International Journal of

Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 6 (1): 82-92, 2011
ISSN 1816-4897 / DOI: 10.3923/ijar.2011.82.92

Effects of Chitosan Coating to Some Postharvest Characteristics of *Hylocercus undatus* (Haw) Brit. and Rose Fruit

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ABSTRACT

Pitaya fruit is a popular fruit in Thailand due to its attractive reddish colour and highly delicate flavour. However, the shelf life of this fruit is rather short under ambient condition. The objective of this research was to extend the fresh quality of pitaya fruit by using a natural coating substance, chitosan. The effects of chitosan coating in extending the postharvest life of pitaya fruits relating to weight loss, stomatal conductance, stomatal size in terms of stomatal width and stomatal length, stomatal aperture, wilting percentage and shelf life were investigated. The experiment was arranged in a completely randomized design, composed of coating with chitosan at four levels (0, 1, 2 and 3%) and then stored under ambient temperature. Each treatment consisted of four replicates, ten fruits per replication. The results indicated that fruits treated with 3% of chitosan had the least attributes of stomatal conductance, stomata size in terms of stomatal width, stomatal length and stomatal aperture. In addition, treatment of 3% chitosan also showed the lowest wilting percentage and gave the maximal postharvest life of 8.17 days.

Key words: Chitosan, *Hylocercus undatus* (Haw) Brit. and Rose, stomata, storage life, wilting

INTRODUCTION

Hylocereus undatus (Haworth) Britt and Rose, or Pitaya fruit is in the family of Cactaceae and originated in North, Central and South America. They are widely planted and consumed throughout the world as a fruit crop. Currently, they are being cultivated commercially in at least 22 countries of the tropics, including Thailand (Diczbalisand and McMahon, 2004). Pitaya fruit is also a health fruit, which has high nutritional value including beta-carotene, lycopene and vitamin E, with average concentrations of 1.4, 3.4 and 0.26 μg/100 g edible portion, respectively (Charoensiri *et al*., 2009). These attributes have important roles for reducing cholesterol, activating the digestive function and preventing colon cancer and diabetes (Ariffin *et al*., 2009). In Thailand, a popular grown variety of this fruit is the red pitaya with white flesh. This fruit variety is oblong-shaped about 7 to 10 cm wide, 10 to 15 cm long and weighs 300 to 700 g with leathery, thin, brilliant reddish color with several greenish scales on the fruit skin (Ariffin *et al*., 2009). The internal flesh is pure white, soft, juicy with numerous small black seeds. Both the flesh and seeds are edible with a delicious taste and aroma making it pleasant to eat especially during hot weather. Because the attractive appearance with its red skin colour, delicately sweet flavour and high nutritional supplement, the pitaya fruit is a good candidate for export beyond the local Asian markets of Singapore, Hong Kong, Taiwan, Philippines, Malaysia and Thailand (Hoa *et al.*, 2006; Mizrahi *et al.*, 1997; Le Bellec *et al.,* 2006; Luders and McMahon, 2006; Wichienchot *et al.*, 2010). Generally, pitaya fruit have a very short postharvest life of 3-4 days at ambient temperatures. This short postharvest period limits consumption, visual appeal and causes lower pricing at market. One of the most important problems in marketing pitaya fruit is rapid withering a few days after harvest (Jiang *et al.*, 2002). Ariffin *et al.* (2009) reported that after cutting, its shelf life is rapidly shortened by weight loss and desiccation as a result of transpiration. These characteristics cause a loss of the attractive qualities, shortens storage life and reduces marketability. To delay loss of fresh quality, postharvest treatments need to be applied, including coating. Chitosan, a natural polysaccharide, consists of (1,4)-linked 2-amino-deoxy-β-d-glucan. It is derived from chitin, found in nature and has a great potential for a wide range of postharvest applications to improve the storability of perishable fruits and vegetables (Del-Valle *et al.*, 2005; Hagenmaier, 2005) due to its biodegradability, biocompatibility and non-toxicity (Dutta *et al.*, 2009). In addition, this natural substance also has semi-permeable properties of giving a modified atmosphere of CO_2/O_2, retarding water loss, oxygen and solute transports during storage and thus, maintains the quality of postharvest fruits (Gennadios and Weller, 1990). El Ghaouth *et al.* (1991a) and Jiang *et al.* (2005) indicated that chitosan coating had the potential to improve the quality maintenance, prolong storage life and decrease the decay of strawberry and longan fruit. In addition, Qiuping and Wenshui (2007) reported that application of chitosan coating on Indian jujube fruit cv. Cuimi showed the beneficial effects on reducing weight loss, maintaining fruit quality and extending storage life. As far as we know, there is little available scientific literature about the use of chitosan coating for maintaining the quality and extending the shelf life of fresh pitaya fruit. Consequently, the objective of this study was to determine the effects of chitosan coating on the weight loss of fruit, stomatal conductance, stomata size, wilting appearance and postharvest life of pitaya fruit during storage at ambient temperature.

MATERIALS AND METHODS

Commercial mature pitaya fruits (*Hylocercus undatus* (Haw) Brit. and Rose) cv. Vietnam were harvested at the commercially mature stage from an orchard in Sakonnakorn, in the Northeast of Thailand in September 2009. Each fruit was wrapped with a foam sheath and packed carefully in a fiberboard carton then transported in an air-conditioned vehicle to Mahasarakham University. After they arrived at the laboratory, the fruits were selected again for uniformity of size, shape and freedom from external damage before being coated with chitosan. The experiment was carried out at the laboratory of the Division of Agricultural Technology, Faculty of Technology, Mahasarakham University, in the Northeast of Thailand. A completely randomized design was arranged into coating the pitaya fruit by using chitosan solutions containing four levels: 0% (control), 1, 2 and 3%. Each treatment was carried out in four replicates, ten fruits per replication. Solutions of 0, 1, 2 and 3% of chitosan were applied as a full cover paint to the fruits. After air-dried, all treatments were placed under ambient temperature at 27°C and 88% Relative Humidity (RH) for progressive assessments every 2 days. The following determinations were assessed as follows: (1) weight loss of fruit was calculated as the percentage loss of the initial weight (2) stomatal conductance was measured by using the porometer type AP4 (Delta-T Devices, Burwell, Cambridge, UK). This tool is used for measuring the resistance to loss of water vapour through the fruit stomata in order to be an indicator of the physiological state of the fruit and expressed as in unit of cm sec^{-1}. (3) stomatal size in terms of stomata width, stomata length and stomata aperture by using free hand section methods. The explants of peel were cut from pitaya fruit into size of 5×5 mm and 5-10 mm depth. The explants were dyed with sulfanin 1% for 15 min then dehydrated the tissues with alcohol series at concentrations of 15, 30, 50, 70, 95 and absolute alcohol, respectively, then diped in absolute alcohol mixing with xylene ratio 1:1 and pure xylene, respectively. The slides were sealed by xylene, dibutyl phthalate (DePeX) and compared structure of tissues with the use of optical microscopy ZEISS model Axiostar Plus (serial 3108007777), China with the objective lense of 40x. Size of these attributes were recorded as micron (μm). (4) Wilting percentage was evaluated as percentage, (5) storage life (days) was judged to have terminated when 30% of the fruits had withered. The collected data were statistically analyzed using the SPSS Computer Program, Version 6 (SPSS, 1999).

RESULTS

After coating pitaya fruit with different concentrations of chitosan four levels (0, 1, 2 and 3%), samples were then stored at ambient temperature. The recorded data composed of:

Weight loss: The result from Table 1 showed the changes in weight loss of pitaya fruit. Weight loss of all samples increased continuously with storage time. Both coated and control fruits showed the similar fruit weight loss during storage at ambient temperature. Though, upon subsequent storage, chitosan coated fruits had a lower fruit weight loss compared to the control but they were not significantly different (Table 1).

Stomatal conductance: During the first six days of storage, the amount of stomatal conductance was similar between treatments. The highly significant differences of stomatal conductance between treatments with 0, 1, 2 and 3% chitosan were observed at $p \leq 0.01$ level when the fruits were stored for 8 Days After Harvest (DAH). The lowest amount of stomatal conductance of 0.0513 cm sec^{-1} obtained from fruits treated with 3% chitosan was observed on eight days of storage (Table 2).

Stomatal size: From the free hand section technique, comparing the changes of stomatal size in terms of stomatal width and stomatal length from pitaya fruit, presented that the both above stomatal size slightly decreased on day 4 and then gradually increased throughout the storage period (Table 3, 4). During storage, there were highly significant differences among treatments ($p<0.01$) in stomatal size, both stomatal width and stomatal length. Throughout storage, the smallest size of stomata, both width and length, from fruits treated with 3% chitosan was observed.

Table 1: Weight loss of Pitaya fruit during storage

	Weight loss (%) at different DAH			
Treatments	2 days	4 days	6 days	8 days
Chitosan 0%	1.84	3.12	7.62	5.28
Chitosan 1%	1.79	2.76	5.04	4.98
Chitosan 2%	1.61	2.71	4.22	4.91
Chitosan 3%	1.59	2.67	3.94	4.27
F-test	ns	ns	ns	ns
C.V. (%)	9.70	6.21	9.22	3.87
LSD	0.5232	0.5524	1.6741	1.0321

ns: Non significant

Table 2: Stomatal conductance of Pitaya fruit during storage

	Stomatal conductance (cm sec^{-1}) at different DAH			
Treatments	2 days	4 days	6 days	8 days
Chitosan 0%	0.0020	0.1438	0.1927	0.3283a
Chitosan 1%	0.0018	0.0928	0.1093	0.1433b
Chitosan 2%	0.0015	0.0763	0.1023	0.0920bc
Chitosan 3%	0.0010	0.0685	0.0817	0.0513c
F-test	ns	ns	ns	**
C.V. (%)	0.00	7.27	3.75	3.12
LSD	0.0004	0.0424	0.0447	0.0277

Letters within columns indicate least significant differences (LSD) at **p = 0.01, ns: Non significant

Table 3: Stomatal width of Pitaya fruit during storage

	Stomatal width (µm) at DAH			
Treatments	2 days	4 days	6 days	8 days
Chitosan 0%	30.50a	25.63	27.88a	38.63a
Chitosan 1%	30.38a	23.50	24.75ab	35.88a
Chitosan 2%	26.38ab	21.63	23.13b	26.25b
Chitosan 3%	24.50b	19.88	22.63b	20.63c
F-test	**	ns	*	**
C.V. (%)	3.72	6.42	2.17	2.48
LSD	0.5927	0.7381	0.4765	0.6719

Letters within columns indicate least significant differences (LSD) at **p = 0.01, *p = 0.05, ns: Non significant

Table 4: Stomatal length of Pitaya fruit during storage

	Stomatal length (µm) at DAH			
Treatments	2 days	4 days	6 days	8 days
Chitosan 0%	58.38a	51.00a	67.13a	54.50a
Chitosan 1%	54.13ab	49.63ab	48.63b	50.00ab
Chitosan 2%	52.63b	44.38bc	47.63b	48.50b
Chitosan 3%	45.00c	42.63c	46.75b	42.38c
F-test	**	*	**	**
C.V. (%)	3.92	2.35	4.30	5.57
LSD	0.6540	0.9375	0.6721	0.6800

Letters within columns indicate least significant differences (LSD) at **p = 0.01, *p = 0.05

Stomatal aperture: Stomatal apertures of pitaya skin irrespective of chitosan concentrations, showed the increase in aperture size as advancing storage. As shown in Table 5, the stomatal aperture from fruits treated with 3% chitosan had the smallest size throughout storage. While control fruits markedly became the biggest stomatal pore.

Wilting percentage: Both of the control and the chitosan-coated fruit began to wither after the initial storage. The results from Table 6 showed that uncoated fruit showed the increases in loss of freshness quality. Fresh appearance of pitaya fruit was improved significantly by the coating with 3% chitosan. After storage 8 days, coating with 3% chitosan maintained the most freshness appearance of 13.75%, while control fruits showed the highest wilting percentage to 28.00% (Table 6).

Table 5: Stomatal pore of Pitaya fruit during storage

	Stomatal pore (μm) at DAH			
Treatments	2 days	4 days	6 days	8 days
Chitosan 0%	14.75a	13.50a	12.63a	16.25a
Chitosan 1%	11.38b	8.89b	12.13a	15.88a
Chitosan 2%	10.38b	4.63c	11.25ab	10.75b
Chitosan 3%	9.50b	4.38c	9.25b	9.50b
F-test	*	**	*	**
C.V. (%)	5.87	8.40	3.13	3.51
LSD	0.4719	0.5883	0.3170	0.4112

Letters within columns indicate least significant differences (LSD) at **p = 0.01, *p = 0.05

Table 6: Wilting percentage of Pitaya fruit during storage

	Wilting percentage (%) at DAH			
Treatments	2 days	4 days	6 days	8 days
Chitosan 0%	8.50a	16.30a	19.63a	28.00a
Chitosan 1%	3.60b	5.20b	17.00a	28.57a
Chitosan 2%	1.80b	5.00b	17.50a	22.00ab
Chitosan 3%	1.30b	3.60b	9.00b	13.75b
F-test	**	**	*	**
C.V. (%)	10.33	7.31	5.22	3.28
LSD	1.2412	1.7385	2.6519	3.0016

Letters within columns indicate least significant differences (LSD) at **p = 0.01, *p = 0.05

Table 7: Storage life of Pitaya after coating with chitosan

Treatment	Storage life (days)
Chitosan 0%	7.02b
Chitosan 1%	7.09b
Chitosan 2%	7.27b
Chitosan 3%	8.17a
F-test	**
C.V. (%)	3.44
LSD	0.2191

Letters within columns indicate least significant differences (LSD) at **p = 0.01

Storage life: Differences in storability among treatments related to chitosan concentration. The results showed that the treatment with 3% chitosan coating improved greatly the best storage life extension of 8.17 days (Table 7).

DISCUSSION

The effects of different concentrations (0, 1, 2 and 3%) of chitosan coating on the pitaya fruit cv. Vietnam was investigated.

For weight loss from all treatments showed a similar pattern of increasing over the storage period. Postharvest treatment with chitosan coating, irrespective of any concentrations, had no significant differences of weight loss between fruits treated with chitosan and control fruits during storage. Generally, loss of weight in fresh fruits mainly due to the loss of water caused by transpiration and respiration processes (Hernández-Muñoz *et al.*, 2006). These similar manners in fruit weight loss throughout storage may be due to the natural characteristic of pitaya fruit which composes of several green large scales or bracts protruding cover on fruit surface (Ariffin *et al.*, 2009). This character causes the pitaya fruit to be vulnerable to dehydration due to several paths for transferring the water vapor from the fleshy fruit to environmental air (Maftoonazad and Ramaswamy, 2005; Wu *et al.*, 2006). While, Dong *et al.* (2004) revealed that chitosan coating had an effective role for retarding weight loss of peeled litchi fruit, cv. Huaizhi. Meng *et al.* (2008) also cited that postharvest chitosan coating treatments significantly decreased the weight loss of table grape fruit stored at 20°C. In addition, Chien *et al.* (2007) cited that the use of chitosan coating could retard the water loss and prolonged the shelf life of sliced red pitayas. Furthermore, keeping the pitaya fruits under the ambient temperature in this study, may promote the internal changes and lead to a similar trend between coated and uncoated samples.

The results on stomatal conductance showed a significant difference between various treatments even under the same ambient temperature. In present studies, the application of 3% chitosan coating markedly protected the pitaya fruit by reducing the stomatal conductance to water vapour transmission at the lowest amount of 0.0513 cm sec^{-1} on 8 DAH while the highest stomata conductance (0.3283 cm sec^{-1}) was found in control fruits. Bahar *et al.* (2009) revealed that stomatal conductance is known for its relevance to stress condition. The stomatal conductance is strongly dependent on water status inside the fruit (Shen *et al.*, 2002). Bragg *et al.* (2004) cited that during storage, the water in terms of gaseous mass transfer through stomata were observed. When, the fruits transpire, water evaporates from cell walls and escapes to the environmental atmosphere by diffusing through stomatal pores, which are distributed on the upper epidermis of surface skin. Generally, stomatas in plant tissues are sensitive to the condition of water stress (Bragg *et al.*, 2004). The ability to control stomata opening and closure is important to avoid drought stress harms. Thus, some plants close their stomatas when they receive stress by reducing the stomatal conductance (Hurd, 1976). This implies that the quiet slow increase in the stomatal conductance from fruits coated with 3% chitosan was an effective practice to retain the water vapour deficit from pitaya fruit (Saei *et al.*, 2006). These results agreed with Bordenave *et al.* (2007) and Sebti *et al.* (2006), who claimed that chitosan coating performed as selective permeability to water transmission. This is probably due to chitosan acting as a water barrier preventing of the internal water. In addition, these results are similar to Luders and McMahon (2006), who found that during drought, the reduction of stomatal conductance was observed. While, Banks *et al.* (1993) found that coated surface can decrease a fruit's skin conductance to vapour diffusion. These positive effects of chitosan coating on transpiration are mainly exerted on limiting a greater proportion of the water vapour out of the fruit surface. This is due to the fact that fruit covered with chitosan blocked the loss of water vapour diffusion. Thus, a possible explanation of the decreased stomatal conductance in the coated fruits is that it is due to chitosan forming a very effective water vapour barrier, probably due to a film barrier to water transmission, in order to preserve the water vapor in pitaya fruit (Wong *et al.*, 1992). However, in the present, research on the physiological mechanisms of chitosan coating related to stomatal conductance is relatively unclear.

For stomatal size, the results showed that both stomatal width and stomatal length of the pitaya skin decreased rapidly during the first four DAH. Afterwards, both of them tended to increase their sizes until eight DAH. The results showed that the decrease of stomatal size, both width and length, were extremely pronounced in coated samples, compared to those of untreated samples. The minimum stomatal size was obtained in pitaya coated with 3% chitosan. These results are in accord with Baldwin *et al.* (1995) who cited that chitosan coating acted as barriers to water loss and gas exchange around the fruit. In addition, Ilgin and Caglar (2009) found that the decrease in stomatal conductance was associated well with the reduction in stomatal size. However, the exact mechanism of the action of chitosan to decrease the stomatal size is still unknown. Further detailed physiological studies are required to determine the mechanisms of these effects in order to gain a better understanding.

For stomatal aperture, throughout storage, the sizes of stomatal aperture from fruits treated with chitosan substances were smaller than control samples. The size of the stomatal aperture from fruit treated with 3% chitosan induced the smallest size as the storage period advanced. Bragg *et al.* (2004) reported that stomatal aperture is the dominant factor, which linked to transpiration from fruit tissues. Thus, the smaller sized stomata has a significantly decreased transpiration rate and contributes to maintain the positive turgor pressure of the cells (Bosabalidis and Kofidis, 2002). Nevertheless, water vapour barrier properties of chitosan-coated fruit have not been assessed yet and there are few references to stomatal changes of chitosan-coated commodities and no published study on the effect of chitosan coatings on the stomatal behaviour of pitaya fruit. It is thus necessary to continue studying the change of the stomatal responses to chitosan coating through other experiments in order to clear the mechanism of these responses.

For wilting percentage, the results showed that fresh appearance of pitaya fruit were delayed after coating with chitosan. The best results for controlling the wilting occurrence of fruit at initial four days storage were obtained with the coating with chitosan coating, irrespective of any concentrations. While, the last eight days of storage, it was found that fruit coated with 3% chitosan showed the least wilting percentage of 13.75%. Maftoonazad and Ramaswamy (2005) reported that the primary mechanism of moisture loss from fresh fruits and vegetables is by vapor-phase diffusion driven by a gradient of water vapor pressure between internal fruit and external environment. The film coating of chitosan on the surface skin would help to slow rates of moisture loss from coated fruits. Saei *et al.* (2006) reported that stomata size related to reduce the water loss from fruit. These results were consistent to the smallest stomatal aperture of fruit treated with 3% chitosan. It is thus fruit coated with chitosan at 3% could reduce transpiration rate and preserve the highest fresh quality during storage. Thus, one of beneficial effects of chitosan coating on controlling the withering occurrence of pitaya fruit was the protective effect in reducing stomatal size, including the stomatal aperture, which slowed down the wilting appearance. Therefore, fruit coated with 3% chitosan could effectively reduce water evaporation and maintained the freshness occurrence. These results are consistent with the observation of Simões *et al.* (2009), who cited that the overall visual quality of carrot sticks were strongly affected by the edible coating with chitosan. In addition, the positive effect of coating with chitosan generated a thin film, which acted as a barrier against water loss between fruit and the environment, thus reducing water transfer to environmental condition (Vásconez *et al.*, 2009). Furthermore, chitosan films have the property of a relatively high water vapour permeability (Wong *et al.*, 1992; Butler *et al.*, 1996; Caner *et al.*, 1998). Thus, lowering the amount of withering fruit with increasing chitosan concentration were observed. These data agreed with earlier reports by Kester and Fennema (1986) and Li and Chung (1986).

Generally, under ambient temperature, pitaya fruits have a storage life of approximately 3-4 days (Barbeau, 1990). For shelf life, the results revealed that chitosan coating appeared to be advantageous to storage life, especially at 3% chitosan. These results corresponded with the application of chitosan treatment at 3% significantly minimized as follows; the stomatal conductance, stomatal size and stomatal pore. Chitosan coating also delayed the increase in wilting appearance and consequently extended the maximal shelf life of pitaya fruit (8.17 days). These results are in agreement with those obtained by Nisperos-Carriedo (1994), who reported the formation of a semi-permeable film on the fruit surface after coating which decreased transpiration losses in fruits by forming a protective barrier and acted as the selective permeabilities to water vapour transference through the hydrophilic part of the chitosan coating (Bautista-Banos *et al*., 2006; Jiang *et al*., 2005). In addition, the beneficial effects of chitosan coatings also acted to modify the internal atmosphere of the fruit and thus, decrease transpiration losses and retard fruit senescence (Jiang *et al*., 2005; Qiuping and Wenshui, 2007). Similar results were also reported for numerous horticultural commodities such as tomatoes, strawberries, longan, apples, mangoes, bananas and bell peppers (El Ghaouth *et al*., 1991b, 1992; Du *et al*., 1997; Jiang *et al*., 2005; Kittur *et al*., 1998). Therefore, coating the pitaya fruit with 3% chitosan should be considered for commercial application to preserve the fresh pitaya for a short time period under ambient temperature. However, further extensive postharvest research should be conducted by using the higher concentration of 3% chitosan in order to search the appropriate concentration for coating pitaya fruit on a commercial scale.

In conclusion, the application of chitosan coating at 3% had the most beneficial effect on reducing the stomatal conductance, smaller stomatal size and stomatal aperture and slowed down the fruit withering. Consequently, it could maintain the fresh appearance and extend the maximal storability of pitaya fruit kept at the ambient temperature.

ACKNOWLEDGMENTS

This research was funded by the Mahasarakham University under project no. 5301042/2553. The authors wish to express their sincere thanks to the Financial Office for financial assistance, Ms. Patchalita Pudkhunthod for her assistance. In addition, we also thank Mr. Paul Dulfer for his kindness in improving this study.

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