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The Use of Bee Wax, Chitosan and BAP to Prolong Shelflife of Mangosteen (*Garcinia mangostana* L.) Fruit

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ABSTRACT

The objective of this research was to determine the effect of coating materials and BAP concentration on inhibition of ripening process of mangosteen. The experiment used factorial completely randomized design with two factors, and three replications. Coating materials as the first factor consisted of control (without coating), bee wax 6%, and chitosan 2%. The second factor was concentration of Benzil Amino Purine (BAP), with 0, 5, 10, 15, and 20 ppm. Non destructive observations were weight loss, diameter decrease, peel and calyx color development. While, destructive observations were fruit hardness, total soluble solid (TSS), total titratable acidity (TTA) and opened ability. The result showed that bee wax was effective to inhibit weight loss. Interaction of bee wax and BAP 20 ppm inhibited peel and calyx color changes during storage.

Key word: coating material, cytokinin, benzil amino purine, calyx, storage

INTRODUCTION

Mangosteen (*Garcinia mangostana*) is known as "Queen of Tropical Fruits". It is originated from Indonesia. Export of Indonesian mangosteen is increasing in the last few years. Production of Indonesian mangosteen is quite high, however, the percentage of exported fruit is quite low because of quality and continuity problems. The quality problem is caused by sub optimum on-farm management and lack of postharvest handling. Export quality of mangosteen is unique, depend on fruit size and weight, calyx color and freshness and number of calyx.

Fruit is a life tissue that continue its metabolism after harvesting, especially respiration and transpiration (Pantastico *et al*, 1989). This process will cause postharvest fruit detioration. Postharvest detioration can not be stop, but can be slower down (Santoso and Purwoko, 1995).

Mangosteen has short shelflife. Few postharvest tehcniques have been used, for example low temperature storage, and using coating material (Purwoko and Fitradesi, 2000). Application of Benzyl Amino Purine (BAP), a cytokinine, as plant hormone to inhibite senescence could be prolong the freshness of calyx and keep the calyx green for longer time. Salunke (1989) cited that cytokinin application has inhibited chlorophyl degradation and senesence of leavy vegetables. The objective of this research was to find the optimum BAP concentration and an effective coating material to delay senescence of fruit calyx and to prolong shelflife of mangosteen.

Chitosan has recently gained more interest due to its applications in food and pharmaceutics, and the antimicrobial activity is one of its most interesting properties (Devlieghere, Vermeulen and Debevere, 2004). They found that the antimicrobial effect of chitosan on lettuce disappeared after 4 days of storage, while it maintained on the strawberries during 12 days. Application of chithosan 2.0% on longan was better than those of 0.5 and 1.0%. Chitosan application reduced respiration rate and weight loss, delay changes in color and eating quality an partially inhibited decay of fruit during storage at 2 °C and 90% RH (Jiang and Li, 2000). Sritanaman *et al*. (2005) found that application of 2% chitosan coating only reduced weight loss of irradiated mangosteen fruit, and maintained fruit softness, but did not have effect on total soluble solid.

MATERIALS AND METHOD

The research was done on April to June 2008 at Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia. The main material of this experiment was mature mangosteen fruit harvested at 103 days after anthesis from orchard at Wanayasa district, Purwakarta, West Java, Indonesia. The fruits for the experiment was uniformly mature with red peel color with few greenish spot, with fresh green calyx, harvested at 103 day after anthesis. The fruits then was grouped based on fruit size (the fruit condition can bee seen at Table 1). The condition of fruit during storage was good enough, only few fruits were attacted by

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Botryodiplodia theobromae that caused fruit rot, and few fruit also has yellow latex or gamboge.

Table 1. Fruit condition at the beginning of experiment

Parameters	Group 1	Group 2	Group 3	Average
Weight (g)	100.99	89.40	66.63	85.67
Diameter (mm)	56.64	54.85	49.58	53.69
Fruit color	5R 5/11.5	5R 5/11.5	5R 5/11.5	5R 5/11.5
Calyx freshness	2.5GY9/6	2.5GY9/6	2.5GY9/6	2.5GY9/6
Fruit hardness (kg sec^{-1})	0.87	0.87	0.93	0.89
Total soluble solid (^{0}brix)	18.28	17.88	17.13	17.76
Total titratable acidity (%)	0.55	0.51	0.46	0.51
Ability of fruit to be opened	3.50	2.50	3.00	3.00

Other materials were bee wax 6%, chitosan 2%, BAP, aquades, and clorox as disinfectant. The materials for observation were analitical scale, calipper, munshel color chart, hand refractometer and hand penetrometer.

The experiment was conducted in a factorial randomized design with two factors, i.e., coating materials and BAP. The first factor consisted of control (without coating), coating with bee wax emulsion 6%, and coating with chitosan 2%. The second factor consisted of control (0 ppm BAP), 5, 10, 15, and 20 ppm BAP. There are 15 combination of treatments, with 3 replications for each, total 45 experimental units. Every experimental unit consist of 10 mangosteen fruits, with total 450 fruits, for destructive and non-destructive observation. At the day one, there was also 27 fruits used for observation of harvested quality of the mangosteen. After application of the treatments, fruits were stored at 15 °C. Data from observation were analyzed using Anova and DMRT (*Duncan Multiple Range Test*) at 5%.

Harvested mangosteen fruits were sortaged for uniform size and peel color, with red color with few green color, flawless peel, fresh and green calyx, fruits have to have 4 calyx for each fruit, fruit stem has to be fresh green with no latex, and free of any pests and deseases. Fruits were washed and then dip in 10% clorox for 30 seconds and then air dried.

Bee wax emulsion of 12% was prepared by heated 120 g of bee wax at 90-95 °C, and then added 20 ml oleat acid and 40 ml triethanolamine litle by litle while continuesly stir uniformly. Having with 820 ml added of boiled water, the emulsion was stirred. The emulsion can be used if it is cooler (25 °C). To get bee wax emulsion of 6%, 0.5 l of 12% bee wax emulsion was added with 0.5 l aquades at room temperature. Benzil Amino Purine (BAP) was first mix with NaOH 1 M till totally soluble, and then it made as the concentration treatments. Then, BAP solution was mixed with the coating materials (bee wax 6% and chitosan 2%). For the treatments, fruits were dipped in the solutions for about 5 minutes, and then air dried.

Non-destructive observations were done on fruit weight and weight loss, fruit diameter and diamter loss, and fruit and calyx color using Munshell Color Chart. While destructive observations were done on fruit hardness using hand penetrometer at 3 points (stem-end, middle, and end of fruit), total titratable acid, total soluble solid using hand refractometer, and test of manual opened ability of the fruit with 6 scores (1=very easy, 2=almost easy, 3=easy, 4=almost difficult, 5=difficult, and 6=very difficult to open).

RESULTS AND DISCUSSION

Weight Loss and Decreasing in Diameter

Weight loss, because of respiration and transpiration, was one of the major component that control fruit quality. Application of 6% bee wax significantly reduced the weight loss at 18, 21, 24, 27, 30, and 33 days after application (DAA), while application of BAP did not significantly reduce weight loss at any observation day (Table 2).

The weight loss actually increased. At the end of observation (33 DAA) the weight loss at 6% bee wax was 15.5%, lower then at the application of chitosan 2% with weight loss as high as 23.8%. So, application of 6% bee wax on magosteen fruit can reduce weight loss as much as 35% compared to that of control. Bee wax can cover fruit peel that reduced respiration and transpiration and finally reduced the moisture of the fruit.

The fruit diameter tended to decrease during storage because of water loss from the fruit. Kader *et al.* (1992) cited that water loss was not only directly

affected weight loss, but also affect on texture, nutrient content, and visual quality as the fruit shrinking. Aplication of bee wax or chitosan did not significantly have effect on diameter loss at any

Table 2. Effect of coating material and BAP on weight loss

Treatments	Weight loss at days after application					
	18	21	24	27	30	33
Coating	..... %					
Control	11.5a	13.1a	15.9a	17.9a	20.7a	23.8a
Bee wax 6%	8.7b	9.8b	11.6b	12.7b	13.7b	15.5b
Chitosan 2%	10.6ab	12.1ab	14.6a	16.5a	19.1a	22.1a
F Test	*	*	*	*	*	*
BAP						
0 ppm	9.2	10.4	12.5	13.8	15.7	17.8
5 ppm	9.4	10.8	13.1	14.5	16.7	19.5
10 ppm	10.5	11.9	14.4	16.3	18.6	20.8
15 ppm	9.9	11.3	13.7	15.8	18.9	22.2
20 ppm	12.3	13.9	16.4	18.0	27.2	21.8
F Test	ns	ns	ns	ns	ns	ns
Coating x BAP						
F Test	ns	ns	ns	ns	ns	ns

Note: Value followed by same letter in the same column is not significantly different on DMRT at 5%;
ns : not significant (P>0.05), * : significant (P>0.05)

Table 3. Effect of coating material and BAP on decreasing in diameter (%)

Treatments	Decreasing in diameter (%) (days after application)					
	18	21	24	27	30	33
Coating	..... %					
Control	4.0	4.7	5.3	5.7	6.3	6.9
Beewax 6%	3.6	4.0	4.6	4.8	5.4	6.2
Chitosan 2%	4.1	4.5	5.3	5.7	6.5	7.6
F Test	ns	ns	ns	ns	ns	ns
BAP						
0 ppm	3.7	4.1b	4.8b	5.0b	5.7b	6.3
5 ppm	3.1	3.8b	4.2b	4.5b	5.1b	6.0
10 ppm	4.0	4.3b	5.1b	5.4b	5.7b	6.5
15 ppm	3.8	4.3b	4.8b	5.3b	6.4ab	7.6
20 ppm	4.7	5.7a	6.4a	6.8a	7.6a	8.2
F Test	ns	*	*	*	*	ns
Coating x BAP						
F Test	ns	ns	ns	ns	ns	ns

Note: Value followed by same letter in the same column is not significantly different on DMRT at 5%
ns : not significant (P>0.05), *: significant (P>0.05)

observation. However, application of Cytokinine BAP was significantly affected of diameter loss at 21, 24, and 30 DAA (Table 3), but at the end of experiment (33 DAA) the effect of BAP was not significant in reducing diameter.

Fruit and Calyx Color

Fruit color was changing with storage from fresh red at the beginning to blackish purple at the end of experiment (33 DAP). Interaction application

of bee wax 6% with BAP 20 ppm can retain the purpleish red color till 24 DAA, while the control can only retain that color till 3 DAA (Table 4).

Calyx color and freshness is one of the visual quality of exported mangosteen. At harvesting, all calyxs are fresh green and each of the all fruit has four calyxs. After storage, the calyx tended to be dry and become gray. Application of bee wax 6% and BAP 20 ppm can be used to retain the dark green color of calyx untill 21 DAA, while control treatment can retain the color only 12 DAA (Table 5). Suyanti et al., (1999) said that commercially fruit calyx of mangosteen harvested at 104 DAA can be still fresh green only until 6 days after storage, while Widiastuti (2006) said that mangosteen harvested 103 days after anthesis can retain its calyx color and freshness until 8 days of storage at room temperature.

Fruit Peel Hardness and the Ability of Fruit to Manually Opened

Application of coating and BAP was not significantly affected fruit peel hardness at all observation. Application of coating material reduced fruit peel hardness at 14 DAA. While BAP application did not showed any effect on fruit peel hardness. The value of fruit peel hardness varied from observation to observation since at each destructive observation (Table 6).

Mangosteen fruit is usually opened manually by hand, and during storage the fruit hardness will increase and its will be more difficult to open. Interaction of coating and BAP application significantly affected the ability of fruit to manually opened. Using score observation, the higher the score will be more difficult the fruit to manually opened. During storage, the score increased and the fruit became more difficult to be opened. The increasing of mangosteen fruit peel hardness and the ability of fruit to be manually opened is caused by moisture loss from the peel and the fruit (Sjaifullah *et al.*, 1998). Sritanaman *et al.* (2005) found that application of 2% chitosan coating reduced weight loss and maintained fruit softness of irradiated mangosteen fruit.

Table 4. Effect of coating material and BAP on fruit color

Treatments		Fruit color					
		Red		Purple		Blackish Purple	
		Beginning	End	Beginning	End	Beginning	End
		5R 5/11,5	5R 4/10	5R 3/10	5R 3/8	6R 3/8	6R 2,3/6
Coating	BAP	.....	.....	.....day.....	.....	.....	.....
Control	0	0	3	6	15	18	33
	5	0	<3*	3	12	15	33
	10	0	3	6	18	21	33
	15	0	3	6	21	24	33
	20	0	9	12	33	~	~
Bee wax 6%	0	0	3	6	15	18	33
	5	0	3	6	9	12	33
	10	0	3	6	9	12	33
	15	0	6	9	21	24	33
	20	0	24	27	33	~	~
Chitosan 2%	0	0	3	6	15	18	33
	5	0	6	9	18	21	33
	10	0	15	18	33	~	~
	15	0	3	6	33	~	~
	20	0	3	6	21	24	33

Note:
R : Purpleish red to blackish purple
~ : at the end of observation (33 DAA), the fruit is still purple.
<3* : at 0 DAA Fruit Color is purple red, after that (>3 DAA) fruit color change to purple, so the color change occur between 0 DAA and 3 DAA

Table 5. Effect of coating material and BAP on fruit calyx color

Treatment		Calyx color					
		Green		Greyish green		Grey	
		Beginning	End	Beginning	End	Beginning	End
		2,5GY	10Y	10Y	7,5Y	7,5Y	7,5Y
		9/10	8,5/9	8,5/12	7,5/11	8,5/9	7,5/13
Coating	BAP			day			
Control	0	0	12	15	24	27	33
	5	0	12	15	24	27	33
	10	0	15	18	27	30	33
	15	0	15	18	24	27	33
	20	0	18	21	24	27	33
Bee wax 6%	0	0	12	15	18	21	33
	5	0	15	18	24	27	33
	10	0	15	18	21	24	33
	15	0	15	18	24	27	33
	20	0	21	>21 *	24	27	33
Chitosan 2%	0	0	12	15	18	21	33
	5	0	15	18	24	27	33
	10	0	18	21	24	27	33
	15	0	18	21	24	27	33
	20	0	15	18	21	24	33

Note:
GY : Light green to dark green
10Y : Dark green
7,5 Y : Greyish dark green to grey
>21 * : at 21 DAA calyx is still green, at 24 DAA calyx is greyish green

Tabel 6. Effect of coating material and BAP on fruit hardness (kg/second)

Treatments	Fruit hardness (days after application)				
	7	14	21	28	35
	kg second^{-1}				
Coating					
Control	0.83	0.82b	0.88	0.96	0.93
Beewax 6%	0.83	0.86a	0.90	0.94	0.92
Chitosan 2%	0.86	0.86a	0.91	0.94	0.96
F Test	ns	*	ns	ns	ns
BAP					
0 ppm	0.84	0.84	0.91	0.96	0.94
5 ppm	0.84	0.84	0.86	0.95	0.97
10 ppm	0.84	0.88	0.88	0.95	0.94
15 ppm	0.84	0.84	0.88	0.91	0.91
20 ppm	0.84	0.84	0.94	0.96	0.93
F Test	ns	ns	ns	ns	ns
Coating x BAP					
F Test	ns	ns	ns	ns	ns

Note: Value with different small letter in the same colom is significantly different on DMRT test at 5%
ns : not significant (P>0.05). *: significant (P>0.05)

Total Soluble Solid and Total Titratable Acid

The sweetness of the fruit is caused by increasing of simple sugar and decreasing of fenolic compounds (Matto et al., 1989). Incresing of the total soluble solid of the fruit will increase the sweetsess of the fruit.

Interaction of coating and BAP application did not significantly affect total soluble solid of the fruit. Application BAP alone only significantly affected on total soluble solid at 28 DAA. The total soluble solid of mangosteen in this experiment varied between observation to observation because of destructive observation used different fruit for each observation (Table 7). Sritanaman *et al.* (2005) found that application of 2% chitosan coating did not have effect on total soluble solid of irradiated mangosteen fruit.

Organic acid in the fruit is one of the energy source for its metabolism, so the higher the acid content is the longer shelflife of that fruit (Wills *et al.*, 1981). In general, organic acid content of the fruit is decrease during maturity because organic acid is used for respiration or it changes into sugar, and the acid content usually increase again at mature stage (Santoso and Purwoko, 1995). Bunsiri, Ketsa and Paull (2003) said that phenolic acid (p-coumarin acid and sinapic acid) content decrease with increasing of maturity level.

Interaction of coating and BAP treatment did not signicantly affect acid content of the fruit at all observation, except at 7 DAA (Table 8). Application of coating or BAP alone is also did not significantly affect total titrable acid. It is possible that the treatments have not affected fruit metabolism yet, so the acid has not been used for substrate of respiration.

Correlation between Variables

Weight loss is usually used as indication of decreasing in fruit quality. Weight loss is caused by respiration and transpiration of the fruit, then it will affect other quality decreasing including visual quality. So, we use weight loss as anchor to correlate with other variables. Weight loss is positively correlated with decreasing of fruit diameter at 5, 21, 27 and 33 DAA. At 27 DAA, coefficient of correlation between weight loss and diameter loss is 50%. So, if weight loss increase will cause increasing in diamter loss.

Table 7. Effect of coating material and BAP on total soluble solid (^{0}brix)

Treatments	Total soluble solid (^{o}brix) at days after application				
	7	14	21	28	35
	^{0}brix				
Coating					
Control	17.84	17.58	16.21	16.28	16.13
Bee wax 6%	17.03	18.21	16.86	16.21	14.90
Chitosan 2%	17.33	17.35	17.3	16.72	16.20
F Test	ns	ns	ns	ns	ns
BAP					
0 ppm	17.23	17.83	16.55	16.03ab	17.16
5 ppm	17.42	17.72	17.72	17.04a	15.06
10 ppm	17.57	17.46	17.44	16.53ab	15.21
15 ppm	17.62	17.97	16.70	16.90a	15.66
20 ppm	17.14	17.58	15.51	15.51b	15.59
F Test	ns	ns	ns	*	ns
Coating x BAP					
F Test	ns	ns	ns	ns	ns

Note: Value followed by same letter in the same column is not significantly different on DMRT at 5%
ns : not significant (P>0.05), *: significant (P>0.05)

Table 8. Effect of coating material and BAP on total titratable acid (%)

Treatments	Total titratable acid at days after application				
	7	14	21	28	35
Coating	.....	.....	.....	%	
Control	0.49	0.49	0.49	0.43	0.44
Bee wax 6%	0.50	0.52	0.53	0.41	0.45
Chitosan 2%	0.50	0.48	0.45	0.45	0.42
F Test	ns	ns	ns	ns	ns
BAP					
0 ppm	0.47	0.44	0.51	0.43	0.48
5 ppm	0.51	0.50	0.49	0.42	0.46
10 ppm	0.50	0.54	0.48	0.43	0.39
15 ppm	0.50	0.50	0.50	0.42	0.44
20 ppm	0.50	0.49	0.47	0.44	0.42
F Test	ns	ns	ns	ns	ns
Coating x BAP					
F Test	*	ns	ns	ns	ns

Note: Value followed by same letter in the same column is not significantly different on DMRT at 5%
ns : not significant (P>0.05), *: significant (P>0.05)

CONCLUSIONS

Application of bee wax 6% had positive effect on several variables including in reducing of weight loss, retained the fruit color longer, retained the green color and freshness of fruit calyx during storage. Combination of bee wax 6% with BAP 20 ppm may be applyed to retain the green color and freshness of calyx till 21 days of storage, although at 15 DAA the calyx start to wringkle. While decreasing in diameter, fruit peel hardness, the ability of fruit to be opened manually, total soluble solid and total titrable acid were not affected by application of coating materials and BAP.

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