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The Effectiveness of Poliamin to Inhibit the Chilling Injury in Various Levels of Maturation and Ripeness of Sugar Banana

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Abstract: A study about the effectiveness of poliamin to inhibit the chilling injury in the sugar banana (Musa Paradisiaca, L.) has been conducted to examine the effectiveness of poliamin (putresin, spermidin, spermin) towards the chilling injury in some levels of maturation and ripeness of the sugar banana. This study employed the sugar banana whose level of ripeness was 85%, fully ripe, over ripe and soaked in 8 min in poliamin solution which consisted of Putresin (Put), Spermidin (Spd), Spermin (Spm) with 1.5 mm concentration. The banana was kept in 10 days with the temperature of 10° celsius. Real-time observation to examine the ethylene emission was conducted every day by using photo acoustic spectrometer while the respiration rate, chilling injury index (necrosis and pitting) and texture were examined every 2 days in the room temperature after the banana was taken out from the freezing room. The research finding showed that soaking the banana in the poliamin solution (putresin, spermidin, spermin) could inhibit the respiration rate, ethylene emission, chilling injury index (necrosis and pitting) as well as soften the texture of the banana. The type of poliamin used to effectively inhibit the chilling injury in all ripeness levels of banana was Putresin substance (Put), followed by Spermidin (Spd) and Spermin (Spm). The spermin substance was very effective to inhibit the chilling injury in the banana whose ripeness was 85%.

Key words: Sugar banana, low temperature, chilling injury, poliamin, ripeness

INTRODUCTION

The sugar banana (Musa Paradisiaca, L.) was one of the leading tropical fruits of Indonesia which has export potential, bear fruits quickly is available along the season is tasty and preferable by many people as well as become the good source of vitamin C and pro vitamin A. However, this fruit is not always available in all areas, so distributions with cold temperature are needed. The sugar banana belongs to climacteric fruits in which the maturation and ripening process does not take a long time. During the maturation and ripening process, the respiration rate and ethylene level will increase, so it can make the fruit decayed and rotten quickly (Wills *et al*., 1998; Kader, 1992). One of the ways to lengthen the storage period of banana is by inhibiting the respiration rate and other metabolism processes which are done by keeping the banana in the cold temperature. However, banana is very sensitive to chilling injury, so some treatments to minimize the chilling injury has to be undertaken. In this research the inhibiting of chilling injury is carried out by soaking the banana in poliamin solution.

Chilling injury is a process by which the cell membrane is decayed or the cell and tissue which are sensitive to cold temperature are damaged as the toxic metabolic such as acetaldehyde, ethanol and oxaloacetate are accumulated. The chilling injury can occur when the temperature of the tropical fruit reaches 5-15° celsius (Kader, 1992). Some symptoms of the chilling injury are the lesion on the surface of the banana, necrosis and pitting (blackish-brown spots in the banana skin), abnormal color changing in the surface and inside the fruit, water soaking, water losing and wrinkled, decayed texture and flavor, the increased rotting as the leaking metabolic allows the microorganism (like fungi) to grow, fasten the ethylene production as the lipid peroxide increases, cut the storage period and the banana fails to be mature and ripe after they are taken out of the freezing room (Lyons, 1973; Skog, 1998; Kuo and Parkin, 1989).

The chilling injury in the fruit storage is not desired as it can lower the quality of the fruit as well as the consumer 's acceptance. Some treatments have been done to prevent the chilling injury. The treatments are wax layering (Lyons, 1973), hypobaric storage, antioxidant

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layering (Kuo and Parkin, 1989), controlled atmosphere and modified atmosphere (Skog, 1998) and gibberellin acid utilization. However, those treatments have the downside such as technically impractical and the ineffective results. As a result, another alternative to improve the weakness of those treatment has to be found. One of the technologies to improve is using poliamin substance. The use of poliamin substance recently is to inhibit the chilling injury in non-tropical fruits. Poliamin is a carbon chain compound whose amine group is >1 the non-protein amino acid compound (Rhodes, 2005) are able to delay the senescence, soften the texture, inhibit the chilling injury, bind the cell wall, pectin at the central lamella and membrane lipid (Leiting and Wicker, 1987; Valero *et al*., 1998). The positively-charged poliamin is able to bind some negatively-charged molecules such as protein, membrane phospholipid and pectin and it has similar characteristic to calcium in a way of its ability to delay the senescence and soften the texture of apple, strawberry and lemon (Valero *et al*., 1988; Martinex-Romero, 2002; Kpawoh *et al*., 2002) as well as inhibit the ethylene in tomato, avocado and pear and inhibit the chilling injury in cucumber (Shen *et al*., 2000; Valero *et al*., 1998).

Poliamin is very effective to strengthen the integrity of central lamella and cell membrane and it is suitable to inhibit the cell damaging. The poliamin group consists of some compounds, namely putresin (two amine groups), spermidin (three amine groups) and spermin (four amine groups). Poliamin is naturally formed in the plant cells and it has regular effect to the cell division. Moreover, the poliamin concentration fully depends on the types and the level of maturation and ripeness of the fruit (Valero *et al*., 1998).

This research aims to find out the effectiveness of poliamin (putresin, spermidin and spermin) to the inhibition of chilling injury in some levels of maturation and ripeness of the sugar banana.

MATERIALS AND METHODS

Materials and equipment: Materials employed in this study was the sugar banana taken from Dusun Tlogowatu, Desa Sluweng, Kecamatan Kemalang, Kabupaten Klaten, Central Java. The banana was harvested directly from the field and the sample was taken from the three layers of the central part of the banana bunches whose ripeness level was 85%, fully ripe and over ripe. Other materials consisted of Putresin (Put), Spermidin (Spd), Spermin (Spm), perchlorate acid ($HClO_4$), 1,6 hexandiamine compound, benzoil chlorida, tween 20, methanol, dietilether, chloroform, trietilamin, nitrogen, helium, carbondioxide, Bromo Thymol Blue (BTB) and other materials to perform the chemical analysis.

Equipment used in this study was Thin Layer chromatography and camag TLC scanner 3 "dummy" S/N 081124 (1.14.16) to perform the poliamin analysis, photo accoustic spectrometer "intra cavity" assembled in the Physics Department of Mathematics and Sciences Faculty of Gajahmada University and 5 watt of tunable laser CO_2 to measure the ethylene emission, 10° celsius freezer, a container equipped with pressure controller to soak the fruit, spectrophotometer, respirator unit, Humbold Universal Penetrometer H-1250 to measure the fruit texture and other equipment to perform the physical and chemical analysis.

The research design: The research design employed in this study was Rancangan Petak Terbagi (RPT). The whole quadrat was the maturation and ripeness levels of the banana (K) which consisted of three stages: K1 = 85% ripe; K2 = fully ripe and K3 = over ripe and the partially quadrat was the type of the Poliamin (P) which consisted of four stages: Po = no Poliamin soaking (control); P1 = Putresin soaking (Put); P2 = Spermidin soaking (Spd); P3 = Spermin soaking (Spm). The finding was carried out by doing variety analysis in the real span of 5%. To differentiate the treatments, Duncan's multiple range test in the real span of 5% was undertaken.

The implementation: The banana was directly harvested from the field then it was sorted based on its maturation and ripeness level: 85% ripe, fully ripe and over ripe. After that, the banana was soaked based on its ripeness level in three different solutions, namely Putresin (Put), Spermidin (Spd), Spermin (Spm) and control. The banana was soaked in the poliamin solution whose concentration was 1.5 mm in which it contained 0.2% tween 20 in the 1 atmosphere pressure +200 mm Hg within 8 min. The soaked banana was 600 g/L of poliamin solution. Additional tween 20 was aimed to improve the absorption of the poliamin solution in the banana skin. After soaking the banana, draining was carried out in the crepe paper. Then, the banana was kept in the freezer with 10° celsius temperature during 10 days and observation was done by observing the ethylene emission rate, respiration rate, texture and chilling injury index (necrosis and pitting). The observation was undertaken every 2 days in the room temperature (±27°C). However, the ethylene emission rate was examined every day by using the spectrometer photo accoustic in the freezer with 10° celsius temperature.

Analysis of the data

The ethylene emission and respiration rates: The measurement of the ethylene emission rate was done by using the spectrometer photo accoustic while the respiration rate was observed by using the spectrophotometer.

Texture: The banana texture was measured by using Humbold Universal penetrometer H-1250. The texture measurement was done in 12 different spots in the edge, center and the base of the banana. After that, the mean score was calculated. The texture was in millimeter.

Chilling injury index: The chilling injury index (necrosis and pitting) was measured according to Kpawoh *et al.* (2002) with some modifications. The necrosis and pitting were recognized by observing the blackish-brown spots in the banana skin (in the edge, center and the base of the banana) which has been stored in the cool temperature. After that the percentage of the spots caused by the necrosis and pitting was determined. The value of necrosis and pitting was expressed in percentage by summing up the percentage of necrosis and pitting in the edge, center and base of the banana. The percentage was converted to certain scores as follows: 0 = no necrosis and pitting symptom, 1 = necrosis and pitting <25% (low symptom) 2 = necrosis and pitting 25-50% (moderate symptom) and 3 = necrosis and pitting >50% (high symptom). The score obtained was expressed as the chilling injury index.

RESULTS AND DISCUSSION

The ethylene emission rate: Figure 1-3 displayed the ethylene emission rate of the different treatment of the poliamin solution in the 85% ripe, fully ripe and over ripe bananas during 10 days of storage in the temperature of 10° celsius.

Figure 1 showed that the control led to the relatively high ethylene emission rate of 85% ripe banana while soaking the banana in the poliamin solution (putresin, spermidin and spermin) was able to inhibit the ethylene emission rate. It was due to the fact that the poliamin compound could bind the cell wall and cell membrane, so the cell was more stable and resistant to the external effect, including the cool temperature (Leiting and Wicker, 1987).

In the control, day 1 until day 6 produced relatively low and stable ethylene emission but starting from day 7 the ethylene emission was highly increased and reached its peak in the day 8. The highly increased ethylene emission was likely because the banana has passed the chilling injury time due to the cool temperature pressure/stress (Lyons, 1973). The chilling injury was able to trigger the ACC enzymeactivity ((1-Aminocyclopropane-1-Carboxylic acid) in ethylene syntesis.

In line with the result of the 85% ripe banana (Fig. 1), the fully ripe banana (Fig. 2) and over ripe banana (Fig. 3)

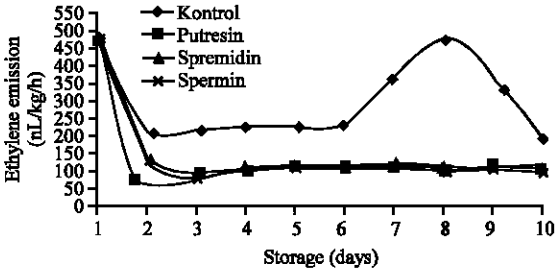

Fig. 1: The ethylene emission (nL/kg/h) of the 85% ripe banana with the treatment of poliamin during 10 days of storage in the temperature of 10° celsius

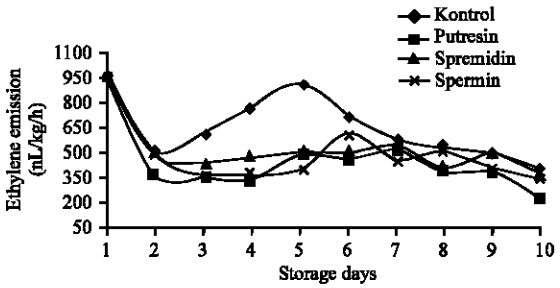

Fig. 2: The ethylene emission (nL/kg/h) of fully ripe banana with the treatment of poliamin during 10 days of storage in the temperature of 10° celsius

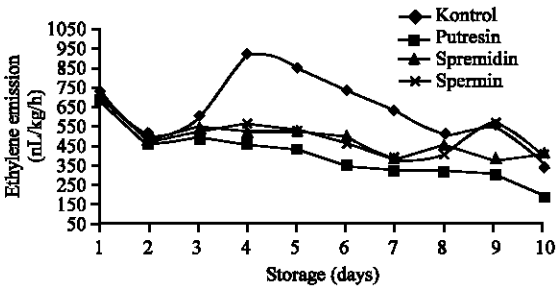

Fig. 3: The ethylene emission (nL/kg/h) of over ripe banana with the treatment of poliamin during 10 days of storage in the temperature of 10° celsius

also had their biggest ethylene emission in the control rather than in the banana treated in the poliamin.

The treatment of soaking banana in the poliamin solution (putresin, spermidin and spermin) could inhibit the ethylene emission of the banana. The inhibition was as follows: the 85% ripe banana (the inhibition by putresin was 56.42%, sperdimin was 51.71% and spermin was 51.68%) the fully ripe banana (the inhibition by putresin was 32.7%, sperdimin was 23.6% and spermin was 19.1%) and the over ripe banana (the inhibition by

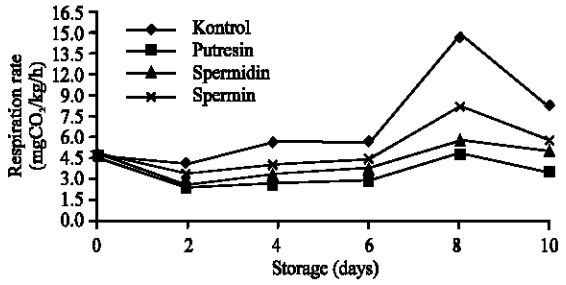

Fig. 4: The respiration rate (mgCO_2/kg/h) of the 85% ripe banana with different poliamin treatment within 10 days of storage

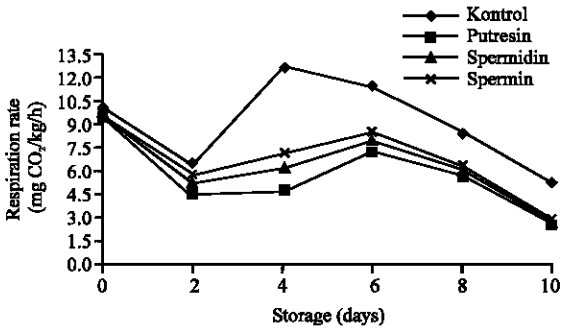

Fig. 5: The respiration rate (mgCO_2/kg/h) of the fully ripe bananas with different poliamin treatment within 10 days of storage

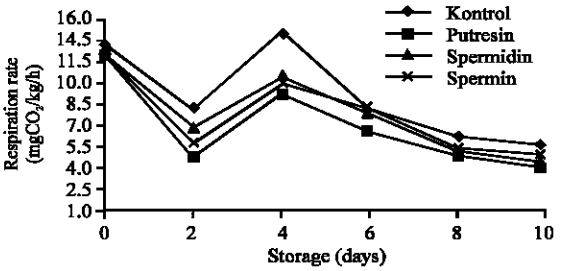

Fig. 6: The respiration rate (mgCO_2/kg/h) of the over ripe bananas with different poliamin treatment within 10 days of storage

putresin was 36%, sperdimin was 18.5% and spermin was 17%). This was due to the fact that the poliamin compound could strongly bind the pectin compound in the central lamella which was between the amine groups of the poliamin and the carboxyl group of the pectin. They formed the pectin-poliamin compound which resulted in the stronger cell wall and resistant to the external effect including the cool temperature so that the chilling injury could be prevented (Shen *et al*., 2000; Valero *et al*., 1998).

The biggest inhibition was made from the Putresin compound (Put). This was because the putresin was one of the poliamin compounds which had the lowest amine group (two amine groups). Therefore, it was easier to bind the central lamella maker (pectin) and cell wall maker (phospholipid) which formed the stronger bind compound compared to the Spermidin (Spd) and Spermin (Spm) whose amine groups were larger, respectively 3 and 4 amine groups (Rhodes, 2005).

Respiration rate: The respiration rate with various poliamin treatments in 85% ripe, fully ripe and over ripe bananas within 10 days of storage in the temperature of 10° celsius is shown in Fig. 4-6.

Figure 4 displayed that the control from day 1 until day 6 made the relatively low and stable respiration rate; while in day 7 the respiration rate increased significantly and reached its peak in day 8. The significant increase of the respiration rate was likely caused by the banana has been in chilling injury due to the pressure or stress of the cold temperature (Lyons, 1973). The increased respiration rate was followed by the the increased ethylene emission rate (Fig. 1-3).

In the 85% ripe (Fig. 4), fully ripe (Fig. 5) and over ripe (Fig. 6) bananas, it showed that the control made the biggest respiration rate compared to the poliamin treatment to all level of maturation and ripeness while the poliamin treatment (putresin, spermidin and spermin) could inhibit the respiration rate. The inhibitation was as follows: in the banana whose ripeness was 85% the inhibition was 45.39% by putresin, 35.95% spermidin and 23.66% by spermin the fully ripe banana the inhibition was 36.06% by putresin, 30.19% by spermidin and 26.48% by spermin and the over ripe banana the inhibition was 25.17% by putresin, 14.58% by spermidin and 14.15% by spermin. This was because the poliamin compound could make a strong bond with the pectin in central lamela and cell membrane so that the cell was more stable and resistant of the external influence such as oxygen and cold temperature (Leiting and Wicker, 1987).

Chilling injury index (necrosis and pitting): The analysis of chilling injury index (necrosis and pitting) of the banana with various poliamin treatment and different level of maturation and ripeness within 10 days of storage in the temperature of 10° celsius was shown in Table 1.

From Table 1, it was found out that the Chilling Injury Index (CI) of the controlled group (with no soaking in the

Table 1: The chilling injury index of the banana in various poliamin treatment and various maturation and ripeness within 10 days of storage

	Chilling injury index (necrosis and pitting)					
	Storage period (day)					
Treatment combination	0	2	4	6	8	10
K_1P_0	0	0	0	1	2	3
K_1P_1	0	0	0	0	0	1
K_1P_2	0	0	0	0	1	1
K_1P_3	0	0	0	1	1	1
K_2P_0	0	0	2	2	3	3
K_2P_1	0	0	0	1	1	2
K_2P_2	0	0	0	1	2	2
K_2P_3	0	0	0	2	2	3
K_3P_0	0	2	3	3	3	3
K_3P_1	0	1	1	2	2	3
K_3P_2	0	1	2	2	2	3
K_3P_3	0	1	1	2	3	3

K_1 = 85% of ripeness; K_2 = fully ripe; K_3 = over ripe; P_0 = Without Poliamin; P_1 = Putresin (Put); P_2 = Spermidin (Spd); P_3 = Spermin (Spm); Score 0 = no symptom of necrosis and pitting; score 1 = necrosis and pitting <25% (low symptom); score 2 = necrosis and pitting 25-50% (moderate symptom); score 3 = necrosis and pitting >50% (high symptom), the texture of the banana (mm) storage period (days), treatment combination

Table 2: The texture (hardness) of the banana in various poliamin treatment and different level of maturation and ripeness within 10 days of storage in the temperature of 10° celsius

	Chilling injury index (necrosis and pitting)					
	Storage period (days)					
Treatment combinations	0	2	4	6	8	10
K_1P_0	1.63	1.86	2.17	2.37	2.71	3.12
K_1P_1	1.61	1.78	1.90	1.96	2.08	2.16
K_1P_2	1.69	1.89	1.98	2.09	2.16	2.29
K_1P_3	1.60	1.97	2.22	2.31	2.37	2.53
K_2P_0	3.82	3.98	4.18	4.38	4.52	4.67
K_2P_1	3.28	3.62	3.69	3.84	3.91	3.99
K_2P_2	3.51	3.74	3.86	3.99	4.07	4.16
K_2P_3	3.63	3.82	3.93	4.06	4.21	4.30
K_3P_0	11.42	11.78	12.13	12.42	12.79	13.02
K_3P_1	10.08	10.37	10.63	10.91	11.15	11.25
K_3P_2	10.23	10.57	10.61	10.82	11.08	12.66
K_3P_3	10.49	10.53	11.15	11.29	11.35	12.92

K_1 = 85% of ripeness; K_2 = fully ripe; K_3 = over ripe; P_0 = without poliamin; P_1 = Putresin (Put); P_2 = Spermidin (Spd); P_3 = Spermin (Spm); The higher the index, the softer the texture

poliamin solution) was higher (high symptom) than the banana soaked in the poliamin solution. This showed that the poliamin could inhibit the necrosis and pitting during the storage. The biggest inhibition was the blackish-brown spots in the banana skin which indicated the chilling injury.

Wills *et al*. (1998) suggested that the chilling injury was caused by the damaged sensitive cells and tissues as the toxic metabolic was accumulated (such as acetaldehyde, ethanol and oxaloacetate). The injury made the necrosis and pitting (the blackish-brown spots), the color changing in the surface and inside the fruit the failed ripeness the development of off flavor (the undesirable flavor) the growth of fungi in the surface and the rotten banana.

Texture (hardness): The texture (hardness) measurement of the banana in various poliamin treatment and different level of maturation and ripeness within 10 days of storage in the temperature of 10° celsius was displayed in Table 2. Table 2 showed that the texture of the banana in the controlled group made the bigger texture index (which means the texture is softer) than the banana treated in the poliamin solution. This applied to all level of maturation and ripeness. This proved that the poliamin compound could inhibit thesoftening process of banana texture during the storage period.

The poliamin treatment (putresin, spermidin and spermin) could inhibit the softening process of banana texture and the biggest inhibition was done by the Putresin compound (Put). This was because the poliamin compound could bond strongly with pectin compound in the central lamela. The amine groups of the poliamin and the carboxil groups of the pectin made the complex compound (pectin-poliamin), so the cell wall was stronger and resistant to the external influences such as cold temperature so that the chilling injury could be prevented (Shen *et al*., 2000; Valero *et al*., 1998). Poliamin also

stimulated the activity of PME (Pectin Methil Esterase) enzyme (Leiting and Wicker, 1997), so "demethylation" (methyl group breakdown) in the pectin compound occured by which made more carboxyl groups bonding with the amine groups of the poliamin (either endogen or exogen poliamin). The positively charged poliamin had similar characteristic with the calcium in a way it could delay the texture softening process and senesence as well as inhibit the chilling injury (Valero *et al*., 1998).

CONCLUSION

Based on the research finding and the discussion, the conclusion drawn is as follows. Soaking the banana in the poliamin solution (putersin, spermidin and spermin) can inhibit the respiration rate, ethylen emission rate, chilling injury (necrosis and pitting) as well as decrease the reduced sugar rate and soften the banana texture in all level of maturation and ripeness. The most effective poliamine to inhibit the chilling injury in all level of maturation and ripeness of banana is putresin, followed by spermidin and spermin. The putresin can effectively inhibit the chilling injury in the banana whose ripeness is 85%.

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