Chapter 3: Physiological and biochemical changes associated with different harvest maturities of *H. polyrhizus*

3.1 Introduction

Ripening of dragon fruit has been reported previously but the work was mainly centered on the well established yellow peel dragon fruit, *Selenicereus megalanthus* (Nerd and Mizrahi, 1998). Apart from the work on yellow peel dragon fruit, some studies were also carried out on the red peel dragon fruit, *H. undatus* and *H. polyrhizus* harvested close to full colour (Nerd *et al.,* 1999). It is known that dragon fruit which is a non climacteric fruit retains its best quality when harvested at advanced colour stage (Le *et al.,* 2000).

Nevertheless, little is known about the optimal harvest stage for prolonged storage. According to Nerd *et al.,* 1999, the first change in peel colour was recorded 24–25 days after anthesis in *H. undatus* and 26–27 days in *H. polyrhizus.* Meanwhile studies with yellow peel dragon fruit showed that the duration of fruit development depends on seasonal temperatures and that the fruits reached the optimal flavour close to full colour stage (Nerd and Mizrahi, 1998).

Thus, postharvest physiology can be affected by cultivar, environmental conditions and also by harvest time (Bron and Jacomino, 2006). Likewise, in Bacuri fruit, fresh mass loss increased and was affected by maturity stage (Teixeira *et al.,* 2005). Duru and Turker, (2005) indicated that cactus pear a non climacteric fruit (*Opuntia* sp) after reaching full maturity, physical losses occurred in which the fruit started to soften and deteriorate that leads to loss of fruit quality. Therefore, harvesting at the proper stage of maturity is crucial for optimum quality and often for the maintenance of this quality after harvest (Sturm *et al.,* 2003).

Therefore the objective of this study was to determine the ripening criteria of *H. polyrhizus* harvested at three different maturity stages by examining a number of physicochemical parameters including weight loss, peel colour, pulp firmness, total soluble solids, pH, total sugars and total reducing sugars.

3.2 Materials and methods

3.2.1 Plant materials

The plant materials used in these studies are of *Hylocereu*s genus and *polyrhizus* species. The fruit were harvested from the mother plants that were nearly 15 years old at Multi Rich Farm an area of 6 hectares located in Mantin (latitude 2˚ 49' 58"N, longitude 101˚ 54' 0"E), Negeri Sembilan in the morning. The climate condition in the farming area was dry with loose soil with 10 to 30% sand while the planting arrangement was in trellis form. As their growth pattern is similar to a vine, thus the plants were supported by vertical concrete to a height of 1m to 1.5m at which point they are allowed to branch and hang down, thus forming a circle of branches all around the concrete. After harvesting fruit were packed unwashed in cartons and transported the same day to Postharvest Biotechnology Laboratory at the University of Malaya in Kuala Lumpur.

The fruits were sorted and graded by size and appearance. Since no quality standard for *H.polyrhizus* in Malaysia is available, the fruit were manually graded into three different groups according to weeks after anthesis: five weeks after anthesis (fruit completely red with an average weight of 550 ± 9 g), four weeks after anthesis (fruit completely green with an average weight of 350 ± 7 g) and three weeks after anthesis

(fruit completely green with an average weight of 270 ± 13 g). This grouping was assisted by the previous preliminary work conducted for *H. polyrhizus* from the same farm of all stages.

Hereafter, *H. polyrhizus* materials used in this study were referred to as below

D 0 : Five weeks after anthesis

D 1 : Four weeks after anthesis

D 2 : Three weeks after anthesis.

After sorting, 15 fruit of each stage were immersed in benomyl solution 0.05 % for 2 minutes washed and air dried overnight before being deployed into experiments. The day-old fruit were placed at room temperature (23 ± 1° C) in respiration jars and ventilated at 0.5 L per hr air flow, with relative humidity of 80 - 85 %. Visual characteristics of fruit were assessed every day from commencement of the period of the observation. Day-old fruit are (for practical purposes) equated to or considered as equivalent to fruit at harvest in the present study. As for biochemical determination, pulp tissue of fruit samples were collected everyday, cut into block sized and frozen in liquid nitrogen. Frozen pulp tissues were kept at -20˚C until biochemical analysis which was carried on the following week. All measurements were carried out in triplicates and standard error was evaluated by means. The data produced were subjected to analysis of variance (ANOVA) using Duncan Multiple Range.

No	Diagram	Description
1		D 0
2		D 1
3		D 2

Figure 3.1: Pictures of *H. polyrhizus* harvested at three different harvesting maturities used for this study.

3.2.2 Weight loss

Fresh weight of fruit samples were measured every day for almost two weeks using a weighing balance (Mettler PJ 3000, Highstown, NJ). Weight loss was expressed as percentage of fresh weight against initial weight at harvest.

$$\text{Weight loss (\%)} = \frac{Wa - Wb}{Wa} \times 100\ \%$$

Wa – weight at day of harvest

Wb – weight at day of sampling

3.2.3 Visual observation (Subjectively)

Fruit were visually assessed for colour changes and any physiological disorders such as sunken area of the peel, discolouration of the peel, water logging and also drying of the tip of the bracts.

3.2.4 Colour Measurements (L*a*/b*)

Bracts/Peel colour index of the fruit samples were objectively determined using a Minolta Chroma Meter (CR-200) in L*a*/b* coordinates in the Munsell Colour System. The colour was evaluated by measuring *L* (brightness, 100 = white, 0 = black), *a* (+, red; -, green) and *b* (+, yellow; -, blue) (McGurie, 1992). A white tile (No: 21733001) was used to calibrate the instrument. L*a* / b* values at the stalk end, middle and blossom end of five fruit were measured and the average recorded. Colour measurements were obtained from the day of harvest until the fruit reached the over-ripe stage. The method of calculating L*a*/b* coordinates is as follows:

$$L^*a^*/b^* = \frac{L \times a}{B}$$

3.2.5 Determination of pulp firmness

Fruit softening was determined by measuring firmness during ripening of fruit. Firmness was measured by puncture analysis at the stalk end, middle and the blossom end of each fruit (without peel) using a Fruit Hardness Tester, a custom-made compression meter. Fruit firmness was expressed in kilogram force (kg f).

3.2.6 Determination of total soluble solids

Total soluble solids (TSS) were measured using a refractometer (Atago LCD digital refractometer Model PR-1, Japan) with sap squeezed from the pulp of the *H. polyhrizus*. The TSS content was expressed as standard ˚ Brix unit.

3.2.7 Determination of pH

pH of the fruit sap was measured using a digital pH meter (Hanna Instrument, 211, Italy).

3.2.8 Determination of sugar

3.2.8.1 Preparation of reagents

Glucose stock solution (100ug/ml)
100mg of glucose was dissolved in 900 ml of distilled water. Final volume was made up to 1000ml and stored at 4^{o}C.

Sodium hydroxide (NaOH) stock solution (0.5M)
10 g of NaOH was dissolved in 450 ml of distilled water. Final volume was made up to 500ml.

Acetic acid (CH_3COOH) stock solution (0.5M)
14.3ml glacial CH_3COOH was added into 450ml of distilled water. Final volume was made up to 500ml.

Copper reagent
Reagent A
25g of potassium sodium tartarate ($C_4H_4O_6KNa.4H_2O$)
25 g sodium carbonate (Na_2CO_3)
20g sodium hydrogen carbonate ($NaHCO_3$)
200g sodium sulphate (Na_2SO_4)
All the above were dissolved in 800 ml of distilled water. Final volume was made up to 1000ml solution by adding distilled water.

Reagent B
0.3g of cupric sulphate ($CuSO_4 \circ 5_2 0$) was dissolved in 20ml of distilled water. Copper reagent was prepared by adding 1.0ml of copper reagent A to 25ml of copper reagent B.

Arsenomolybdate stock solution
25.0 g of $(NH_4)_6\ MO_7.4H_2O$ was dissolved in 450ml of distilled water. Then 21.0 ml of concentrated H_2SO_4 was added slowly while mixing the solution. Finally, 3g of Na_2HAsO_4 was added and incubated at 37°C oven. Solution stored at room temperature.

Table 3.1: Preparation of standard curve for total sugar and total reducing sugar

Glucose Stock solution (µl)	0	100	200	400	500	600	800	1000
Distilled water (µl)	1000	900	800	600	500	400	200	0
Content (µg)	0	10	20	40	50	60	80	100

3.2.8.2 Preparation of sample, extraction of sugar.

1g of the pulp tissue (refer 3.2.1 page 31) of each ripening stage was ground in liquid nitrogen using a chilled mortar and pestle. The ground powder was then added to 4ml of 0.5 M NaOH. The homogenate was centrifuged using a Sorvall centrifuge (RC5C) at 5,500 rpm for 20 minutes at 4°C. The supernatant was then neutralized with 0.5 M CH_3COOH. The volume was then made up to 20ml using distilled water and stored at 4°C until further analysis.

3.2.8.3Total sugar assay

Total sugar content was determined using the Phenol-sulphuric method (Dubois *et al*., 1956). In this method, 1 ml of the sample solution (refer 3.2.8.2) was added to 0.1 ml of 80% phenol, followed by the addition of 5 ml of concentrated sulphuric acid. The mixture was allowed to stand at room temperature for 10 minutes and then incubated in a 25-30 ^{0}C water bath (Pharmacia Biotech Multi Temp III) for 20 minutes. The absorbance at 490 nm was recorded using spectrophotometer (Ultra Spec II) and the sugar content was obtained by referring to the glucose standard graph. The standard

graph was obtained by adding phenol and sulphuric acid to a standard glucose solution with concentrations between 0-100 μg/ml. Total sugars was expressed in mg/g fresh weight.

3.2.8.4 Total reducing sugar assay

Reducing sugars were assayed as described by Somogyi, (modified method in 1952). 1 ml of the sample solution (refer 3.2.8.2) was added to 1 ml of copper reagent in a test tube and vortexed. The tube was covered with Al foil and the mouth was closed with a marble and incubated in a boiling water bath for 20 minutes. The mixture was cooled on ice for 10 minutes and 0.1 ml of arsenomolybdate solution was added and mixed immediately. 5 ml of distilled water was added and mixed thoroughly. The absorbance at 520 nm was recorded using spectrophotometer (Ultra Spec II) and the total reducing sugar content was obtained by referring to the glucose standard graph. The standard graph was obtained by adding copper reagent and arsenomolybdate solution to a standard glucose solution with concentrations between 0-100 μg/ml Total reducing sugars was expressed in mg/g fresh weight.

3.3 Results

3.3.1 Weight loss

Rate of weight loss was higher in D 1 and D 2 fruits compared to D 0 fruit. The weight loss was only 3 % for D 0 fruit as opposed to D 1 and D 2 fruits which was nearly 6 %. (Fig 3.2)

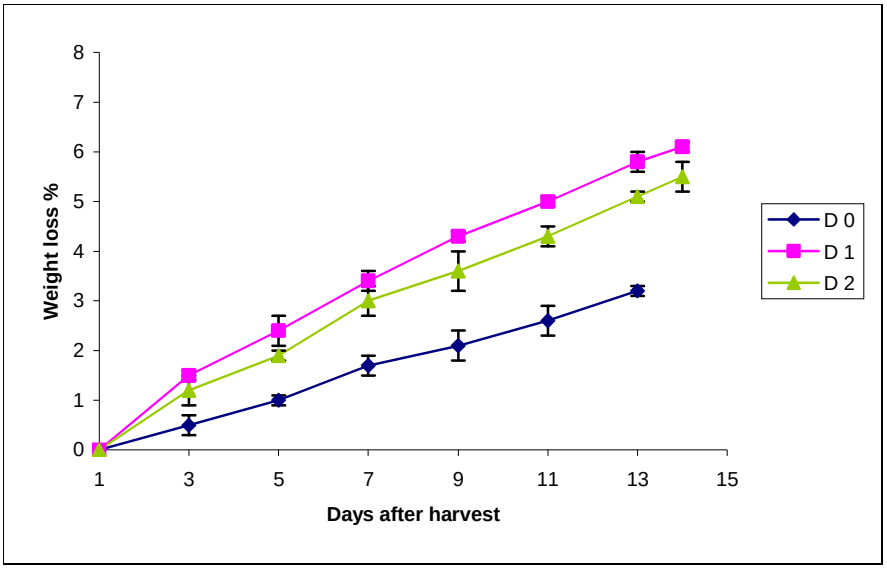


Figure 3.2: Percentage of weight loss of *H. polyrhizus* harvested at three different harvesting maturities at room temperature.

3.3.2 Visual observation

The bracts/peel colour of D 0, D 1 and D 2 fruits held at RT was monitored daily for 2 weeks. The physical appearance (whole fruit) and pulp colour (transversely cut fruit) of representative samples of D 0, D 1 and D 2 fruits over the 2-week period are as shown in Figure 3.3 and 3.4. D 0 fruit remained red throughout storage with the bract tips turning yellow only after 2 weeks. As for D 1 and D 2 fruits, the colour change in the same time interval was similar. The colour of D 1 and D 2 fruits changed from green to red within one week while the bract tips remained green. The colour development was

initiated from the basal part of the bracts. However, the fruit colour continued to develop more intense red colouration after a fortnight. Yellowing of the bract tips was also observed in D 1 and D 2 fruits at the end of the 2-week storage with higher intensity on D 1 fruit.

In terms of pulp intensity, D 0 fruit had more intense colour from day 4 till day 8 but on day 13, the pulp colour became translucent red. As for D 1 fruit, the colour of the pulp was uneven purple with insignificant white appearance. But on day 13, the appearance looked the same as in D 0 fruit. On the other hand, D 2 fruit pulp colour appeared to be white on day of harvest. However, the colour of the pulp changed from purplish to translucent red earlier compared to D 0 and D 1 fruits.

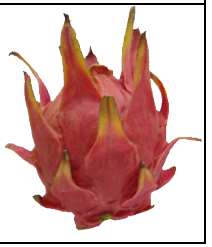
Harvest maturity	1	3	5	7	9	11	13	14
D 0								
D 1								
D 2								

Figure 3.3: Visual observation of whole fruit of *H. polyrhizus* harvested at three different harvesting maturities at room temperature

Harvest maturity	D 0	D 1	D 2
Day 1			
Day 2			
Day 4			
Day 6			
Day 8			
Day 13			
Day 14			

Figure 3.4: Visual observation of transversely cut fruit of *H. polyrhizus* harvested at three different harvesting maturities at room temperature

3.3.3Colour measurements

L*a*/b* value for D 0 fruit showed that the colour of the peel remained within the red colour range from day of harvest (175.1) until day 11 then after dropped to 120.9 on day 13 (Fig 3.5). D 1 fruit showed changes from negative L*a*/b* value on day of harvest (-32.9) to positive value day 3 onwards to 122.1 on day 13. Almost identical pattern was observed in D 2 fruit, with L*a*/b* value of -30.0 on day of harvest to positive value of 139.2 value on day 14.

3.3.4Pulp firmness

Fig 3.6 shows that pulp firmness for D 0 fruit was lower than D 1 and D 2 fruits at day of harvest. However the reduction of pulp firmness was sharper in D 1 and D 2 fruits compared to D 0 fruit which showed slower rate of firmness reduction from day of harvest.

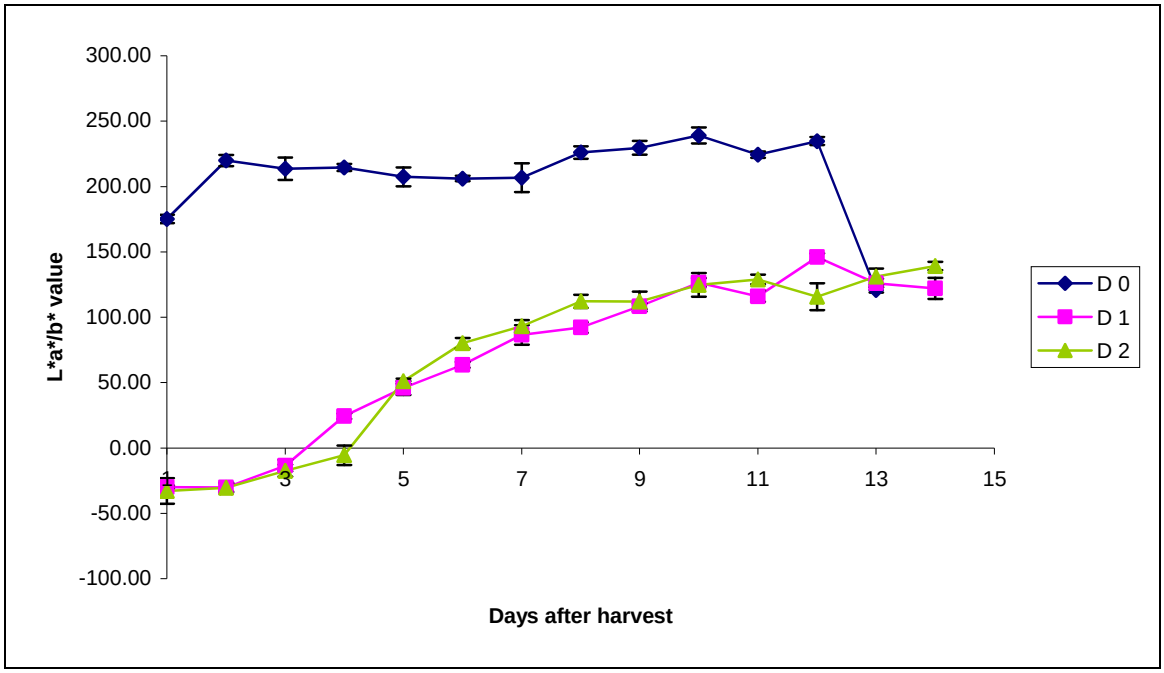


Figure 3.5: L*a*/b* measurement of *H. polyrhizus* harvested at three different harvesting maturities at room temperature

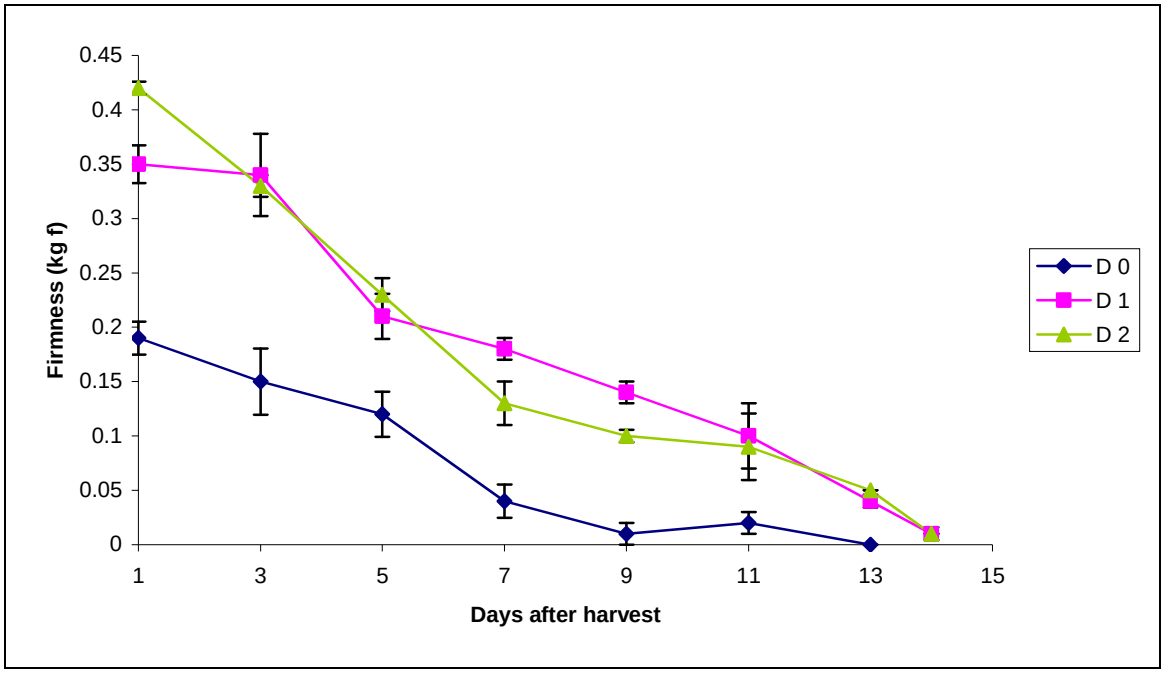


Figure 3.6: Pulp firmness of *H. polyrhizus* harvested at three different harvesting maturities at room temperature

3.3.5 pH measurement

Figure 3.7 shows that pH reading for all fruits were not same at harvest. The trend for the fruit appears to suggest that pH increased from pH 4 to around pH 5.5 over the 2-week period for D 2 and D 0 fruits except for D 1 fruit. D 1 fruit showed inconsistency in the pH values.

3.3.6Total soluble solids

Higher amounts of total soluble solids were obtained from D 0 fruit (13.5 ˚Brix) followed by D 1 fruit (10.7 ˚ Brix) and D 2 fruit (7.3 ˚Brix) on day of harvest (Fig 3.8). Total soluble solids of D 0 fruit were inconsistent from day of harvest till day 13 with reading of 13.5 ˚Brix. As for D 1 fruit, the TSS level was around 10 to 11 ˚Brix for a week and then declined to 8.2 ˚Brix on day 13. D 2 fruit had a gradual increase of total soluble solids from harvest to day 9 (10.9 ˚Brix) but the TSS level maintained to a value of that (8 ˚ Brix) till day 14.

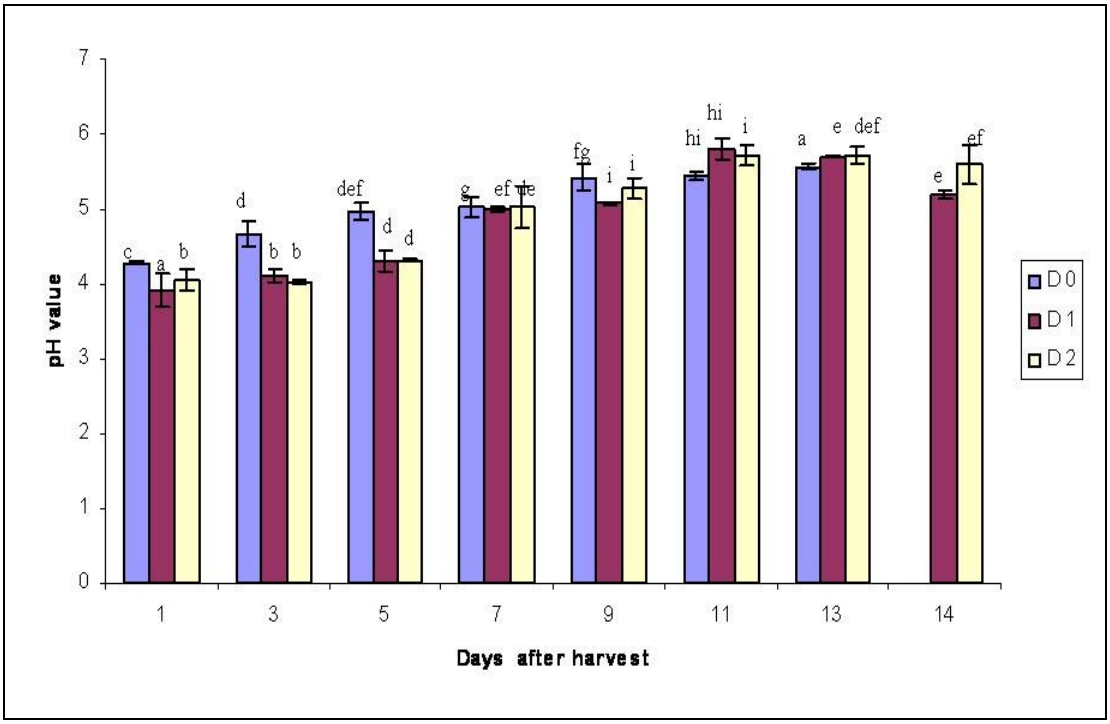


Figure 3.7: pH value of *H. polyrhizus* harvested at three different harvesting maturities at room temperature.

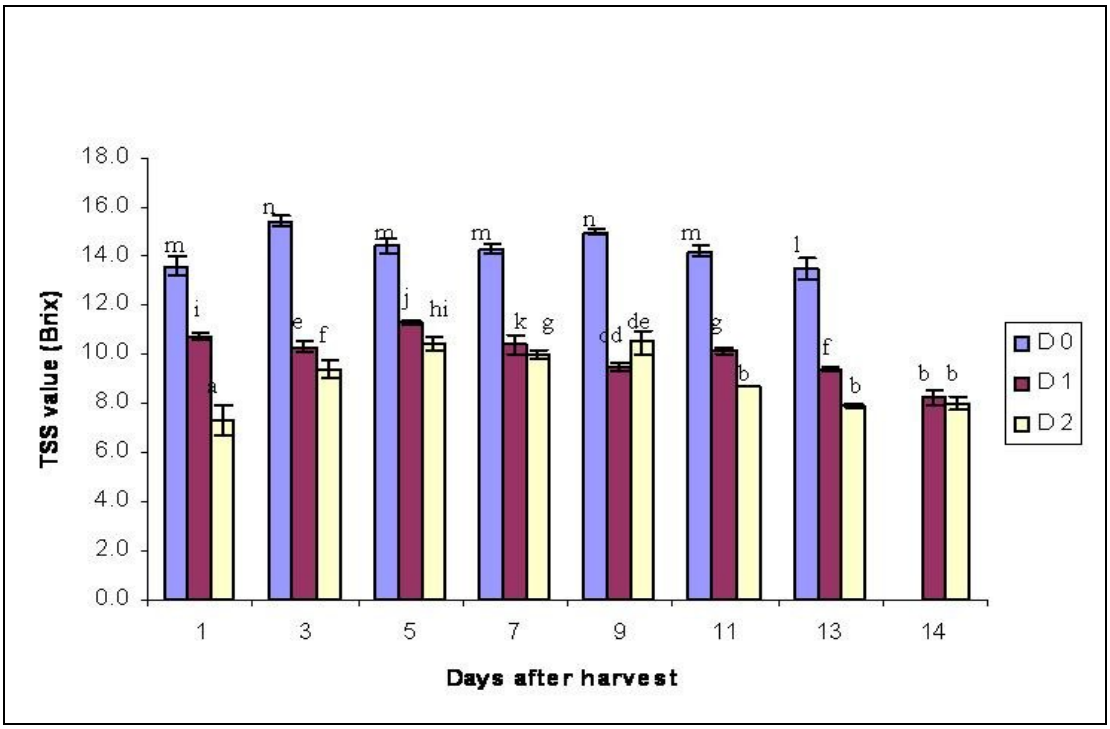


Figure 3.8: Total soluble solids of *H. polyrhizus* harvested at three different harvesting maturities at room temperature

3.3.7Total sugars

Total sugar content was higher in D 0 fruit than D 1 fruit while D 1fruit had slightly more than D 2 fruit at harvest. On day of harvest, the total sugar for D 0 fruits was 81.58 mg/g which maintained until day 9 and then fell to 63.33 mg/g till day 13 (Fig 3.9). However, for D 1 fruit the total sugar remained initially and then decreased within the range of above 40mg/g till day 14 with amount of 39.07 mg/g. As for D 2 fruit, the amount maintained up to day 11 but eventually fell to 28.53 mg/g on day 14.

3.3.8Total reducing sugars

At harvest the average of total reducing sugars in D 0 fruit was 2 to 3 times that was found in D 1 fruit and almost 4 times than that in D 2 fruit at harvest. The amount of total reducing sugar for D 0 fruits maintained for a 1-3 days and decreased continuously (Fig 3.10). The total reducing sugars of D 1 fruit maintained for first 2 days and then fell to a value that was lower than the initial. As for D 2 fruit, an increase was observed until day 9 although the amount of total reducing sugar gradually decreased on day 11 and remained to 21.10 mg/g till day 14. Statistically, the total reducing sugars seems that there is difference between D 1 fruit and D 2 fruit at day 7 and till the end of the experiment.

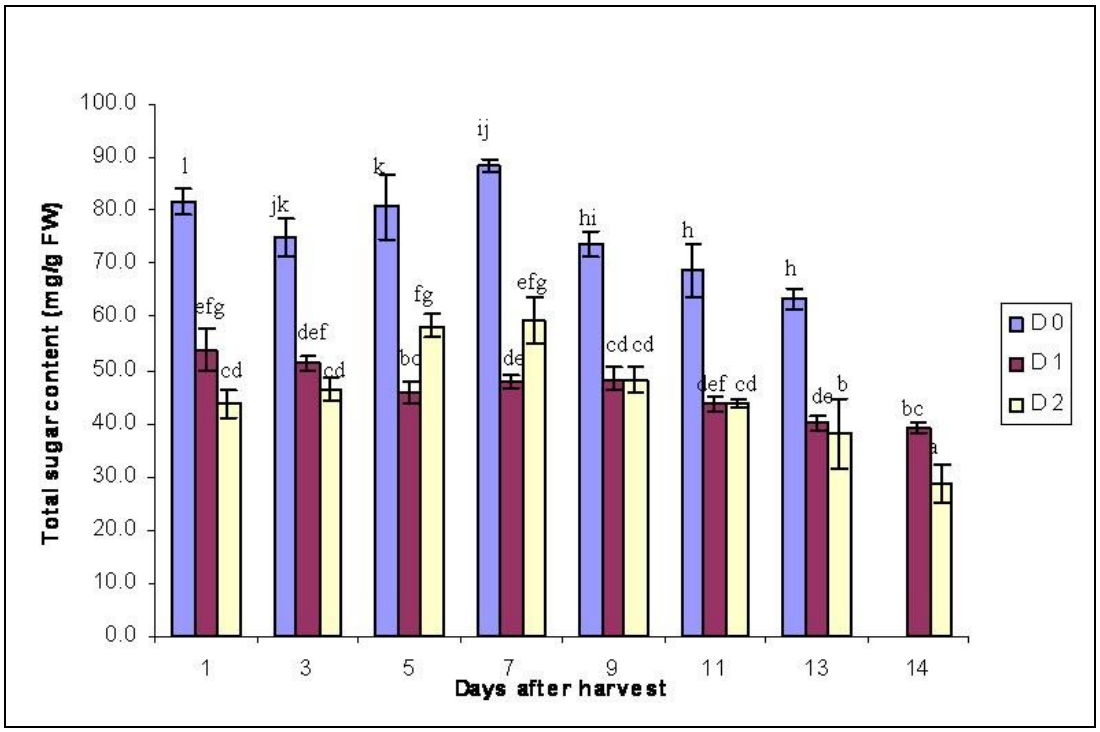


Figure 3.9: Total sugar measurement of *H. polyrhizus* harvested at three different harvesting maturities at room temperature.

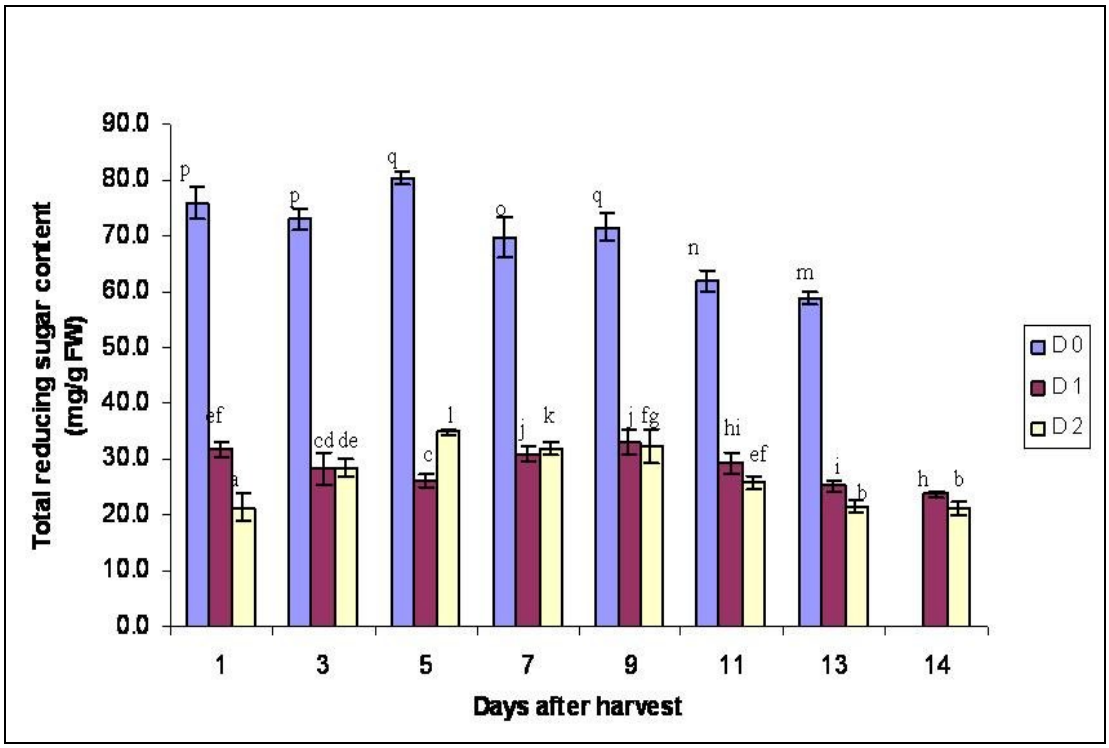


Figure 3.10: Total reducing sugar measurement of *H. polyrhizus* harvested at three different harvesting maturities at room temperature.

3.4 Discussion

Postharvest quality of fruit is generally dependent on the stage of maturity at harvest. From the result obtained, there is enough evidence to indicate that D 1 and D 2 fruits are inferior in quality to D 0 fruit; reflecting condition at harvest. Changes following harvest such as bracts/peel colour, firmness, total soluble solids and sugar level however found to be inconsistent.

During postharvest ripening, fresh weight decreased and was affected by the maturity stage. The rate of weight loss was greater in D 1 and D 2 fruits compared to D 0 fruit. Fruits lose their weight, basically by losing water vapour through respiration and also through transpiration; this is an important characteristic since high water loss affects visual appearance (Kader, 1992). Lower weight loss of D 0 fruit could be due to reduced rate of water vapour loss. A similar trend was also observed in cactus pear (*Opuntia* sp) (Cantwell and Suslow, 2002). This is because generally for cactus, mature fruit contains thick wax for the protection purposes and thus the moisture loss is trapped. Besides that, it was reported by Teixeira *et al*. (2001) that bacuri immature fruit tend to lose higher weight than more mature fruit.

In many fruits, bracts/peel colour is a major determinant for consumers to decide whether the fruit have ripened or not (Kays, 1999). Most obvious change of the bracts/peel colour is the loss of chlorophyll and at the same time unmasking and/or accumulation of other pigment within the fruit. In D 0 fruit, there was no change in colour suggesting the stability of the pigment and also no degradation processes initiated. The subjective

evaluation indicated that bracts/peel colour changes from green to red for D 1 and D 2 fruits during storage. As an outcome, there was a significant increase in the L*a*/b* readings from initial negative value to positive value for D 1 and D 2 fruits stored at room temperature. This objective method was used to enhance accuracy and precision of the subjective evaluation of the fruit peel colour (Chandran, 1998). According to Mizrahi and Nerd (1999) the bracts/peel colour change is initiated between four and five weeks after anthesis and fruit turns red 4-5 days later. Appearance of first bracts/peel colour is observed from the basal part of the bracts of the fruit; this suggests that fruit at one point during ripening can be partially red and partially green. Eventually when the fruit ripens, the fruit surface will be uniformly red. These observed colour or pigment changes occur as a series of coordinated biochemical processes that result in the synthesis and degradation of pigments during ripening of fruits (Sturm *et al.,* 2003). As *H. polyrhizus* fruits ripen, the degradation of chlorophyll is often observed with the accumulation of pigments such as anthocyanin or betacyanin (Stintzing *et al.,* 2002). The changes in bracts/peel colour also reported to be parallel with pulp colour and both bracts/peel and pulp contain the same betacyanin pigment (Wybraniec and Mizrahi, 2002). As for D 1 and D 2 fruits, the lack of precursors such as cyclo DOPA aromatic structure, betalamic acids and enzymes for betacyanin pigment synthesis could be the reason of the fruit not reaching the red intensity of D 0 fruit (Esquivel *et al.,* 2007).

Changes in fruit texture that result in fruit softening are responsible in turning hard fruit into soft edible fruit during ripening (Prasanna *et al.,* 2007). Tissue firmness is influenced by various factors like structural integrity of the primary cell wall and the middle lamella,

accumulation of storage polysaccharides, and the turgor pressure generated within cells by osmosis (Jackman and Stanley, 1995). Fruit softening during ripening generally regarded to result from reduction in firmness can be attributed to several factors such as hydrolysis of starch, water loss by transpiration and pectin degradation of the cell walls of the pulp (Arenas-Ocampo *et al.,* 2003) that eventually bring about deliquescent texture. Besides that, pulp firmness loss is also associated with solubilisation and depolymerisation of cell wall polysaccharides (Rosli *et al.,* 2004). In this study, pulp firmness for all stages of fruit decreased following days after harvest. Nevertheless, the reduction was slower in D 0 fruit compared to D 1 and D 2 fruits. Possibly in early maturity stages, the enzymes related to softening such as pectin methylesterase, polygalacturonase and so on were still not completely synthesized or activated (Johnston *et al.,* 2002). In addition, Barrett and Gonzalez, (1994) stated that high firmness loss in mature cherry was because it contained greater activity of softening enzymes. Furthermore, unmasking of betacyanin pigment in the pulp could possibly affect the pulp firmness however little information is available relating pulp firmness with pulp pigment development.

The initial value of pH 4 of the 3 groups of fruit would suggest that fruit harvested at three maturities were equally acidic. The pH values increase for all stages of fruit during storage was within the range of 3– 5. The pH values are similar to the cactus pear (Mobhammer *et al.,* 2006), thus confirming that this fruit is a low acidic food. Since betalains typically maintain their colour throughout the pH range from 3 to 7 (Stintzing *et al.,* 2002), the pH cannot be the only factor responsible for the differences observed

during colour development for both pulp and peel. Besides that, the increase in pH is probably due to organic acids being metabolized by the fruit during ripening and storage (Marsh *et al.,* 2004). This is a general behaviour for many other fruits (Almeida and Huber, 1999).

Total soluble solids (TSS) for D 1 and D 2 fruits showed inconsistent trend following days after harvest. The findings by Nerd and Mizrahi*.,* (1998) reported that TSS increased significantly as the peel colour developed in yellow pitaya. In the present study, the TSS is positively correlated with maturity of fruit at harvest (i.e. increasing amount of TSS as fruit progressively matures) since TSS in D 0 fruit higher than TSS in D 1 fruit which was higher than D 2 fruit. The different level of TSS is mainly due to contribution by sugar content, organic acids, soluble pectins and other dissolved substance with different refractive indices from water (Holcroft and Kader, 1999). The TSS according to MacRae and Redgewell, (1992) may increase or decrease during postharvest storage as carbohydrate is utilized for respiration purposes and furthermore pectin solubilisation also contributes to TSS value. Fruit harvested at a later stage seems to have higher ˚ Brix value that is maintained even after harvest probably due to longer period of attachment to mother plant because of the constant supply of sugar translocation. However, fruit harvested at early stage will eventually increase but not to the extent of fruit left longer on tree (Le *et al.,* 2000).

Total sugar that was measured in D 0 fruit was higher than fruit harvested earlier. However following days after harvest, total sugar for D 0 fruit decreased as opposed to those harvested earlier which showed increased sugar level. The amount of total reducing

sugar in D 0 fruit accounts for around 80% to 90% of the overall total sugar compared to fruit harvested earlier. This indicates that the predominant sugars found in this fruit are glucose and fructose which coincide with the findings by Wu and Chen, (1997). Apart from that, cactus pear which is closely related to this fruit is also shown to contain glucose and fructose while sucrose is present in a very low amount (Cantwell *et al.,* 1995; Gurrieri *et al.,* 2000).

Organoleptic observation from this study indicates that *H. polyrhizus* were most palatable for fruit harvested between 4 to 5 weeks after anthesis suggesting that D 0 fruit. At the latter stage, the fruit had turned completely red, contains high TSS and sugar levels. In addition, shelf life of D 0 fruit was 13 days at room temperature confirms that non climacteric fruit can be stored for 14 days provided that it is harvested at the proper stage of ripening (Wang, 1997).

On the other hand, D 1 and D 2 fruits also showed changes in properties such as peel and pulp colour, firmness and also sugar level. However the changes only led to an inferior fruit. Therefore, D 0 fruit which kept its visual acceptance and marketing quality for more than one week would be suitable harvesting maturity. Apparently, harvesting fruit at earlier stages is possible but there is a cut off point such as low sugar level and non-attractive appearance which must be determined.

Following the determination of physiological and biochemical attributes of three different harvest maturity, experiment on the activity of the cell wall degrading enzymes were carried out in profiling the trend of the fruit softening during ripening of *H. polyrhizus*.