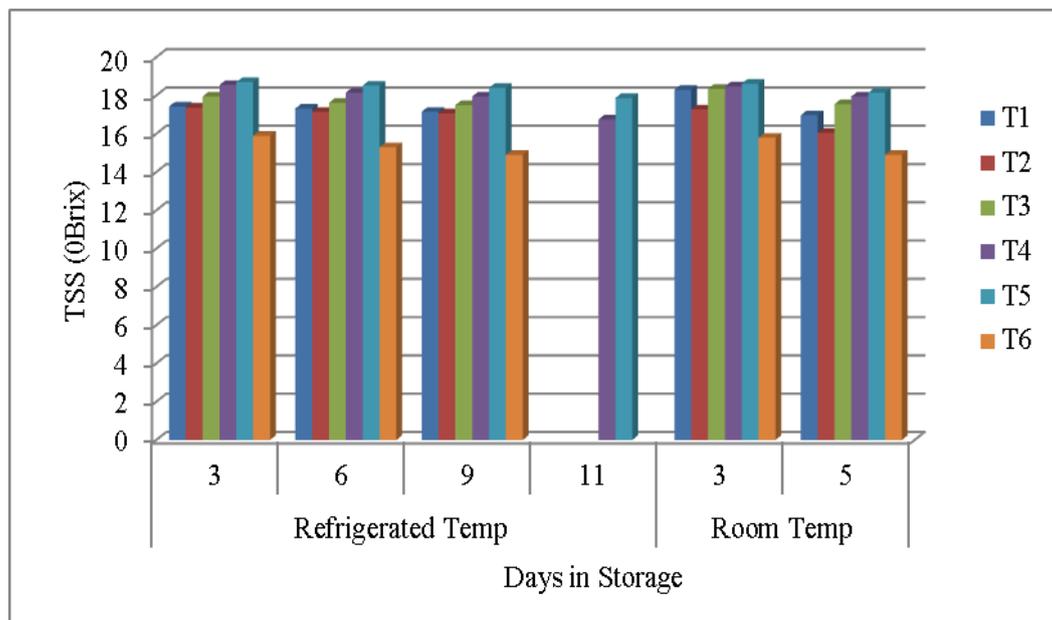


Fig 1. Effect of different treatments on Total soluble solids (°Brix) content of litchi fruit at refrigerated and room temperature

T₁= Polypropylene packet, T₂ = Corrugated fiber board box, T₃ = Corrugated fiber board box + polypropylene packet, T₄ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet, T₅ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet + corrugated fiber board box, T₆ = Control

Total soluble solids

The results presented in figure 1 revealed that total soluble solids contents of fruit decreased significantly during storage in all the treatments at refrigerated and room storage conditions. However, fruit treated with treatment T₅ significantly retained maximum

TSS after 11th day of storage followed by T₄, while minimum TSS was recorded in control i.e. T₆ after 9th day after storage at refrigerated temperature and after 5th day after storage at room temperature. At room temperature, the rate of TSS was decreased very rapidly as compared to refrigerated temperature.

Total sugar

Results of the effect of different treatments on total sugar content of litchi fruits were summarized in figure 2. Result revealed that, total sugar content significantly decreased gradually during storage in both conditions in all treatments along with control. Among the different treatments, fruits treated with treatment T₅ exhibited the higher total sugar content followed by T₄ after 11th day with the minimum was observed in control (T₆) after 9th day of storage at refrigerated temperature. The same trend was also observed in room temperature samples. The loss rate of total sugar content was much faster in room temperature samples as compared to refrigerated samples.

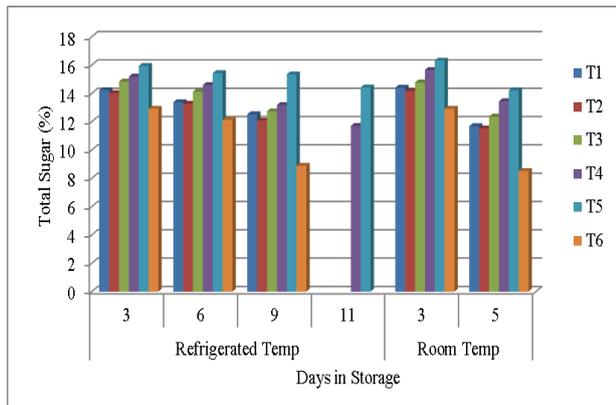


Fig 2. Effect of different treatments on total sugar (percent fresh weight) content of litchi fruit at refrigerated and room temperature

T₁= Polypropylene packet, T₂ = Corrugated fiber board box, T₃ = Corrugated fiber board box + polypropylene packet, T₄ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet, T₅ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet + corrugated fiber board box, T₆ = Control

Titrateable acidity

Figure 3 showed that the acidity percentage of all the treated and untreated fruits were significantly decreased rapidly in both storage conditions. Fruits treated with application of glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + corrugated fiber board box + polypropylene (T₅) showed maximum acidity followed by T₄ after 11th day in storage at refrigerated condition. The untreated (T₆) fruits showed minimum acidity after 9th and 5th day

in both storage conditions, respectively as compared to treated fruits.

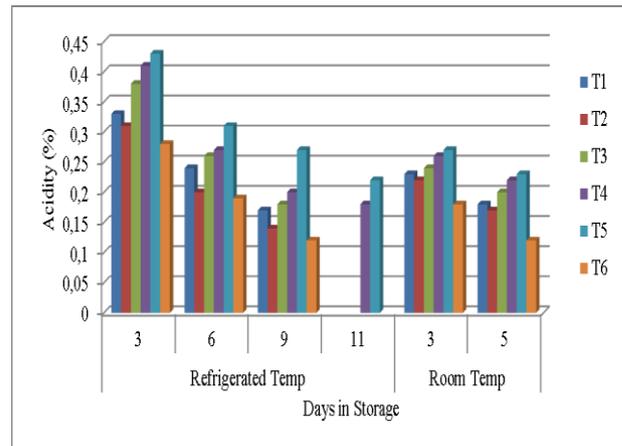


Fig 3. Effect of different treatments on titrateable acidity (percent fresh weight) content of litchi fruit at refrigerated and room temperature

T₁= Polypropylene packet, T₂ = Corrugated fiber board box, T₃ = Corrugated fiber board box + polypropylene packet, T₄ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet, T₅ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet + corrugated fiber board box, T₆ = Control

Ascorbic acid (Vit. C)

Ascorbic acid reduced drastically in all the treatments with the advancement of storage duration (Figure 4). However, ascorbic acid content was always significantly higher in treatment T₅ followed by T₄ after 11th day and lower in T₆ after 9th day in storage at refrigerated temperature. The same trend was also observed in room temperature samples. Samples at room temperature contained lower amount of vitamin C as compared to refrigerated samples.

Anthocyanin

The levels of anthocyanin contents of the fruit decreased during storage. The rate of decrease was found to be higher in T₆ in comparison with other treatments (Table 4). The decrease rate was found to be slow in treatments T₅ and T₄ and consequently the red colour pigment and quality was maintained during extended storage. Samples at room temperature showed rapid decrease of anthocyanin content as compared to refrigerated samples throughout the storage period.

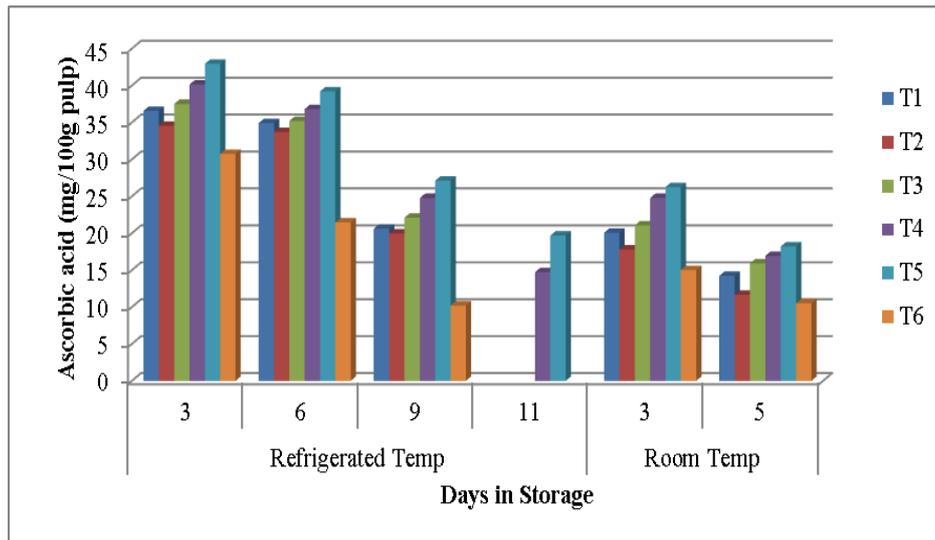


Fig 4. Effect of different treatments on ascorbic acid (mg/100g pulp) content of litchi fruit at refrigerated and room temperature

T₁= Polypropylene packet, T₂ = Corrugated fiber board box, T₃ = Corrugated fiber board box + polypropylene packet, T₄ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet, T₅ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet + corrugated fiber board box, T₆ = Control

Table 4. Effect of different treatments on anthocyanin (mg/100g peel) of litchi fruit

Treatments	Days in storage					
	Refrigerated Temperature (10°C)				Room Temperature	
	3 DAS	6 DAS	9 DAS	11 DAS	3 DAS	5 DAS
S. T ₁	16.07 ^c	15.69 ^c	14.63 ^{bc}	--	13.13 ^c	10.23 ^c
T. T ₂	15.42 ^{cd}	13.57 ^d	12.42 ^c	--	12.92 ^c	9.89 ^c
U. T ₃	17.17 ^{bc}	16.56 ^c	15.38 ^b	--	13.59 ^c	10.48 ^c
V. T ₄	19.85 ^{ab}	19.44 ^b	16.41 ^b	13.03 ^b	17.64 ^b	14.99 ^b
W. T ₅	21.63 ^a	21.23 ^a	20.07 ^a	18.57 ^a	24.71 ^a	20.91 ^a
X. T ₆	12.83 ^d	9.82 ^e	7.5 ^d	--	10.89 ^c	6.10 ^d

T₁= Polypropylene packet, T₂ = Corrugated fiber board box, T₃ = Corrugated fiber board box + polypropylene packet, T₄ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet, T₅ = Glutathione (10 mmol/litre) + citric acid (100 mmol/litre) + polypropylene packet + corrugated fiber board box, T₆ = Control. Values followed by different superscript letters are significantly ($P < 0.05$) different from each other. DAS: Days in Storage

It was evident from the results that, weight loss increase over storage time. The similar result was also recorded by Sivakumar and Korsten (2006) in litchi cv. Mauritius and Somboonkaew and Terry (2008) in cv. Kom. Somboonkaew and Terry (2004) observed that, plastic films affected weight loss of litchi at 13°C when stored for 9 days. Higher temperature causes water molecules to have more free energy, which can accelerate their movement which is important for exchange to the atmosphere around the produce resulting in faster evaporation (Kays and Paull, 2004).

Browning grade increases with increased in storage duration. This finding was concomitant with the finding of Sun *et al.*, (2011). Browning occurs due to the dehydration of pericarp tissues with excessive loss of water. Cell breakdown during harvesting, grading and packing may also enhance browning (Khan *et al.*, 2012). Browning and discolouration correlated well with PPO activity and phenolics concentration (Sun *et al.*, 2009; Mishra *et al.*, 2011). The use of 10 mmol/litre glutathione and 100 mmol/litre citric acid managed browning of litchi fruit and also inhibited the PPO

activity by 80-85% and maintained the better appearance of the fruit (Jiang *et al.*, 1999; Jiang and Fu, 1998).

Disease incidence checked in storage with glutathione treatment and polypropylene packaging. Decay reduction, maintenance of good quality and extension of storage life of litchi can be possible by controlled atmospheres with low O₂ and high CO₂ (Mahajan, 2001). Storage for long duration (over 14 days) lead increased disease occurrence because of tremendous physiological stress (Sivakumar *et al.*, 2007; Aklimuzzaman *et al.*, 2011).

The total soluble solids and total sugar content of treated and untreated fruits decreased with progress of storage period at refrigerated as well as at room temperature.

This might be due to consumption of sugar for respiration and utilization of sugars by growth of microbes (Tassou and Boziaris, 2002). The loss of ascorbic acid during storage may be ascribed to its higher oxidation rate, which consequently increased its degradation and browning (Gimnez *et al.*, 2003). This result was also in complete agreement with the results reported by Mitra *et al.*, (1996) and Aklimuzzaman *et al.*, (2011). Like ascorbic acid, titratable acidity also showed a declining trend in storage as had also been reported by Huang and Scott (1985). The decline in acidity might be due to the fact that in storage conditions, organic acids were utilized in the process of respiration.

A continuous decrease in peel anthocyanin was also observed during storage in all treated and untreated fruits. This observation was completely corroborates the findings of Mangaraj *et al.*, (2012) and Zhang *et al.* (2000). Brownmiller *et al.* (2008) and Harbourne *et al.* (2008) reported that anthocyanins were relatively unstable due to their degradation or the formation of condensed polymers. The anthocyanin and phenolics levels contribute to browning of litchi pericarp. An oxidation of endogenous or exogenous phenolics by PPO can degrade litchi anthocyanin rapidly (Ruenroengklin *et al.*, 2009).

4. CONCLUSIONS

Biotic and abiotic factors can affect pericarp browning of litchi fruit, which causes a series of physico-chemical changes in fruit, such as enzymatic browning, senescence and degradation of anthocyanin pigment. On the basis of results of this experiment, we suggest that the combined application of glutathione and citric acid with packaging materials could be advantageous in the control of browning and to some extent beneficial in decay control and weight loss of litchi fruit at refrigerated and also at room temperature. Using these treatments, it might be suitable for treatment of fruit stored for shorter period (eg. 2 weeks). We recommend the combined application of glutathione and citric acid with different packaging materials to control pericarp browning, weight loss, disease incidence and other quality parameters in litchi fruit.

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