International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 4 (11): 349-361, 2009
ISSN 1816-4897

Minimally of Polyphenol Oxidase Activity and Controlling of Rotting and Browning of Longan Fruits cv. DAW by SO_2 Treatment under Cold Storage Conditions

[1]Wilasinee Chitbanchong, [1]Vicha Sardsud, [2]Kanda Whangchai,
[3]Rumphan Koslanund and [4]Pitipong Thobunluepop
[1]Postharvest Technology Research Institute, Chiang Mai University,
Chiang Mai, Thailand
[2]Department of Biology, Faculty of Science, Chiang Mai University,
Chiang Mai, Thailand
[3]Postharvest Products and Processing Research and Development Office,
Department Of Agriculture, Bangkok, Thailand
[4]Department of Agricultural Technology, Faculty of Technology,
Maha Sarakham University, 44000, Thailand

Abstract: The effects of sulphur dioxide, in combination with, storage temperatures on postharvest decay, pericarp browning and physiological ultrastructure changed of the Longan fruit cv. daw were studied. The treatment of fresh the Longan fruit with SO_2 fumigation combined with the suitable storage condition improved the overall the Longan fruit quality, especially on inner and outer peel tissue and aril color than no SO_2 treatment, while no SO_2 treatment showed the dark color of inner and outer peel of the Longan fruit was appeared, this was correlated with the increasing of polyphenol oxidase (PPO) activity. Moreover, the main factor affected Longan fruits quality was storage duration, the increasing of weight loss, pH value of both peel and aril, PPO activity, especially on the changing of dark-red color of peel was observed after long term of storage. However, the sulphite residues could detect immediately after SO_2 treatment in all part of the Longan fruit, especially on peel tissue, but the residues was significantly decreased along the storage durations. On the other hand, Scanning Electron Microscope (SEM) evaluation found that the surface cracking was also impair the physiological function of the cuticle and increasing water permeability, which may cause water soaking at the inner side of the peel. The injured cell would accelerate the oxidation of phenolic substances and the oxidative products resulted in dark color of inner and outer peel. Therefore, the combination sulphur dioxide fumigation with controlling the optimum of storage temperature could control of postharvest decay and browning.

Key words: Longan (*Dimocarpus longan* Lour.), SO_2 treatment, storage, browning, PPO

INTRODUCTION

Longan (*Dimocarpus longan* Lour.) is a tropical fruit in the *Spindaceae* family. In Thailand, the Longan is a most extensive production and one of the most economically important fruits that has exported fresh Longan to China, Hong Kong, Malaysia, Singapore,

Corresponding Author: Dr. Pitipong Thobunluepop, Department of Agricultural Technology, Faculty of Technology, Maha Sarakham University, 44000, Thailand

Indonesia and Canada (Tongdee, 1997). The cultivated areas are in the Northern region of Thailand. In the year 2008, dried and especially fresh fruit of the Longan is mostly marketed locally and export of the fruit has been increasing rapidly, the exported of fresh Longan is about 168,286 tons and frozen Longan at 346 tons (Lin *el al*., 2001). However, the quantity of domestic and export Longan has been limited by its highly perishable nature, short storage life and susceptibility to postharvest diseases, as a result of bacterial, yeast and fungal infections (Tongdee, 1997). Color deterioration causes the fruit to fetch a lower price at market and even be unmarketable (Smith and McWilliams, 1978). Rapid pericarp browning during storage is the main problem resulting in restrictions on the export of Longan to long-distant markets (Sardsud *et al*., 1994). The fresh Longan fruit could be stored for only 2-3 days at ambient temperature, which caused of discoloration and disorder by postharvest disease including chilling injury and especially on the pericarp browning (Martinez and Whitaker, 1995). Pericarp browning has been attributed to oxidation of phenolics by PPO, producing brown coloured by-products (Ferrar and Walker, 1996). The PPO has been widely studied in various fruits such as apple, grape, litchi and plum (Lin *et al*., 1994) but little is known about the Longan.

For many years, the recommended method to control postharvest decay and prevent pericarp browning in the Longan has been sulfur dioxide (SO_2) treatment. The use of SO_2 fumigation has been the most effective practical postharvest treatment for control of quality during storage (Deng *et al*., 2005). It is currently commercially used in many countries. Recently, importing countries such as China and Singapore have restricted the import of Longan product and other fruits and reduced the maximum permitted residual level of SO_2. Longan consumers are becoming cautious regarding SO_2 residues, due to allergenic symptoms and caused of off-tasted (Whangchai *et al*., 2006).

The storage of Longan fruit under cold condition and the treatment of fresh Longan by using sulfur dioxide is very effective application in browning prevention on the pericarp of the fruits (Jiang *et al*., 2004). However, due to the restriction of the import countries, sulfur dioxide is less use due to allergic to humans (Underhill *et al*., 1997). There is a need to find out the suitable of sulfur dioxide concentration and the storage conditions which are the effective and could be prevented the browning and prolonged the storage shelf life of fresh Longan. Thus, the aim of this study was to evaluate the effect of sulfur dioxide treatment and storage conditions on browning prevention and prolonged the storage shelf life of fresh the Longan fruit to provide the better appearance and safe for consumers.

MATERIALS AND METHODS

Longan (*Dimocarpus longan* Lour.) fruit cv. Daw was harvested from Chiang Mai province, Thailand in the year 2007. The fruits were then separated into bunches with selected homogenous size and grading. The fruit bunches without defects and spoilage was used for the experiments. The experimental design in this study was laid out in 2×2×5 Factorial in CRD with 4 replications. Treatments were including with and without SO_2 treat Then, the treated fruits were store at 2±2 and 7±2°C. Finally, fruits were stored for 0, 2, 4, 6 and 8 weeks. The fruits were sampling immediately after SO_2 treatment at the rate 4.50 tons per 2.5 kg SO_2 and then every twice month after stored under various storage temperatures as discussed above.

Weight Loss Percentage

The fresh weight of the fruit was determined for all treatments as an index of desiccation rate. Weight loss was calculated as following;

$$\text{Weight loss} = (W_f - \text{Weight of sample})/W_f \times 100$$

where, W_f was weight of fresh fruit and Ws was weight of sample.

Polyphenol Oxidase Activity and Determination of Pericarp pH (unit)

Three fruits per treatment were thawed and peeled. And 2 g of pericarp tissue was homogenized in 0.1 M phosphate buffer, pH 6.6 and 0.5 g of insoluble polyvinyl pyrollidone (Merck) for 30 sec with a polytron homogenizer (Kinematica GmbH, Kreins, Luzern, Switzerland; probe diameter, 20 mm). The homogenate was centrifuged for 10 min at 8000x g in a Sorvall rotor SS-34 at 4°C. The supernatant was collected and centrifuged repeated in 1.5 mL tubes at 20 000x g for 10 min at 4°C. The supernatant was collected into a fresh tube and 0.75 mL was used for the PPO assay in duplicate. The PPO assay was conducted by adding 0.12 mL 4-methyl catechol (Sigma, St. Louis, MO, USA) freshly dissolved (0.25 g) in 2 mL of ethanol and 10 mL of distilled water (final concentration, 23 mM). A contron spectrophotometer was used to follow changes in absorbance at 410 nm over 2 min and the linear progress of the reactions was recorded between 30 and 90 sec. Protein content was determined according to Bradford with bovine serum protein as the standard. Results were calculated as in Δactivity mg^{-1} protein x1000. The change in the pH of the buffer was determined in duplicate. To determine the pH of pericarp and aril tissue, extraction was carried out as described, but without PVP and with distilled water instead of phosphate buffer.

Peel and Aril Color

The pericarp (peel) and aril color of the Longan fruit were analyzed initially and after various storage duration. The color was measured on opposite sides of the fruit using (colourQuest XE, Hunter Associates Laboratory, Inc., New York, USA) Minolta chromameter (model CR-200; Minolta, Ramsey, NJ) which provided CIE L^*, C^*, H^*, A^* and B^* values.

Preparation of Longan Pericarp for Scanning Electron Microscope (SEM)

The Longan pericarps were cut into 5 mm squares for SEM evaluation. The pericarps were cut in a dish of 0.1 M phosphate buffer pH 7.3. The pieces were transferred immediately after they were cut into a primary fixative. The Longan pericarp pieces were fixed in a fixative solution as described by Bozzola and Russell (1999) with slight modification for anatomical study. The pericarp specimens were fixed with a primary fixative containing 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 at 4°C for 2 h. After that the tissue was usually washed in the same buffer vehicle used in the glutaraldehyde fixation step. Next, the specimens were post-fixed in 1% osmium tetroxide in the same buffer for 2 h. Then, the specimens were dehydrated stepwise by exposure to ethanol-buffer mixture (30, 50, 70, 80, 90 and 100%) allowing 15 min in each and critical point drying with liquid CO_2. This is a critical drying technique, as it achieves a phase change from liquid to dry gas without the effects of surface tension and is, therefore, suitable for delicate biological specimens for removal of water from the specimens. For SEM, the dried specimen was mounted on specimen studs and sputter coated with gold. Finally, the specimens were viewed with a scanning electron microscope (JEOL, JSM-5910LV, JEOL Ltd., Tokyo, Japan) at 15 kV.

Sulphite Residual (mg kg^{-1})

A sample of 50 g from the whole fruit, aril and peel was obtained from a minimum of 30 fruits and stored overnight at -70°C. Sample were then examined in duplicate for sulphite residual according to De Vries *et al*. (1987).

Statistical Analysis

The statistical analysis was carried out using a statistical software Statistic version released 8.0 and Least significant different test at 95% was used to determine significant difference among the treatments.

RESULTS AND DISCUSSIONS

The ANOVA analysis indicated that SO_2 treatment changed pH value of peel tissue significantly (Table 2). The pH value of peel tissue decreased significantly after treated the Longan fruit with SO_2 (4.30), when compared with non SO_2 treatment (5.36). However, SO_2 treatment did not affected on pH value of aril. The storage temperature did not effect on pH value of peel and aril changed. On the other hand, the storage duration was the main factor that affect on the change of peel and aril pH. The pH value of both part of the Longan fruit increased significantly in the long term of storage (Table 1). Additionally, ANOVA analysis indicated that the storage duration did not affect only pH value of peel and aril changed but also affected on weight loss of the Longan fruit (Table 2), while the weight loss

Table 1: The effects of sulphur dioxide treatments, storage temperatures and storage durations on Longan cv. Daw fruit quality changes

					C*			H*		
	pH		Weight loss		Peel			Peel		
Treatments	Peel	Aril	(%)	PPO#	Aril	Outer	Inner	Aril	Outer	Inner
No SO_2	5.36a	6.71a	10.58a	9.50a	7.41a	28.10b	21.58a	67.25a	66.20b	76.37b
SO_2	4.30b	6.72a	10.13a	8.05a	7.24a	32.90a	22.19a	66.42a	70.07a	82.88a
LSD (0.05)	0.03	0.03	0.89	1.91	0.37	0.46	1.67	1.32	0.49	0.48
Temperature (°C)										
2	4.82a	6.72a	10.08a	8.30a	7.55a	34.42a	22.43a	67.43a	69.31a	79.26b
7	4.84a	6.72a	10.63a	9.25a	7.10b	26.6b	21.34a	66.24a	66.95b	80.00a
LSD (0.05)	0.03	0.03	0.89	1.91	0.37	0.46	1.67	1.32	0.49	0.48
Storage durations (weeks)										
0	4.78b	6.51e	0.00e	9.58a	5.88d	27.87c	20.95b	54.77e	69.10b	70.00e
2	4.86a	6.81b	9.25d	9.05a	5.88d	28.33c	20.42b	65.23d	67.14c	78.52d
4	4.84a	6.66d	11.32c	7.41a	6.86b	31.75b	22.54ab	67.86c	70.70a	80.97c
6	4.83a	6.75c	14.32b	7.60a	7.74b	31.21b	23.88a	71.27b	63.64c	82.78b
8	4.83a	6.88a	16.88a	10.20a	8.44a	33.37a	21.67ab	75.04a	70.08a	85.87a
LSD (0.05)	0.05	0.04	1.41	3.02	0.59	0.72	2.65	2.08	0.77	0.75

	L*			A*			B*		
		Peel			Peel			Peel	
Treatments	Aril	Outer	Inner	Aril	Outer	Inner	Aril	Outer	Inner
No SO_2	40.74a	55.67b	60.90b	2.84a	11.14a	5.09a	6.75a	25.73b	20.81a
SO_2	40.72a	60.66a	76.2a	2.77a	10.95a	2.79b	6.57a	30.94a	21.90a
LSD (0.05)	0.54	0.49	1.75	0.14	0.3	0.52	0.37	0.44	1.00
Temperature (°C)									
2	40.94a	58.59a	67.06b	2.85a	12.01a	4.22a	6.89a	32.17a	21.86a
7	40.52a	57.77b	70.04a	2.76a	10.08b	3.66b	6.43b	24.51b	20.85a
LSD (0.05)	0.54	0.49	1.75	0.14	0.3	0.52	0.37	0.44	1.60
Storage durations (weeks)									
0	37.28d	54.75c	74.02a	1.96c	9.89c	1.50d	5.47b	31.39a	20.86a
2	38.47c	56.35b	72.29a	2.10c	10.69b	2.74c	6.45b	30.01b	19.92a
4	40.59d	66.01a	69.03b	2.12c	10.10c	3.34bc	7.04ab	28.08c	22.29a
6	41.13b	56.68b	65.26c	3.05b	13.49a	4.03b	6.93ab	26.20d	22.43a
8	46.18a	57.02b	62.14d	4.78a	11.07b	8.08a	7.42a	26.02d	21.27a
LSD (0.05)	0.85	0.77	2.76	0.22	0.47	0.82	0.59	0.70	2.53

The different letters indicate the statistically significant difference by LSD at 5% level. *Polyphenol enzymatic activity (PPO) was described in Δactivity mg^{-1} protein$\times 10^3$

Table 2: Effects of sulphur dioxide treatment, storage temperatures and storage durations on the changes of peel and aril pH and weight loss of Longan cv. Daw

SO_2 treatment	Temperature (°C)	Time (weeks)	pH Peel	pH Aril	Weight loss (%)	PPO# (Δactivity mg^{-1} protein)
No	2	0	5.36a	6.61gh	0k	9.78ab
		2	5.38a	6.80de	8.98ij	9.22ab
		4	5.36a	6.60h	11.38fghi	7.60b
		6	5.34a	6.62gh	14.19cdef	7.30b
		8	5.34a	6.83cd	16.50abcd	10.10ab
	7	0	5.36a	6.62gh	0k	9.78ab
		2	5.42a	6.84bcd	10.52hij	9.46ab
		4	5.37a	6.73ef	12.17efgh	8.93ab
		6	5.35a	6.69fg	14.94abcde	9.01ab
		8	5.33a	6.84bcd	17.10a	13.71a
SO_2	2	0	4.20c	6.41i	0k	9.39ab
		2	4.32b	6.82cd	8.24j	8.60ab
		4	4.30b	6.72ef	10.61hij	6.41b
		6	4.31b	6.89abc	13.88defg	6.73b
		8	4.33b	6.96a	17.02ab	7.75ab
	7	0	4.20c	6.41i	0k	9.39ab
		2	4.33b	6.80de	9.27ij	8.94ab
		4	4.33b	6.60h	11.13ghi	6.70b
		6	4.35b	6.80de	14.26bcde	7.40b
		8	4.33b	6.91ab	16.92abc	9.26ab
LSD (0.05)			0.098	0.083	2.83	6.05
Source of variation						
SO_2			*	ns	ns	ns
Temperature			ns	ns	ns	ns
Time			*	*	*	ns
SO_2 × temperature			ns	ns	ns	ns
SO_2 × time			*	*	ns	ns
Temperature × time			ns	ns	ns	ns
SO × temp × time			ns	*	ns	ns

The different letters indicate the statistically significant difference by LSD at 5% level. ns: not significantly different
*: Polyphenol enzymatic activity was described in Δactivity mg^{-1}protein x 10^3

increased significantly during the long term of storage (Table 1). However, weight loss of the Longan fruit did not affected by SO_2 treatment and storage temperatures (Table 1). Table 1 and 2 indicated that all treatments not affected on the change of PPO activity.

The ANOVA analysis indicated that SO_2 did not affect on aril color changed. However, the storage duration was the main factor affected on the change of aril color (Table 3) (Fig. 1a-d). The aril was bright orange-yellow color after stored for 8 weeks (C^*, H^*, L^*, A^* and B^*were increased significantly) (Table 1). Moreover, the storage temperature, interaction between SO_2 and storage duration and interaction between all treatment significantly increased C^* and B^* values (Table 3), which was indicated that the aril became dull yellow color (Fig. 1g, h). The ANOVA results indicated that SO_2 treatment, storage temperature and storage duration significantly affected on H^*, L^* and A^* values (Table 4). The SO_2 treatment and storage temperature significantly increased H^* and L^* values, while A^* value significantly decreased (Table 1). The results showed that inner part of peel color of no SO_2 treatment and stored under 7°C was more darkened than that SO_2 treatment and stored under 2°C (L^* value decreased), which was more bright green-yellow color (A^* decreased) (Table 4) (Fig. 1c, d). For the storage duration, H^* and A^* values significantly increased, while L^* value decreased after stored for 8 weeks (Table 1). This results indicated that the inner part of peel color was became orange-yellow darkness color after stored for 8 weeks (Fig. 1e-g). Interestingly, all treatments were SO_2 treatment, storage temperature and duration was the main factors affected on the outer peel color (Table 5). The outer peel red

Table 3: Effects of sulphur dioxide treatment, storage temperatures and storage durations on the changes of aril color of Longan cv. Daw

			Aril colour parameters				
SO_2 treatment	Temperature (°C)	Time (weeks)	C*	H*	L*	A*	B*
No	2	0	5.93g	70.20bcd	37.04h	2.02ef	5.56ef
		2	7.68abcde	71.17bcd	41.03bcd	2.43de	7.27bcd
		4	7.97abcd	63.90ef	45.96a	3.33b	7.15bcd
		6	8.84a	55.07g	38.40fgh	4.50a	7.27bcd
		8	7.46cdef	76.39a	41.73bc	1.80f	7.21bcd
	7	0	5.93g	70.20bcd	37.04h	2.02ef	5.56ef
		2	6.68efg	69.72cd	40.16cde	2.17ef	6.27def
		4	8.57abc	67.60de	47.01a	3.14bc	7.93ab
		6	8.13abc	54.20g	38.13gh	4.66a	6.61cde
		8	6.94defg	74.07ab	40.94bcd	1.79f	6.68cde
SO_2	2	0	5.83g	65.51ef	37.51gh	2.17ef	5.38f
		2	6.90defg	73.68abc	41.06bcd	1.97f	6.58cde
		4	8.12abc	67.78de	46.34a	2.98bc	7.50abc
		6	8.06abcd	54.26g	38.30fgh	4.61a	6.58cde
		8	8.77ab	76.33a	41.99b	2.17ef	8.46b
	7	0	5.83g	65.51ef	37.51gh	2.17ef	5.38f
		2	6.2og	70.5obcd	40.10cde	1.93f	5.71ef
		4	6.32fg	61.63f	45.43a	2.77cd	5.58ef
		6	8.74ab	55.56g	39.05efg	4.84a	7.26bcd
		8	7.64bcde	73.39abc	39.85def	2.07ef	7.33abcd
LSD (0.05)			1.18	4.17	1.70	0.44	1.18
Source of variation							
SO_2			Ns	ns	ns	ns	ns
Temperature			*	ns	ns	ns	*
Time			*	*	*	*	*
SO_2 × temperature			ns	ns	ns	ns	ns
SO_2 × time			*	ns	ns	ns	*
Temperture × time			ns	ns	ns	ns	ns
SO × temp. × time			*	ns	ns	ns	*

The different letters indicate the statistically significant difference by LSD at 5% level. ns: Not significantly different

color of no SO_2 treatment were scarlet than orange-red (H^* decreased), became darkened (L^* decreased), less intensely red (C^* decreased) and blue-yellowish (B^* decreased) color (Table 1). Moreover, under high storage temperature (7°C) and long term of storage (8 weeks), the outer peel color became blue-yellowish (B^* decreased), darkened (L^* decreased) and more scarlet than orange-red (hue angle; H^*, decreased) or changed to cloudy and dark or scarlet, which was showed in browning (Table 1) (Fig. 1e, h). Pericarp browning increased with increasing of storage period. Fruit fumigated with SO_2 did not show any pericarp browning throughout this investigation. According to Duan *et al.* (2004) the major factors reducing the storage life and marketability of the Longan fruit are microbial decay and pericarp browning. Low temperature storage at 1-5°C is used to reduce pathological decay, but has only a limited role in reducing pericarp browning. In this study, the SO_2 treatment inhibited browning and decreased PPO activity of Longan pericarp during storage. Low PPO activity correlated with low browning appearance. According to Jiang and Fu (1998), the sulfur dioxide application gave better results in controlling litchi browning and 80-85% inhibition of PPO (Jiang, 1999). Moreover, the fruit deteriorates rapidly when removed from cold storage. It was observed that under the refrigeration conditions Longan fruits have a storage life of approximately 30 days. Pulp quality and disease development are generally stable during cold storage until such time as fruits become visually unacceptable from pericarp browning (Jiang and Li, 2001). Sulfur dioxide fumigation has been the most effective postharvest treatment for control of pericarp browning in the Longan fruit and is used extensively in commercial situations at present. However, there is increasing consumer and regulatory resistance to the use of this chemical (Jiang *et al*., 2002).

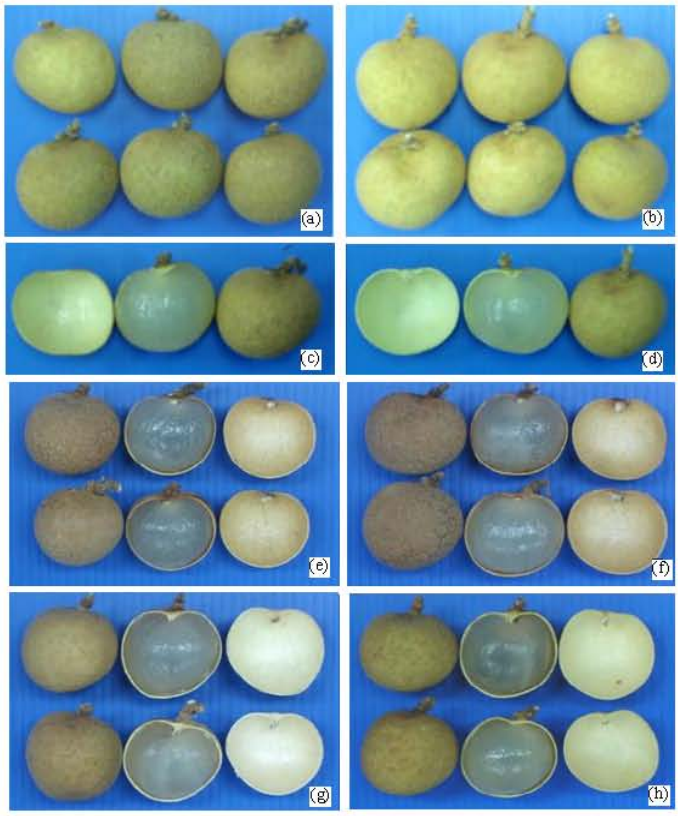

Fig. 1: The effects of SO_2 treatments, storage temperature and storage duration on the changing of inner and outer peel tissue and aril color of Longan cv. daw. (a) No SO_2 treatment at the initially of storage, (b) SO_2 treatment at the initially of storage, (c) inner and outer peel tissue and aril color changing by no SO_2 treatment at the initially of storage, (d) inner and outer peel tissue and aril color changing by SO_2 treatments at the initially of storage, (e) and (f) the changing of inner and outer peel tissue and aril color by no SO_2 treatments stored at 2 and 7°C for 8 weeks, respectively, (g) and (h) the changing of inner and outer peel tissue and aril color by SO_2 treatments stored at 2 and 7°C for 8 weeks, respectively

The pearson correlation coefficients analysis showed that SO_2 treatment and storage temperature factors resulted weight loss, the changing of peel and aril tissue pH and polyphenol enzymatic activity were had the positive correlation between them (Table 6, 7). Moreover, the effect of storage duration had positive correlation between peel tissue pH-PPO enzymatic activity and weight loss-aril tissue pH but stated the negative correlation between aril pH and peel tissue pH (Table 8). Meanwhile, the experiment found that a lower pH in the peel kept in SO_2 treatment might be beneficial in preventing browning. The rapid increase in the browning index of the Longan fruit stored in SO_2 treatment after long term of storage may be due to the senescence and fruit decay, indicated by increases in pH value, which was agreed with Tian *et al.* (2002). According to Solomon *et al.* (1992) reported that PPO catalyzed browning of fruit could be prevented by several application such as; heat inactivation of enzymes, exclusion or removal of one or both of the substrates (O_2 and

Table 4: Effects of sulphur dioxide treatment, storage temperatures and storage durations on the changes of inner part of peel color of Longan cv. Biewkaew

			Inner part of peel colour parameters				
SO_2 treatment	Temperature (°C)	Time (weeks)	C*	H*	L*	A*	B*
No	2	0	21.45abc	86.69ab	74.08bcd	1.25g	21.39abc
		2	23.87ab	72.65h	52.12hi	7.13b	22.75ab
		4	22.54abc	77.68f	60.43fg	4.83de	22.01ab
		6	22.47abc	66.77i	49.83i	8.81a	20.67abc
		8	21.63abc	75.65g	59.30g	5.35cd	20.95abc
	7	0	21.45abc	86.69ab	74.08bcd	1.25g	21.39abc
		2	17.27c	75.55g	56.13gh	4.25de	16.73c
		4	20.54bc	79.86e	65.10ef	3.59ef	20.21abc
		6	23.48ab	67.11i	57.41gh	9.10a	21.64abc
		8	21.7abc	75.08g	60.48fg	5.39cd	20.36abc
SO_2	2	0	20.44bc	85.05c	73.96bcd	1.76g	20.33abc
		2	20.89abc	82.95d	71.54cd	2.41fg	20.72abc
		4	22.53abc	85.60bc	76.16bc	1.75g	22.45ab
		6	26.01a	73.13h	75.87bc	7.52ab	24.89a
		8	22.50abc	86.43bc	77.27b	1.40g	22.45ab
	7	0	20.44bc	85.05c	73.97bcd	1.76g	20.33abc
		2	19.65bc	82.93d	68.79de	2.34fg	19.48bc
		4	24.53ab	87.98a	87.44a	0.82g	24.48ab
		6	23.57ab	72.99h	77.95b	6.91bc	22.53ab
		8	21.36abc	86.72ab	79.56b	1.20g	21.31abc
LSD (0.05)			5.29	1.50	5.52	1.63	5.06
Source of variation							
SO_2			ns	*	*	*	ns
Temperature			ns	*	*	*	ns
Time			ns	*	*	*	ns
SO_2 × temperature			ns	ns	ns	ns	ns
SO_2 × time			ns	ns	*	*	ns
Temperature × time			ns	ns	*	ns	ns
SO_2 × temp × time			ns	ns	ns	ns	ns

The different letters indicate the statistically significant difference by LSD at 5% level. ns: not significantly different

phenols), adding compounds that inhibit PPO or prevent melanin formation and especially on controlling the pH to be lowering to 2 or more units below the pH optimum, by reaction-inactivation of the browning enzyme. However, experimental results indicated that non-treated and treated Longans fruit with SO_2 provided the pH about 4.30-5.36 in peel and about 6 in arils tissue. This results congruence to Wong (1995) reported that the pH optima to most PPO's activity are near 6. Under this condition, PPO was activated and accelerated the browning of Longan fruits. Moreover, Underhill and Critchley (1992) found that the pericarp browning was correlated with moisture loss. Likewise, it is every likely that the natural cracking of Longan peel facilitates rapid moisture loss and cause surface browning during harvest and storage. The surface cracking was also impair the physiological function of the cuticle and increasing water permeability, which may cause water soaking at the inner side of the peel (Medeira *et al*., 1999). The injured cell would accelerate the oxidation of phenolic substances and the oxidative products resulted in dark color of inner and outer peel (Abe, 1990). The PPO and peroxidase (POD) catalyze the oxidation of phenolics to quinines and then condense tannins to brown polymers. The initiation of the enzymatic browning depends largely on the loss of compartmentation of enzymes and substrates. In this study, there were high activities of PPO and POD in the Longan fruit at harvest, but no skin browning occurred while high ATP production and low malondialdehyde (MDA) content were observed, which further supports the hypothesis that the loss of compartmentation of enzymes and substrates was the key factor for the enzymatic browning reaction of plant tissues. Thus, reduced skin browning of the Longan fruit by pure oxygen treatment could be accounted for maintenance of compartmentation of enzymes and substrates by enhanced respiration and ATP production.

Table 5: Effects of sulphur dioxide treatment, storage temperatures and storage durations on the changes of outer part of peel color of Longan cv. Daw

			Inner part of peel colour parameters				
SO_2 treatment	Temperature (°C)	Time (weeks)	C*	H*	L*	A*	B*
No	2	0	30.30de	70.27c	53.40jk	10.22fgh	28.49ef
		2	31.53d	67.58de	52.03k	12.03c	29.14e
		4	35.51c	70.01c	61.29de	11.60cd	31.43d
		6	34.14c	63.50h	53.52jk	15.22a	30.56d
		8	29.63ef	67.18def	50.60ij	11.48cd	27.31fg
	7	0	30.30de	70.27c	53.40jk	10.22fgh	28.49ef
		2	18.70j	62.64h	56.07h	8.58j	16.60l
		4	22.93hi	66.42efg	65.13c	9.14ij	21.01j
		6	21.50i	58.46i	55.11hi	11.21cde	18.34k
		8	28.50f	65.66fg	53.13jk	11.73c	25.96gh
SO_2	2	0	25.43g	67.93de	56.10h	9.55ghi	23.54i
		2	38.85b	70.34c	58.22g	13.06b	36.57bc
		4	42.38a	75.72a	69.84a	10.40efg	41.02a
		6	40.95a	67.23de	59.85ef	15.82a	37.74b
		8	37.45b	73.35b	59.52fg	10.72def	35.86c
	7	0	25.43g	67.93de	56.11h	9.55ghi	23.54i
		2	24.24gh	68.00d	59.09fg	9.09ij	22.47i
		4	28.20f	70.66c	67.79b	9.28hij	26.58gh
		6	28.23f	65.37g	58.22g	11.71c	25.67h
		8	37.87b	74.14b	61.82d	10.34efg	36.42bc
LSD (0.05)			1.45	1.54	1.53	0.95	1.40
Source of variation							
SO_2			*	*	*	ns	*
Temperature			*	*	*	*	*
Time			*	*	*	*	*
SO_2 × temperature			ns	*	*	ns	ns
SO_2 × time			*	*	*	*	*
Temperature × time			*	*	*	*	*
SO_2 × temperature × time			*	*	*	Ns	*

The different letters indicate the statistically significant difference by LSD at 5% level. ns: Not significantly different

Table 6: Pearson correlation coefficients of SO_2 treatment on the change of polyphenoloxidase enzymatic activity (PPO), Weight Loss (WL) and pH value of peel and aril of Longan fruits cv. DAW

	PPO	WL	pH-peel
WL	0.764		
pH-Peel	0.897	0.864	
pH-Aril	0.896	0.873	0.994

Table 7: Pearson correlation coefficients of storage temperatures on the change of polyphenoloxidase enzymatic activity (PPO), Weight Loss (WL) and pH value of peel and aril of Longan fruits cv. DAW

	PPO	WL	pH-peel
WL	0.783		
pH-Peel	0.910	0.862	
pH-Aril	0.907	0.870	0.994

Table 8: Pearson correlation coefficients of storage duration on the change of polyphenoloxidase enzymatic activity (PPO), Weight Loss (WL) and pH value of peel and aril of Longan fruits cv. DAW

	PPO	WL	pH-peel
WL	ns		
pH-Peel	0.232	Ns	
pH-Aril	ns	0.538	-0.221

The result suggests that SO_2 treatment may be suitable for keeping of the Longan's fruit over a relatively short period, which skin ultrastructure played a role in its storability. However, the suitable concentration and fumigation time is also necessary to point out. The Longan pericarp was thick about 630-700 μm and composed of three layers. The outer layer

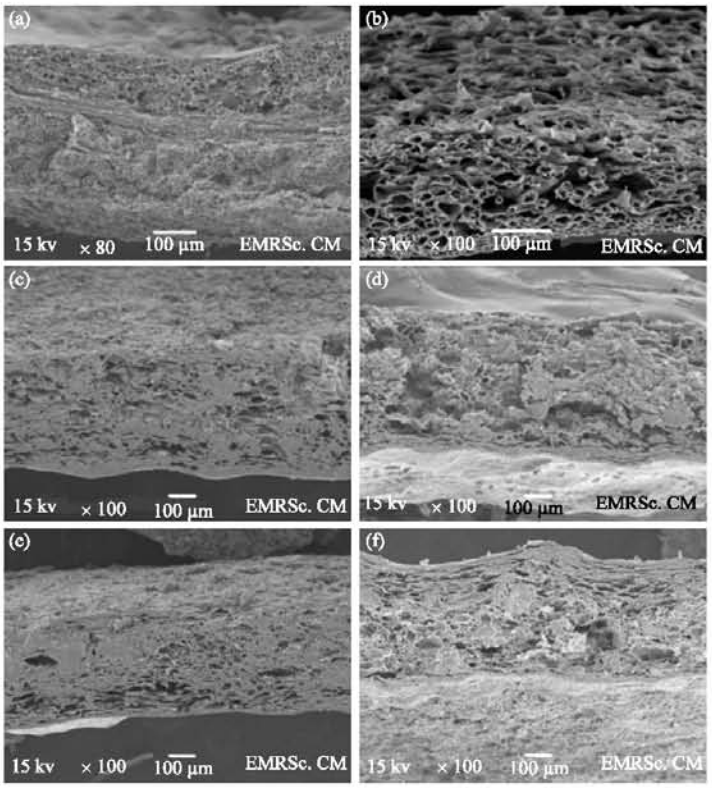

Fig. 2: Transverse sectional micrographs of the Longan fruit pericarps cv. Daw affected by SO_2 treatment and various storage condition. (a, b) Longan pericarps after no SO_2 and SO_2 treatment at the initially of storage, (c, d) longan pericarps affected by no SO_2 treatment after stored at 2 and 7°C for 8 weeks and (e, f) Longan pericarps affected by SO_2 treatment after stored at 2 and 7°C for 8 weeks

is exocarp consisted of natural opening and cracking on the surface. It was covered by thin discontinuous layer of cuticle and brown epidermal hair. The mesocarp, main part of the pericarp consisted of about 70% of the pericarp tissue. It contained elliptical in shape with thick cell walls (Fig. 2a, b). The vascular bundles were tubular and consisted of one layer cell. When the fruit showed during SO_2 treatment, increasing of storage duration and temperatures, the dark color of inner and outer peel of the Longan fruit was appeared. The SEM observation showed a layer of injured cell in the pericarp was fibrous tissues disappeared (Fig. 2c, d). Wax that covered the pericarp and epidermal hair also damaged. The mesocarp cell were also damaged and had collapsed. The destruction of cell membrane was also observed (Fig. 2e, f). Underhill and Critchley (1992) found that the pericarp browning was correlated with moisture loss. Likewise, it is every likely that the natural cracking of Longan peel facilitates rapid moisture loss and cause surface browning during harvest and storage. The surface cracking was also impair the physiological function of the cuticle and increasing water permeability, which may cause water soaking at the inner side of the peel (Medeira *et al*., 1999). The injured cell would accelerate the oxidation of phenolic substances and the oxidative products resulted in dark color of inner and outer peel (Abe, 1990).

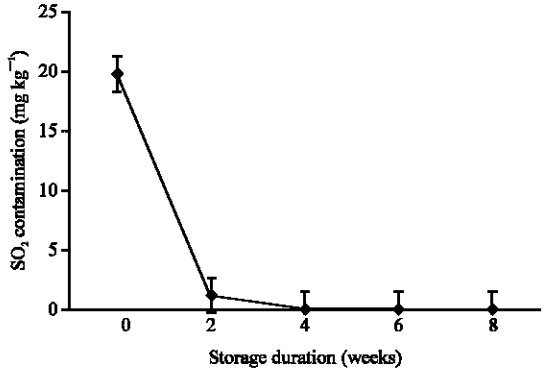

Fig. 3: The effect of storage duration on SO_2 contamination in aril of Longan cv. Daw

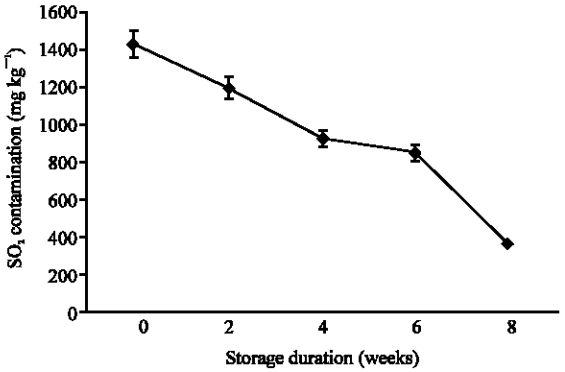

Fig. 4: The effect of storage duration on SO_2 contamination in peel of Longan cv. Daw

At the prior of storage, the contamination of sulphite residue was found highest in both aril and peel tissue. On the other hand, the contamination of sulphite significantly decreased along the storage durations (Fig. 3, 4). However, sulphite contamination still high concentration in peel tissue (350 mg kg^{-1}) after stored for 8 weeks (Fig. 4), while sulphite contamination was not found in aril after stored for 4 weeks (Fig. 3). The fumigation time and concentration are the most important factors affecting the SO_2 residues. Higher concentration and longer fumigation time resulted in higher SO_2 residue (Ye and Ge, 1996) which was mainly located in the peel and much less in the aril and gradually decreased with prolonged storage (Lemmer *et al*., 2000). Han *et al.* (2001) reported that most of the SO_2 residue was located in the pericarp. Appropriate SO_2 treatment lowered the SO_2 residue level in the pulp to as low as 10 μg g^{-1}. The eating quality was maintained during the early stage of storage and the shelf life was extended as compared with the control fruit. If SO_2 concentration and fumigation time were strictly controlled, lower residue and longer storage life could be achieved.

CONCLUSIONS

In conclusion, the combined application of SO_2 treatment and cold storage temperature stored Longan fruits under the cold condition significantly prevented pericarp browning of harvested the Longan fruits. Exposure of the Longan fruits to those conditions enhanced

high color quality, reduced weight loss percentage, prevented cell wall cracking and delayed the activity of PPO and the decompartmentation of PPO and POD and their substrates.

ACKNOWLEDGMENTS

The authors wish to thank for Ministry of Agriculture and Cooperation, Department of Agriculture, Royal Thai government for financial support provided is gratefully acknowledged, Assistant Prof Dr. Vicha Sardsud, Postharvest Technology Research Institute, Chiang Mai University and Dr. Pitipong Thobunluepop, Department of Agricultural Technology, Faculty of Technology, Maha Sarakham University for comment on the manuscript.

REFERENCES

Abe, K., 1990. Ultrastructural Changes During Chilling Stress. In: Chilling Injury of Horticultural Crops. Wang, C.Y., (Ed.). CRC Press Inc., Boca Raton, USA., pp: 71-84.

Bozzola, J.J. and L.D. Russell, 1999. Electron Microscopy: Principles and Techniques for Biologists. 2nd Edn., Jones and Bartlett Publishers, Massachusetts, pp: 634.

De Vries, H.W., T. Patrick and H. Andreas, 1987. Molecular and atomic clouds associated with infrared cirrus in Ursa Major. Astrophys. J., 319: 723-729.

Deng, Y., W. Ying and L. Yuanfei, 2005. Effects of high O_2 levels on post-harvest quality and shelf life of table grapes during long-term storage. Eur. Food Res. Technol., 221: 392-397.

Duan, X.W., Y.M. Jiang, X.G. Su, H. Liu and Y.B. Li *et al*., 2004. Role of pure oxygen treatment in browning of litchi fruit after harvest. Plant Sci., 167: 665-668.

Ferrar, P.H. and J.R.L. Walker, 1996. Inhibition of diphenol oxidases: A comparative study. J. Food Biochem., 20: 15-30.

Han, D.M., Z.X. Wu and Z.L. Ji, 2001. Effects of SO2 treatment on the overal quality of Longan fruits (cv. Shixia) during cold storage. Acta Horticulturae, 558: 375-380.

Jiang, Y. and J. Fu, 1998. Inhibition of polyphenol oxidase and the browning control of Litchi fruit by glutathione and citric acid. Food Chem., 62: 49-52.

Jiang, Y.M., 1999. Purification and some properties of polyphenol oxidase of Longan fruit. Food Chem., 66: 75-79.

Jiang, Y.M. and Y.B. Li, 2001. Effects of chitosan coating on postharvest life and quality of Longan fruit. Food Chem., 73: 139-143.

Jiang, Y., Z. Zhang, D.C. Joyce and S. Ketsa, 2002. Postharvest biology and handling of Longan fruit (*Dimocarpus longan* Lour.). Postharvest Biol. Technol., 26: 241-252.

Jiang, Y., L. Yuebiao and L. Jianrong, 2004. Browing control, shelf life extension and quality maintenance of frozen litchi fruit by hydrochloric acid. J. Food Eng., 63: 147-151.

Lemmer, D., F.J. Kruger and I.J. Froneman, 2000. Evaluation of the postharvest characteristics of litchi fruit from the cultivar development programme. South Afr. Litchi Growers Assoc. Yearbook, 11: 33-36.

Lin, S.X., Y.M. Jiang, Y.B. Li, D.L. Zhang and F. Chen, 1994. Effect of GA3 on colour of plum fruit after harvest. Acta Hortic. Sin., 21: 320-322.

Lin, H.T., S.J. Chen, J.Q. Chen and Q.Z. Hong, 2001. Current situation and advances in postharvest storage and transportation technologies of Longan fruit. Acta Hortic., 558: 343-351.

Martinez, M.V. and J.R. Whitaker, 1995. The biochemistry and control of enzymatic browning. Trends Food Sci. Technol., 6: 195-200.

Medeira, M.C., M.I. Maia and R.F. Vitor, 1999. The first stages of pre-harvest peel pitting development in Encore mandarin: An histological and ultrastructural study. Ann. Bot., 83: 667-673.

Sardsud, U., V. Sardsud, C. Sittigul and T. Chaiwangsri, 1994. Effect of Plant Extracts on the *in vitro* and *in vivo* Development of Fruit Pathogens. In: Development of Post-harvest Handling Technology for Tropical Tree Fruit, Jonhson, G. and I. Highley (Eds.). ACIAR, Canberra, Australia.

Smith, C.W. and E.L. McWilliams, 1978. Chilling, hardening and electrolyte loss in *Scindapsus pictus* and *Maranta leuconeura*. Hortscience, 13: 344-344.

Solomon, E.I., M.J. Baldwin and M.D. Lowrey, 1992. Electronic structures of active sites in copper proteins: Contributions to reactivity. Chem. Rev., 92: 521-542.

Tian, S.P., Y. Xu, Q.Q. Gong, A.L. Jiang, Y. Wang and Q. Fan, 2002. Effects of controlled atmospheres on physiological properties and storability of Longan fruit. Acta Hortic. (ISHS), 575: 659-665.

Tongdee, S.C., 1997. Postharvest Physiology and Storage of Tropical and Sub-Tropical Fruits. CAB International, USA., pp: 335-345.

Underhill, S.J.R. and C. Critchley, 1992. The physiology and anatomy of lychee (*Litchi chinensis* Sonn.) pericarp during fruit development. J. Hortic. Sci., 67: 437-444.

Underhill, S.J.R., L.M. Coates and Y. Saks, 1997. Litchi. In: Postharvest Physiology and Storage of tropical and Subtropical Fruits, Mitra, S.K. (Ed). CAB International, Wallingford, UK., pp: 191-208.

Whangchai, K., K. Saengnil and J. Uthaibuta, 2006. Effect of ozone in combination with some organic acids on the control of postharvest decay and pericarp browning of Longan fruit. Crop Prot., 25: 821-825.

Wong, D.W.S., 1995. Food Enzymes, Structure and Mechanism. Chapman and Hall, New York pp: 284-320.

Ye, Q. and Y.Q. Ge, 1996. Several questions of grape postharvest diseases and SO_2 treatment. Xinjiang Agric. Sci., 4: 184-184.