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FRUIT WATER LOSS IN RELATION TO PEEL SURFACE MORPHOLOGY AND PHYSICAL PROPERTIES OF *MUSA* AAA 'BERANGAN' AND 'WILLIAM CAVENDISH' DURING DEGREENING

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ABSTRACT

Water loss from the stomata of banana fruit has been thought to hasten fruit ripening. A study has been carried out to determine fruit water loss in relation to peel surface morphology and physical properties of *Musa* AAA 'Berangan' and 'William Cavendish' during degreening at ripening temperature of 18±2 °C and 27±2 °C. Water loss, stomatal density, length and opening, and peel thickness were determined in this study. There was a significant difference in water loss in all the bananas determined if water loss expressed in correspondence to days of ripening. The distribution of stomatal density in a finger differs from each regions and faces. The distribution seemed identical among the bananas studied. The thickness of the peel decreased significantly as ripening progressed and correlated negatively with water loss in all the bananas determined. Fruit water loss did not correlate significantly with stomatal density and opening. Probably stomatal is not the principal route for water loss in Berangan and Cavendish bananas. Further studies need to be carried out to look into the characteristics of cuticle and epicuticular wax on these two bananas.

Keywords: Stomata density, stomata length and opening, peel thickness, ripening duration, surface morphology

INTRODUCTION

The most abundant constituent of a banana fruit is water. The peel of the green fruit has 90% water, while the pulp has about 73% (Marriott 1980). One characteristic feature of banana fruits is the relatively large proportion of peel (exocarp) tissue which makes up about 80, 40 and 33% of the fresh weight of juvenile, mature and fully ripe fruits, respectively. The peel undoubtedly makes a significant contribution to the overall metabolism of the banana fruit (Palmer 1971). The peel of banana protects the pulp from the surrounding environment and stomata which is thought to be the principal route for gaseous exchange (Banks 1984). Water loss (WL) from the stomata of the fruit peel has been attributed to reducing the green-life of plantain fruit (Burdon et al. 1993), and in some instances hastens fruit ripening (George & Marriott 1985; Burdon et al. 1994a).

To our knowledge, no work has been done to examine the effect of WL on the failure of 'Cavendish' banana to degreen at tropical temperature of 27±2 °C. Cavendish banana could only degreen normally at 18±2 °C. In contrast, cultivars like 'Berangan' banana can undergo natural degreening at tropical temperatures where the green peel changes to yellow. Since WL is across the epidermis, it is important to understand the morphology of the peel surface and physical properties of fruits. Thus, the objective of this study is to characterize peel morphology and fruit physical properties in relation to fruit WL during degreening of Berangan and Cavendish at 18±2 and 27±2 °C.

MATERIALS AND METHODS

Mature green *Musa* AAA Berangan and William Cavendish banana were obtained from a fruit distributor and transported to Postharvest Laboratory, Faculty of Agriculture, Universiti Putra Malaysia, Selangor Malaysia. Fruits that free from any form of injuries and damages were selected for the experiments. Vaseline was applied to the cut surface to avoid WL. The carton containing the fruit and calcium carbide (CaC_2) (10 g $CaC_2.kg^{-1}$ fruit) was placed in a chamber of 18±2 °C (relative humidity (RH) 90-95%) and room of 27±2 °C (RH 75-80%) for ripening initiation. After 24 h, the cover of the carton was removed and the fruit was allowed to continue ripening in the respective temperatures.

Berangan banana ripened at 18±2 °C did not degreen and fail to ripen normally, thus the fruit was discarded. Berangan banana ripened at 27±2 °C (B27) degreened and ripened normally. An evaluation of up to six stages of ripening, based on Federal Agriculture Marketing Authority (FAMA), visual score indices was carried out on the fruits. The scale is as follows: 1 = mature green, 2 = green with trace of yellow, 3 = more green than yellow, 4 = more yellow than green, 5 = yellow with green tips and 6 = full yellow. Cavendish banana that ripened at 18±2 °C (C18) degreened and ripened normally, and thus an evaluation up to six stages of ripening based on FAMA visual score scale was carried out. The peel of Cavendish banana ripened at 27±2 °C (C27) failed to degreen. Therefore, the evaluation was carried out on a daily basis with 24 h interval for 5 days until brown specks appeared on the peel of the fruit. Day 0 was considered as the day the fruit was initiated to ripen using CaC_2. The duration for peel of B27 and C18 fruit to change from one ripening stage (RS) to another, was recorded in hours.

Weight loss measurements were made at every stage of ripening (B27 and C18) or day (C27) for the period of ripening by weighing individual fruit. Fruit WL was calculated as a percentage reduction from the initial fruit weight. To account for differences in surface area (SA) and duration of ripening from one RS to another fruit, WL was also expressed as per cm^2 of fruit peel SA and per h of ripening duration. Peel SA was determined by peeling off the peel and traced on a paper. Then the area (cm^2) of cut-traced paper was determined using a leaf area meter (LI-3100, LICOR Nebraska, USA). Stomatal densities/unit area of fifteen different locations on the peel of a fruit was assessed using nail varnish technique (Johnson

& Brun 1966) and counted with optical compound microscope (Lietzs M. Lux, Leica, German). The peel thickness was measured at the mid region of fruit using vernier caliper.

Samples of the peel measuring 0.5 cm x 0.5 cm x 0.5 cm, were cut from the mid region of the fruit and fixed in Karnovsky's fixative (Karnovsky 1965). The tissues were post-fixed in 1% osmium tetroxide dissolved in 1% cacodylate buffer and dehydrated through graded series of ethanol to absolute ethanol. The tissues were dried in a Blazer CD 30 critical point dryer and subsequently prepared for SEM viewing under JOEL 6400 scanning electron microscope (SEM) (JEOL, Japan) under high vacuum at an acceleration voltage of 15kV with a working distance of 39 mm. Stomatal length referred to the distance of the long axis of the two guard cells, while the stomatal opening referred to the widest distance transversely across the two guard cells. Ten stomata per replicate were determined from each RS or ripening day after the acetylene treatment.

The experimental design was a RCBD with four replications of five fruits per replicate except for the stomatal density where a factorial arrangement was used. When the F values of ANOVA showed significance at $p \leq 0.05$, a DMRT was used to separate the means. A correlation analysis by means of Pearson's correlation matrix was also performed to establish the association between WL, stomatal density and opening, peel thickness and ripening duration.

RESULTS AND DISCUSSION

Water loss

There was a significant difference in WL as ripening progressed in all the bananas determined (Table 1). The total WL of B27 throughout the six ripening stages was 5.43%. Similarly, C18 also showed significant increase of WL as the ripening progressed. C27 also showed significant increase of WL as days after the acetylene treatment progressed (Table 1). A loss of 11.19% occurred as the ripening progressed from day 0 to 5 after the acetylene treatment.

When the values of WL were expressed in correspondence to the peel SA (cm^2) and ripening duration (h), there was no significant change in the rate of WL in all the three groups of bananas studied throughout the ripening process (Table 1). However, B27 lost more water than C27 and C18. This is due to higher SA to volume ratio of Berangan banana (1.24) than Cavendish banana (1.08) which has larger dimension (unpublished data). According to Burdon et al. (1993), the manner by which WL is expressed might influence its interpretation, and fruit dimensions are important when WL is expressed as a percentage of the initial weight.

It has been demonstrated experimentally that the green-life of a plantain fruit was shortened in low humidity (George & Marriott 1983). It has been postulated that RH (Paull 1996; Semple & Thompson 1988) and high ripening

at their apex than at their basal) which reduced clustering of leaves around the plant stem which in turn lowered the c.v. of leaf area density. This would mean that, all properties being equal, plants having leaf shapes that are broad at the apex such as oblanceolate, obovate and spatulate (Glattstein 2003) would intercept more solar radiation than plants having leaf shapes that are broad at the basal such as ovate and cordate. Leaf petioles also increase solar radiation interception in particular by reducing leaf clustering around the plant stem, but the advantages of having leaf petioles are least pronounced for plants that have long, narrow leaves. This may indicate why plants having such leaves rarely have petioles (e.g. maize and oil palm) as they already capture solar radiation effectively.

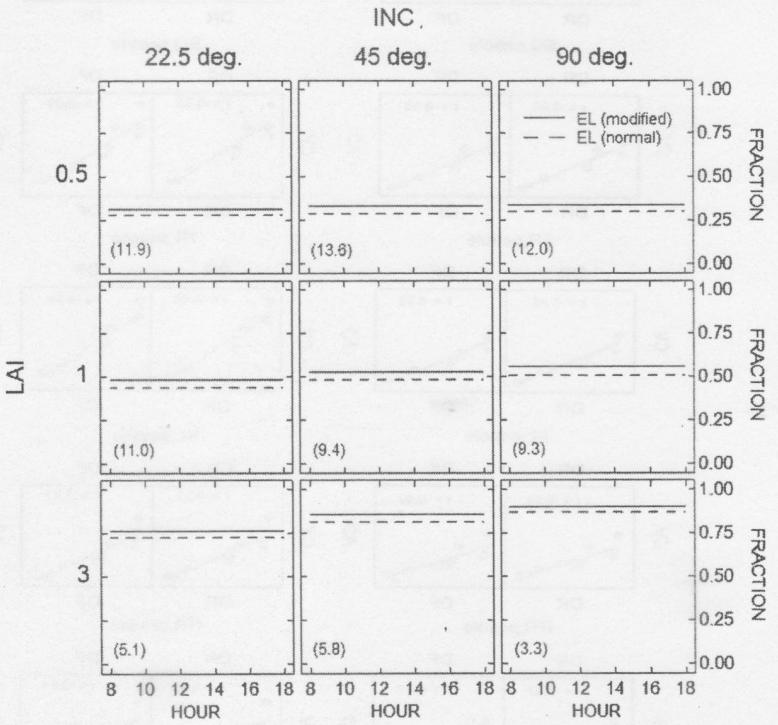

Figure 3. Comparisons between the fraction of intercepted diffuse solar radiation by the EL (modified) and normal EL prototypes. Note: values in brackets indicate the mean hourly difference (in percent) between the fraction of intercepted solar radiation by the EL (modified) and EL (normal) prototypes. LAI and INC are the leaf area index and solar inclination, respectively.

Results presented here indicated that leaf shape does have an effect on solar radiation interception (though to a rather small extent) by altering the spatial distribution of leaf area density. Solar radiation interception is augmented for plants having leaves that are shaped in such a way that causes the canopy to be "spread out" more uniformly or homogenously; thus, reducing self-shading or clustering of leaves. Plants with long, narrow leaves, for example, increases solar radiation interception as compared to plants with short, wide leaves. This indicates that, all

temperature (New & Marriott 1984) of a ripening room could induce banana finger drop. In this study, the low RH and tropical temperature has induced finger drop of C27 by day 4 (unpublished data). The low RH and high ripening temperature could also cause a failure in Cavendish banana to degreen (Dadzie & Orchard 1997). This could be one of the reasons that Cavendish banana failed to degreen in low RH and high tropical ripening temperature of 27±2 °C.

Table 1. Effects of ripening stage or days after acetylene treatment on water loss and water loss rate of Berangan banana ripened at 27±2 °C and Cavendish banana ripened at 18±2 °C and 27±2 °C.

Ripening stage or days after acetylene treatment[z]	Water loss (%)	Progression ripening stage or days after acetylene treatment		Rate water loss ($g.cm^{-2}.h^{-1}$)
	Berangan banana ripened at 27±2 °C			
1	-[y]	From	To	
2	0.96 e[x]	1	2	2.76×10^{-4} a
3	2.12 d	2	3	6.28×10^{-4} a
4	2.92 c	3	4	5.60×10^{-4} a
5	4.16 b	4	5	6.73×10^{-4} a
6	5.43 a	5	6	1.09×10^{-3} a
	Cavendish banana ripened at 18±2 °C			
1	-	From	To	
2	1.34 d	1	2	1.84×10^{-4} a
3	2.62 cd	2	3	1.77×10^{-4} a
4	4.20 bc	3	4	1.68×10^{-4} a
5	5.36 ab	4	5	2.48×10^{-4} a
6	6.27 a	5	6	1.27×10^{-4} a
	Cavendish banana ripened at 27±2 °C			
0	-	From	To	
1	1.26 e	0	1	3.19×10^{-4} a
2	3.97 d	1	2	6.71×10^{-4} a
3	6.26 c	2	3	5.28×10^{-4} a
4	8.63 b	3	4	5.98×10^{-4} a
5	11.19 a	4	5	6.63×10^{-4} a

[z]Ripening stage refers to Berangan and Cavendish bananas ripened at 27±2 °C and 18±2 °C, respectively; days after acetylene treatment refer to Cavendish banana ripened at 27±2 °C.

[y]No water loss was detected.

[x]Mean separation in columns within banana cultivars and ripening temperatures followed by the same letter are not significantly different by DMRT, $p \leq 0.05$.

Stomatal density

Stomatal densities of B27, C18 and C27 were not significantly changed as ripening progressed (Table 2). The stomatal density on the concave face of B27 was significantly highest among five faces in a fruit with 5.1 stomata per mm^2. The bottom-left and bottom-right faces did not show any significant differences in stomatal density and had the least density as compared to other faces. The stomatal density at the top right and top left faces of B27 was almost similar. A similar result was obtained in C18 and C27. Among three regions of stem end, mid region and floral end in a fruit, the stomatal density at the stem end of B27 was significantly higher by 6.8% than those at the mid region and floral end. Similarly the stomatal density at the stem end of C18 and C27 was significantly higher than the mid region and floral end by 14 and 10%, respectively.

Despite the ripening temperature, Cavendish banana had denser stomatal densities than Berangan banana (Table 2) indicating stomatal density varied in cultivars. A similar observation was found by Zakaria & Razak (1997) where the stomatal density of 'Novaria', 'Intan' and 'Jari Buaya' bananas at the mid region of the concave face was 488, 244 and 576 per cm^2, respectively. The banana stomatal density at the mid region of 'Ihitisim', 'Ubok Iba', 'Agbagba', 'Obino L'Ewai', 'Bobby Tannap', 'Bluggoe', 'Pelipita', 'Fougamou 582.4.486 hybrid fruit' and '612,74 hybrid fruit' was 654, 528, 430, 529, 571, 437, 503, 511, 302 and 267 per cm^2, respectively (Burdon et al. 1993). The stomatal density of a banana fruit, however, was very low if compared to the leaves for which Simmonds (1962) gave values of between 13,500 and 46,900 per cm^2. The very low stomatal density on the fruit surface compared to the leaves suggests that the amount of water lost via the fruit stomata may be relatively small.

The distribution of stomatal density at different faces of a finger seemed identical among the three groups of bananas studied. The concave face, which was shaded from the sun during its development as a bunch on the tree showed the highest density compared to other faces. The bottom left and right faces of the finger were exposed to sun when it was in bunch arrangement on the tree. As a result, the density was the lowest among the faces evaluated. The faces of the top left and top right faced neighbouring fingers in a hand, thus the microclimate was shadier than the bottom left and right. This finding is in line with the findings of Zakaria & Razak (1997). The stomatal density at the mid region of a concave face of Novaria, Intan and Jari Buaya bananas was 488, 244 and 576 per cm^2 compared to those at the bottom face with 268, 172 and 428 per cm^2, respectively (Zakaria & Razak 1997).

In this study, among the stem end, mid region and floral end of a banana fruit, the stem end has the highest stomatal density. Zakaria & Razak (1997) revealed that the stomatal density at the mid region was denser than the floral end. Unfortunately, no result was published for stomatal density at the stem end by Zakaria & Razak (1997). The differences of stomatal density distribution in stem

end, mid region and floral end of banana indicating microclimate in stem end is more favourable for stomata development.

Table 2. Main and interaction effects of six ripening stages (RS) or days after acetylene treatment (D), five faces (F) and three regions (R) of Berangan banana ripened at 27±2 °C and Cavendish banana ripened at 18±2 °C and 27±2 °C on stomatal density per mm^2.

Factor	Berangan banana ripened at 27±2 °C	Cavendish banana ripened at 18±2 °C	Cavendish banana ripened at 27±2 °C
Ripening stage (RS) or days after acetylene treatment (D)[z]			
1 or 0	4.0 a[y]	4.5 a	4.5 a
2 or 1	4.1 a	4.7 a	4.5 a
3 or 2	4.0 a	4.4 a	4.6 a
4 or 3	4.1 a	4.6 a	4.7 a
5 or 4	4.0 a	4.4 a	4.5 a
6 or 5	4.0 a	4.6 a	4.6 a
Face (F)			
Concave	5.1 a	5.3 a	5.4 a
Top right	4.2 c	4.5 b	4.5 b
Bottom right	3.6 d	4.2 c	4.3 c
Top left	4.3 b	4.5 b	4.5 b
Bottom left	3.6 d	4.2 c	4.2 c
Region (R)			
Stem end	4.4 a	5.0 a	4.9 a
Mid region	4.1 b	4.3 b	4.4 b
Floral end	4.1 b	4.3 b	4.4 b
Interaction			
RS or D x F	NS	NS	NS
RS or D x R	NS	NS	NS
F x R	**	**	**
RS or D x F x R	NS	NS	NS

[z]Ripening stage refers to Berangan and Cavendish bananas ripened at 27±2 °C and 18±2 °C, respectively, while days after acetylene treatment refer to Cavendish banana ripened at 27±2 °C.

[y]Mean separation in columns within banana cultivars and ripening temperatures followed by the same letter are not significantly different by DMRT, $p \leq 0.05$.

NS, **Non-significant or significant at $p \leq 0.01$, respectively.

Stomatal length and opening

The stomatal length of B27, C18 and C27 did not show any significant changes during ripening (Table 3). In contrast, the stomatal opening showed significant changes as ripening occurred. The stomatal opening of B27 increased by 61% as the fruit ripened from RS 1 to 3. Then, the opening decreased significantly by 26 and 41.3% as the fruits ripened from RS 3 to 4 and RS 3 to 6, respectively. Similar to B27, the stomatal opening of C18 decreased significantly in parallel with the ripening process. As the fruit ripened from RS 1 to 2, 2 to 3 and 3 to 4, the decrease was by 15, 14 and 30%, respectively. Similarly, the stomatal opening of C27 also decreased significantly by 44% as ripening advanced from day 0 to 4.

Table 3. Effects of ripening stage or days after acetylene treatment on stomatal length and opening of Berangan banana ripened at 27±2 °C and Cavendish banana ripened at 18±2 °C and 27±2 °C.

	Berangan banana ripened at 27±2 °C		Cavendish banana ripened at 18±2 °C			Cavendish banana ripened at 27±2 °C	
Ripening stage	Length[z] (μm)	Opening[y] (μm)	Length (μm)	Opening (μm)	Days after acetylene treatment	Length (μm)	Opening (μm)
1	17.43 a[x]	2.29 c	17.07 a	6.42 a	0	16.94 a	6.46 a
2	17.29 a	2.48 c	18.03 a	5.48 b	1	17.46 a	6.11 ab
3	17.05 a	3.68 a	16.72 a	4.72 c	2	17.75 a	5.49 b
4	18.02 a	2.74 bc	17.11 a	3.30 d	3	17.23 a	5.54 b
5	16.23 a	3.37 ab	16.14 a	3.33 d	4	16.85 a	3.62 c
6	16.96 a	2.16 c	17.75 a	3.46 d	5	16.16 a	4.20 c

[z, y]Mean of 40 observations.
[x]Means for each column followed by the same letter are not significantly different by DMRT, $p \leq 0.05$.

Williams et al. (1989) observed that the stomata of a Cavendish banana fruit are well developed with prominent guard cells and subsidiary cells at 14 d before bunch emergence. The length of stomata for Berangan and Cavendish bananas seemed almost alike in this study (Table 3). Zakaria & Razak (1997) found that the stomatal length of Novaria and Intan bananas was 16.4 and 17.8 μm, respectively. Burdon et al. (1993) discovered that the stomata length of 10 banana cultivars differ from each other. Besides, the stomatal length at stem end, mid region and floral end also varied among cultivars. Burdon et al. (1993) reported that there were changes in the banana stomatal length as ripening progressed. The result obtained from B27, C18 and C27 of this study was not in agreement with the finding of Burdon et al.

(1993). The stomata were well developed before harvest as reported by Williams et al. (1989). The finding of Burdon et al. (1993) where the stomatal length of a banana fruit varied at each RS could probably due to the different chronological ages of the fruits used.

In this study, WL of B27, C18 and C27 was not significantly correlated with stomatal density and opening (Table 4). This finding is in accordance with the finding of Burdon et al. (1994b). In this study SEM micrographs revealed that the stomata were at varying degrees of opening at each RS. Since WL was not significantly correlated with stomatal density and opening, the precise role of the stomata in WL from banana fruits was unclear, it seemed likely that the principal route of the WL might be cuticular (Burdon et al. 1994a). This would not be unusual since in some fruits (such as sultana and grapes) with no stomata on the fruit surfaces, all transpiration is through cuticles (Possingham et al. 1967). The alternative to bulk water loss through the stomata is for water to move at the molecular level across the cuticle (Juniper & Jeffree 1983). The rate of cuticular transpiration is dependent on the cuticle and the epicuticular wax. The total amount of epicuticular wax may in itself not indicative of the potential for transpiration loss rates across the cuticle. It is the wax composition and formation which creates the barrier to water vapour diffusion.

Table 4. Correlation coefficients (r) for water loss (WL), stomatal density (SD), stomatal opening (SO) and peel thickness (Thick) in Berangan and Cavendish bananas during ripening.

	WL	SD	SO	Thick
	Berangan banana ripened at 27±2 °C			
WL	--			
SD	0.25	--		
SO	0.08	-0.22	--	
Thick	-0.996**	0.18	-0.09	--
	Cavendish banana ripened at 18±2 °C			
WL	--			
SD	0.10	--		
SO	0.23	0.10	--	
Thick	-0.99**	0.11	0.91*	--
	Cavendish banana ripened at 27±2 °C			
WL	--			
SD	0.39	--		
SO	0.10	-0.04	--	
Thick	-0.97**	-0.50	0.87*	--

n = 24; *, **Significant at $p \leq 0.05$ or $p \leq 0.01$, respectively.

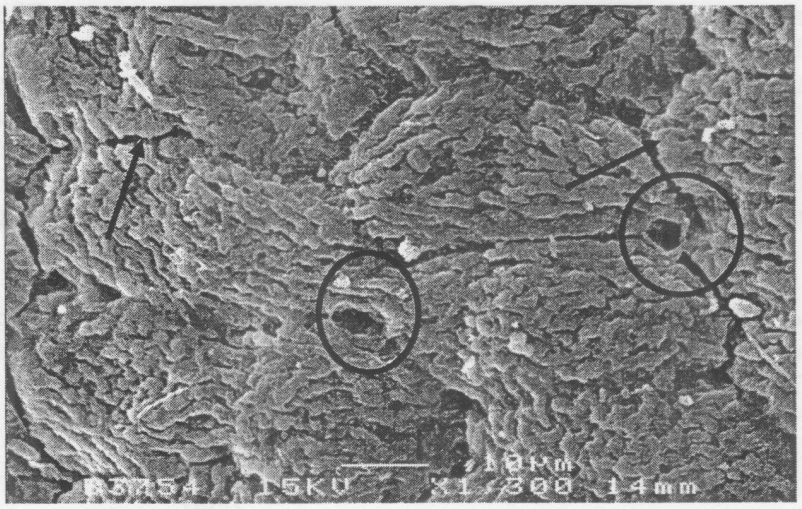

Figure 1. SEM micrograph of pores between epidermal cells (circle) and fractures (arrow) at ripening stage 6 of Cavendish banana ripened at 18±2 °C. x 1, 300.

The SEM micrographs of peel of B27, C18 and C27 reveal the existence of pores and cuticular fractures (Figure 1) which may attribute to WL. According to Burdon et al. (1994b), numerous small pores are relatively more effective than a few large ones in losing water, as long as they are not too close. Stomata, lenticels and superficial fractures of the cuticle modulate the permeance of cuticular membranes (Glenn & Poovaiah 1989). Closed stomata may have conductances of the same magnitude or greater than the permeance of the cuticular membrane (Kerstiens 1996). In 'd'Agen' plums, the large cuticular fractures surrounding the area of stomata collapse the dermal layers of fruits and allow movement of water from dermal cells to the atmosphere (Storey & Price 1999). In grapes, stomata may degenerate and form lenticels which may further modify the local permeance of the cuticle to water (Commenil et al. 1997). The pored and fractures of banana peel epidermal cell found in this study might contribute to WL from the fruit.

Peel thickness

The thickness of the B27 peel decreased by 27.6% as the fruit ripened from stage 1 to 6 (Table 5). Similarly, C18 also showed significant decrease of 38.6% in peel thickness as ripening advanced which was much more than that of B27. The peel thickness of C27 also experienced a significant decrease of 40.3% as days after the acetylene treatment progressed from 0 to 5.

The peel of both ripening temperature of Cavendish banana was thicker than B27 by about 11.5% at RS 1. The difference in peel thickness could be contributed by variation in cultivars. Kachru et al. (1995) reported that peel thickness of 'Dwarf Scavendish' and 'Nendran' cultivars of banana was not the same. In the present study, as ripening progressed to RS 6 or day 5 after the

acetylene treatment, Cavendish banana had thinner peel than Berangan banana. This indicated the thinning of the Cavendish banana peel occurred at a faster rate than Berangan banana once ripening took place. Within a cultivar, the changes in peel thickness of C27 and C18 differed whereby the decrease rate for C27 was 0.0322 $cm.d^{-1}$, while for C18 was 0.0172 $cm.d^{-1}$. Thus, the decrease of the peel thickness in C27 was greater than C18. This could probably due to higher metabolism rate in C27 as fruit ripened at 27 °C as compared to C18 which ripened at 18 °C.

Table 5. Effects of ripening stages or days after acetylene treatment on peel thickness of Berangan banana ripened at 27±2 °C and Cavendish banana ripened at 18±2 °C and 27±2 °C.

Peel thickness (cm)			Peel thickness (cm)
Ripening stage	Berangan banana ripened at 27±2 °C	Cavendish banana ripened at 18±2 °C	Days after acetylene treatment	Cavendish banana ripened at 27±2 °C
1	0.355 a[z]	0.402 a	0	0.400 a
2	0.334 b	0.369 b	1	0.361 b
3	0.316 c	0.334 c	2	0.301 c
4	0.294 d	0.316 d	3	0.285 d
5	0.276 e	0.279 e	4	0.265 e
6	0.257 f	0.247 f	5	0.239 f

[z]Mean separation in columns within banana cultivars and ripening temperatures followed by the same letter are not significantly different by DMRT, $p \leq 0.05$.

According to Asiedu (1987), peel thickness reduction is associated with ripening, probably accounted for by the loss of water in the peel. He found that the peel became senescent after the 250th h of ripening, and the fruit diameter and length shrinkage exceeded by 10% in a single fruit. This finding was clear with high negative significant correlation between peel thickness and WL of the three bananas studied (Table 4), indicating more WL when the peel of fruit is thinner. This was true in this study where the fruits of C27 that had thicker peel experienced higher rate of water loss than B27 (Table 1).

CONCLUSION

The manner by which WL is expressed could influence its interpretation. If the WL was expressed in correspondence to days of ripening, C27 lost more water than B27 and C18. If WL was expressed in correspondence to surface area and ripening

duration, B27 lost more water than C27 and C18. The WL of these three bananas was not affected by the stomatal density and opening. Thus, stomatal is not the principal route for WL in Cavendish and Berangan bananas. Further studies need to be carried out to look into the characteristics of cuticle and epicuticular wax on these two banana fruits. The WL has decreased the fruit peel thickness. Thus, it is very clear that WL has altered the fruit metabolism, quality and hence ripening characteristics of Berangan and Cavendish bananas. The failure of C27 to degreen naturally under a tropical temperature could be due to excess WL during ripening.

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