

Chapter 2: Literature review

2.1 Introduction to climbing cacti

Generally, cacti that can be consumed are divided into two groups; climbing cacti and columnar cacti based on the nature of stem habit. However, further classifications into different genera are according to the fruit description. Climbing cacti are perennial, epiphytic with triangular, fleshy, jointed green stems with aerial roots. Two genera that belong to the climbing cacti are *Hylocereus* and *Selenicereus* (Balerdi and Crane, 2005). The fruit of *Selenicereus megalanthus* are small and because of their peel colour, they are known as yellow pitayas (Mizrahi *et al.*, 1997). They are not grown commercially, except in Colombia. *Hylocereus* is a small genus that contains about 16 American species (Barthlott and Hunt, 1993) and has already received worldwide recognition as an ornamental plant for the large, scented, night-blooming flowers. Its fame is now spreading throughout the world for its fruit, especially in Israel, Vietnam and Australia.

2.2 *Hylocereus* varieties

Stem characterization is the most important feature followed by flower characteristics for the identification of *Hylocereus* species (Onecimo *et al.*, 2007). Among the known species are *H. purpusii* (Weing) with oblong fruit that is covered with large bracts and *H. ocamponis* (S.D) which is closely related to *H. purpusii*. *Hylocereus* species are mainly differentiated by the numbers of spines per areole and colour of the stem. Fruit of *H. trigonus* (Haw) is also oblong shaped but smaller in size while that of *H. costaricensis* (Web) is round and the stoutest of the genus (Le *et al.*, 2006). However, in Malaysia the two commonly cultivated *Hylocereus* species are *H. undatus* (Haw) (red peel with white pulp) and *H. polyrhizus* (Berger) (red peel with red pulp)

(www.doa.gov.my). There are considerable variation in fruit size and shape within the species. Fruit shape ranges from nearly round to an oblong shape with different bracts lengths.

2.2.1 Botanical description

2.2.1.1 Plant

H. polyrhizus as a climbing plant (Figure 2.1) is capable of living as an epiphyte on trees. Like any other cacti, it does not have leaves. The triangular-shaped, segmented and green succulent stems takes on the role of leaves. The segments have one row of small depressions along each of their three edges. These depressions are known as areoles and they occur only on cacti. They bear spines, branches, flowers and fruits. In *H. polyrhizus*, the segments are 15 to 60 cm long, areoles are spaced at 3 to 4 cm and in each areole there are 2 to 3 small spines. In other *Hylocereus* species the segments may be shorter or longer, the areoles closer or further apart, shallower or deeper, the spines fewer or more numerous and smaller or larger in size (Balerdi and Crane, 2005).



Figure 2.1 : Climbing cacti plant of *H. polyrhizus*

2.2.1.2 Floral

According to Le *et al.*, (2006) flowers of the genus *H. polyrhizus* appear under the areoles and in the shape of a funnel (Fig 2.2). The flowers are nocturnal and they only bloom for one night. The white flowers of *Hylocereus* species are hermaphrodite, large and extremely showy (Merten, 2004). The ovary is located at the base of a long tube carrying the foliaceous scales to the exterior. Flowers of this genus are either self incompatible as in *H. polyrhizus* or self compatible as in *H. undatus*. Differences between the pollination systems in the two *Hylocereus* species are due to its morphological differences in the position of anthers and stigma that prevent /allow automatic self pollination (Weiss *et al.*, 1994). Self compatible flowers are also autogamous and will set fruit without the need of pollen vector. Besides that, autogamous flowers possess anthers and stigma on the same level and touches as the flower closes (Merten, 2004). However, the only disadvantage to autogamous varieties is that the fruit is often smaller than if the flowers are cross-pollinated with pollen from

a different clone or different species. This is similar to what has been demonstrated in Israel where self-compatible clones when self pollinated produced smaller fruits than when cross pollinated (Nerd and Mizrahi, 1997; Lichtenzweig *et al*, 2000). The periods between the appearance of floral buds and flowering (1st Stage), and between flower anthesis and fruit harvest are very short (2nd Stage) : around 15 to 20 days for the first stage and 30 days for the second stage (Nerd *et al.*, 1999; Pusphakumara *et al.*, 2005).



Figure 2.2: *H. polyrhizus* flower

2.2.1.3 Fruit

Fruit of *H. polyrhizus* (Figure 2.3) that can weigh up to 800g develop from both the ovary and the receptacle surrounding the ovary and exhibit a positive correlation between weight of the fruit and numbers of seeds embedded within pulp (Weiss *et al*, 1994; Nerd and Mizrahi, 1997). The fruit become bright red in colour when mature, contain white, crimson or pale yellow flesh depending on the cultivar, have large bracts and numerous small soft black digestible seeds embedded within the pulp (Nerd and Mizrahi, 1997). When the lower part of a non-fertilized flower becomes yellowish, the flower falls off 4 to 6 days later, while for fertilized flower the lower part remains greenish and increases enormously in volume, indicating that the fruit has set (Le *et al.*, 2006).



Figure 2.3: *H. polyrhizus* or red dragon fruit

2.2.1.4 Taxonomy

The nomenclature of *H. polyrhizus* is as follows:

Kingdom	Plantae (plants)
Subkingdom	Tracheobionta (vascular plants)
Super division	Spermatophyta (seed plants)
Division	Mangoliophyta (flowering plants)
Class	Magnoliopsida (dicotyledons)
Order	Caryophyllales
Family	Cactaceae (cactus family)
Subfamily	Cactodeae
Tribe	Hylocereae
Genus	Hylocereus (Berger) Britt & Rose
Species	<i>Hylocereus polyrhizus</i> (Haw) Britt & Rose

Sources: Britton and Rose, 1963

2.2.2 Origin and distribution

Although the main origin in which most of the *Hylocereus* sp. came from is Latin America but they appear to also originate from the West Indies (Britton and Rose, 1963 ; Barthlott and Hunt, 1993). Presently, they are distributed all over the world within the tropics and subtropics and grown commercially in Nicaragua, Columbia, Vietnam, and Israel, with the Australian and US industries just beginning (Merten 2004). Furthermore, *Hylocereus* sp. is produced commercially in the Far East (Vietnam, Thailand, Malaysia and China) and marketed under the name ‘dragon fruit’ (Mizrahi and Nerd, 1999; Nobel *et al.*, 2002).

2.3 Commercial cultivation and production

As a fruit crop, 50% of the production in metric ton for both *H. undatus* and *H. polyrhizus* have been marketed in European market and 50 % in Asia market. The biggest producer in Asia is Vietnam followed by Nicaragua for Central America and Colombia for South America (Le *et al.*, 2006). It is also noted that 40% of the fruits imported into European market comes from Vietnam. According to Department of Agriculture in Ho Chi Minh City, the area for *Hylocereus* sp. plantation was about 13,500 hectares with a production of about 211,000 tones in the year 2007 (Nguyen, 2007).

Under the local name dragon fruit, *H. undatus* and *H. polyrhizus* are considered to be a promising new fruit crop in Malaysia. As shown in the Figure 2.4 below, the production of this fruit has increased since 2006 to a value of 8000 metric ton in year 2008. However, the area for cultivation of this fruit remains at about of 900 hectares.

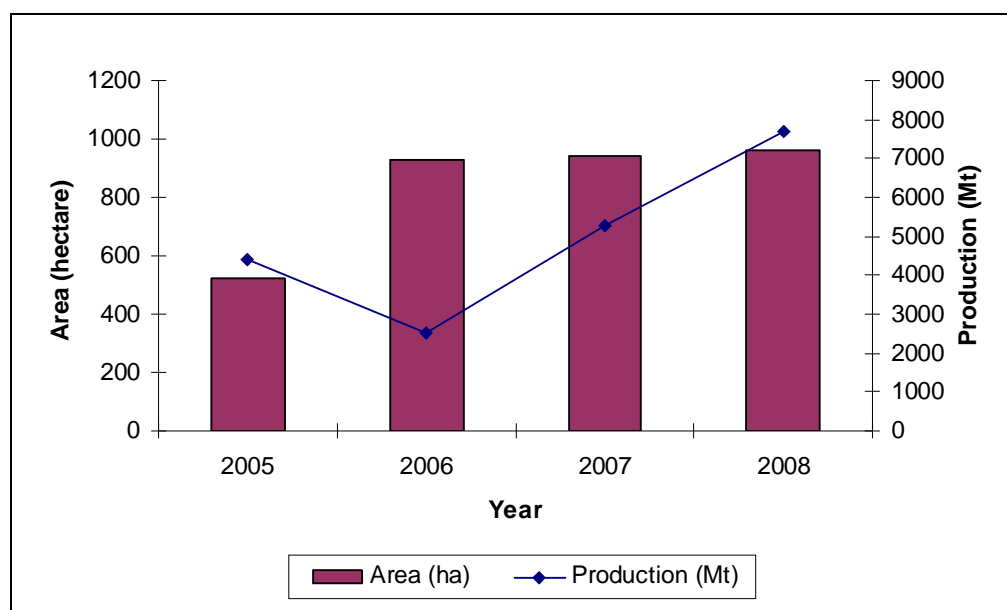


Figure 2.4: Area of Cultivation and Amount of Dragon Fruit Produced in Malaysia

Source : www.doa.gov.my

2.3.1 Commercial value of *H. polyrhizus*

Unlike the majority of fruit crops, plants of the *H. polyrhizus* grown in Vietnam and also in Nicaragua begin to produce significant crops two to three years after planting and reach full production after five years (Jacobs 1999). This result in quick returns for farmers who have a high initial setup cost for this type of crop. Furthermore, the fruit of *H. polyrhizus* are highly appealing because of their coloured flesh comparable to red beet (Stintzing *et al.*, 2002). The red pigment could replace the colourant from red beet which contains carcinogenic compound and undesirable flavour due to the presence of geosmin and pyrazine derivatives (Esquivel *et al.*, 2007). The stability of this colourant, known as betalain, in the pH range of 3 to 7 gives it a great potential in colouring a wide range of food. Besides that, betalain can also act as an antioxidant agent (Pedreno and Escribano, 2001). On the other hand, the fruit itself is nutritious (Table 2.1) and has high level of moisture and lower energy value than banana but the carbohydrate content is much lower than most other fruits (Le *et al.*, 2000). As reported by Ariffin *et al.*, (2008), the seeds of *H. polyrhizus* can also be beneficial as they contain essential fatty acids mainly linoleic acid that cannot be synthesized *in vivo*. Recently in Malaysia, it was reported that the bracts/peel of *H. polyrhizus* can be used as a value-added food ingredient for dietary purpose (Norziah *et al.*, 2008) and for fiber which is highly in demand.

Table 2.1: Nutritional value of *H. polyrhizus*

Component	Value (/100g edible portion)
Moisture (%)	84.00 – 86.00
Energy (kcal)	64.40– 75.30
Protein (g)	0.93 – 1.33
Fats (g)	0.40 – 0.73
Carbohydrates (g)	10.40 – 12.30
Fibre (g)	0.88 – 1.84
Calcium (mg)	8.00 – 15.60
Phosphorus (mg)	22.80 – 31.80
Sodium (mg)	5.00 – 13.50
Magnesium (mg)	29.50 – 42.40
Potassium (mg)	236.00 – 321.00
Iron (mg)	2.41 – 4.04
Zinc (mg)	0.26 – 0.38
Vitamin C (mg)	1.00 – 6.30
Vitamin A (mg)	0.005 - 0.018

Source : (Le *et al.*, 2000)

2.4 Fruit ripening

Fruit which are seed vessels or receptacles developed from a mature, fertilized ovary undergo a series of changes that occur from the stages of growth and development through to the early stages of senescence (Brady, 1987). This process is known as fruit ripening that involves major transitions in fruit development and metabolism. As a genetically-programmed process, fruit ripening culminates in dramatic changes in colour, texture, flavor, and aroma of the fruit flesh (Alexander and Grierson, 2002) leading to the development of a soft edible fruit of desirable quality.

2.4.1 Climacteric and Non climacteric fruit

In terms of ripening physiology, fruits are generally classified into two groups: climacteric, where ripening is accompanied by a peak in respiration and a concomitant burst of ethylene, and non-climacteric, in which respiration shows no dramatic rise and ethylene production remains at a very low level (Alexander and Grierson, 2002). A climacteric fruit can be distinguished from a non-climacteric fruit by analyzing this response to exogenous ethylene application. In climacteric fruit exposure to ethylene will advance the climacteric peak and autocatalytic production will continue even after the exogenous ethylene source is removed (eg. bananas, apple and tomatoes). On the other hand, non-climacteric fruit exposed to ethylene will exhibit a respiration rise but will soon fall immediately after the removal of ethylene (Watkins, 2002). In addition, fruit such as cactus pear and strawberry have low respiration rates in comparison to those of other common fruits (Cantwell, 1995). However, although ethylene is the dominant trigger for ripening in climacteric fruit, it has been suggested that both ethylene-dependent and ethylene-independent gene regulation pathways coexist to coordinate the process in climacteric and non-climacteric fruit (Lelievre *et al.*, 1997). It is known that dragon fruit is a non-climacteric fruit (Nerd *et al.*, 1999). Non-climacteric fruits are capable of ripening in the absence of increased ethylene synthesis (Giovannoni, 2004). Non-climacteric is also characterized by lack of starch as a carbohydrate reserve, hence no significant increase in sugar content of non climacteric fruit is observed after harvest (Tucker, 1996).

2.4.2 The Quest for a Ripening Index

Fruit quality and storage life can be affected by many factors; however the major factor is harvest maturity. Fruit harvested immature are more subjected to physiological disorder and normally ripen to an inferior quality than fruit harvested mature or ripe (Crisosto *et al.*, 1994; Kader, 2002) meanwhile overripe fruit will become very soft and has very short shelf life after harvest. Hence, immature or overmature fruit may not last long and more likely to develop physiological disorders than fruit harvested at proper maturity.

In general two different terms used to refer stages of fruit development are ‘mature’ and ‘ripe’ as stated by Reid, (2002). Physiologically ‘mature’ refers to fruit stage which will ensure proper ripening after harvest. As for postharvest technologists, they define ‘mature’ as a sufficient stage of development that will develop at least the minimum acceptable quality after harvesting and postharvest handling. Conversely, horticultural maturity has been defined as the stage of development at which a plant or plant part possesses the necessary energy substrates for consumption. Therefore, a fruit may be horticulturally mature at any stage of development. In order to determine proper time to harvest, many maturity indices have been developed.

Along with developing maturity indices, physical features such as size, colour, texture, titratable acidity and changes in total soluble solids and physiological features such as respiration and ethylene production have been employed (Reid, 2002). Similarly, the objective determination of harvest maturity on banana using peel colour index was reported by Chandran, (1998) that could describe the timing for banana to reach table ripe stage. Like any other fruits, dragon fruit undergoes a variety of physiological and

biochemical changes during ripening. However, a standard maturity index has yet to be developed for this new crop.

2.4.3 Physiological and biochemical changes during ripening

As ripening progresses, changes in skin and/or flesh colour occur from green to red and these changes are linked to chlorophyll degradation and synthesis/unmasking of pigment(s) readily present within the fruit (Speirs and Brady, 1991; Dolenc-Sturm *et al.*, 1999; Sturm *et al.*, 2003). In *H. polyrhizus*, which has a red–violet pulp, the development of peel colour is accompanied by an increase in the water-soluble pigment(s) in the pulp (Nerd *et al.*, 1999). Dragon fruit contains betacyanin pigments which are responsible for the colour of the peel and pulp (Forni *et al.*, 1992). The known betacyanin pigment of *H. polyrhizus* flesh belongs to betalain pigments (Wybraniec *et al.*, 2001, Wybraniec and Mizrahi, 2002). The first change in peel colour was recorded 26 to 27 days after anthesis and the peel turned fully red 4-5 days after the first colour change.

Pulp firmness decreased to less than 0.90 kg f 25 days after anthesis (Le *et al.*, 2000). Pulp firmness is determined mainly by the physical anatomy of the tissue, mainly cell size, shape and packing, cell wall thickness and strength and the extent of cell-to-cell adhesion, together with turgor status. During fruit ripening, there is a decline in turgor which contributes to textural changes (Shackel *et al.*, 1991; Harker and Sutherland, 1993), probably due partly to an accumulation of osmotic solutes in the cell wall space (Almeida and Huber, 1999), and to postharvest water loss from the ripening fruit. Besides turgor pressure, the changes of pulp firmness are also often attributed to the

enzymatic degradation and modifications of the pectin fraction which are the most common changes that take place during fruit ripening (Rodriguez, 2002; White, 2002).

The decrease of acidity with ripening is also a general behaviour in most fruits, including apples but excluding lemons (Marsh *et al.*, 2004; Blanco *et al.*, 1992). Organic acids show a decline during ripening in an ethylene independent manner, due to the utilization of organic acids in the tricarboxylic acid cycle, for respiration (Kader, 2002; Moretti and Sargent, 2002). There is a sudden surge of acidity in dragon fruit prior to colour change which indicates the beginning of ripening processes. Acidity of dragon fruit is high in the early stages of fruit development, which could be due to the process of biosynthesis of organic acids taking place resulting in an increased H⁺ concentration (Le *et al.*, 2000). However, the acidity of dragon fruit tends to decrease 25 days after anthesis, resulting in very low acidity which characterizes this fruit as a low-acid food (pH > 4.5).

In other fruits such as melons which are also in the market for its sweetness, the pH showed a declining trend in the first part of development after which it recovered to almost up to 6.05 (Villanueva *et al.*, 2004). Malic and citric acid are the most abundant acids in fruit with the exception of grapes and kiwifruit (*Actinidia deliciosa*) in which tartaric acid and quinic acid, respectively, represent the most abundant organic acids (Kader, 2002).

Apart from peel colour and acidity, total soluble solids (TSS) also show changes during ripening of fruit. TSS consists of organic acids, soluble pectins and other dissolved substances which have different refractive indices from water (Holcroft and Kader, 1999). The decrease in pulp firmness often leads to accumulation of soluble pectins and

hence to the increase of TSS value. According to Laskhminarayana *et al.*, (1979), cactus pear soluble solids levels are correlated closely with sugar content. The TSS value for dragon fruit increases from 5.4 °Brix up to 15.2 °Brix indicating that TSS increases during the early stages of fruit development and maintained thereafter (Le *et al.*, 2000) There is a significant increase in concentration of soluble solids during colour change reaching its maximum when the peel fully changes colour. This also correlates with the increase in soluble sugar concentration (Nerd *et al.*, 1999).

In dragon fruits, sugar concentration increased significantly during colour development reaching almost 8%-9% once fully ripen. Sugar and acids are the principle contributors to flavour in fruits and their ratio is often used as harvest and quality indices in different fruits (Kader, 1992). In general, during the ripening of fruits, the pulp accumulates sugar rapidly during the later stages of development (Barbera *et al.*, 1992; Kuti, 1992; Lakshminarayana *et al.*, 1979). This fact is well known by consumers and has been widely documented (Belitz and Grosch, 1987; Ackermann and Amado, 1992). However, the fruit sugar differs between the various parts of the fruit pulp with predominant soluble sugars being glucose and fructose; accounting to higher sugar content in core and lower sugar content at the peripheral part of the fruit pulp (Chang and Yen, 1997). On the other hand, sucrose accounts only 2.5 % to 7.8 % of the total sugar compared to glucose and fructose (Wu and Chen, 1997).

2.4.4 Cell wall degrading enzymes activity during ripening

As previously mentioned, fruit pulp firmness decreases during ripening. This is partly due to selective disassembly of the cell wall components and cell to cell separation which is very pronounced during fruit ripening and is thought to be a key ripening –

associated metabolic event that determines the timing and extent of loss of cell adhesion which leads to fruit softening (Goulao *et al.*, 2007). Although turgor loss and starch degradation and subsequent decline in its content during ripening might contribute, however, enzyme –catalysed changes to cell wall structure and composition are considered the major factor of softening of fruits (Lazan *et al.*, 1995). Attempt to understand the mechanism of fruit softening has led to the investigation of cell wall polymers, their compositional changes and the related cell-wall degrading enzymes during ripening (Knee, 1978).

Under the structural elements of the cell wall, three major classes of polysaccharides are cellulose, hemicellulose and pectins (Perez *et al.*, 2001; Willats *et al.*, 2001; Prasanna *et al.*, 2007) with pectic polysaccharides being the most abundant in fruit cell walls (Willats *et al.*, 2001). Fruit ripening and softening is mainly accompanied by the modification of the composition and amount of pectic and hemicellulosic polysaccharides which take place as a coordinated series of assembly and disassembly steps. Along with that, three major events in the cell wall that occur during ripening are solubilisation and depolymerisation of polyuronides (Brummell and Labavitch, 1997), depolymerisation of hemicelluloses (Maclachan and Brady, 1994) and the loss of some neutral sugars (Gross and Sams, 1984).

Despite the fact that fruits contain more or less similar make up of cell wall enzymes, the manner of the various cell wall carbohydrate components are modified. This suggest that there are subtle regulatory mechanism at the level of enzyme concentration, the type of enzymes isoforms present, and the timing of appearance of those different isoforms might be important in orchestrating wall disassembly and fruit softening (Ali *et al.*, 2004). The enzymes that regulate cell wall changes are generally classified as

pectolytic and non pectolytic enzymes. The pectolytic enzymes such as polygalacturonase (PG), pectin methylesterase (PME), pectate lyase (PL) cleave or modify the nature of the polysaccharide backbone or remove neutral sugars from branched chains. On the other hand, an example of non pectolytic enzyme is endo-1, 4- β -glucanase which is responsible for hemicellulase modifications (Guolao and Oliveira, 2008). Although the presence of these enzymes in various climacteric fruits have been correlated with softening, reports of such activities in non climacteric fruits are scarce (Nunan *et al.*, 2001).

Economically, fruit softening is an extremely important postharvest factor because physical injury that occurs during handling of fruits and their susceptibility to diseases increase proportionally with softening. It has been well established that texture changes in fruits are a consequence of modifications undergone by component polysaccharides that, in turn, give rise to disassembly of primary cell wall and middle lamella structures (Jackman and Stanley, 1995). Such disassembly has been postulated to be the consequence of hydrolytic enzyme activity on carbohydrate polymers (Seymour and Gross, 1996; Tucker, 1996). Experiments to modify the ripening profile of some fruits (especially tomato) through hydrolases activity regulation have demonstrated that the relationship between cell wall disassembly and texture may be very complex (Brownleader *et al.*, 1999).

2.4.4.1 Polygalacturonase (PG)

Polygalacturonase (PG) was thought to be the primary enzyme responsible for pectin breakdown and subsequent loss of firmness (Giovannoni *et al.*, 1991; Brummell and Harpster, 2001). PG is a hydrolytic enzyme which hydrolyses the α -1, 4-glycosidic

bonds between the galacturonic acid residues in galacturonans (Prasanna *et al.*, 2007). Rapid increase in PG activity during ripening along with increase of solubilisation of pectic substances and progressive loss of tissue firmness has been reported in many fruits (Brady, 1987; Fisher and Bennett, 1991; Tucker, 1996). It is believed that PG enzyme is involved in the breakdown of insoluble complex polysaccharides by reducing the length of the chains cross linked by calcium (Jayani *et al.*, 2005). Initiation of softening was shown to correlate with presence of PG in fruits like guava (El-Zoghbi, 1994), apple (Siddiqui *et al.*, 1996) and grape (Nunan *et al.*, 2001), banana (Ali *et al.*, 2004) and many more. As for PG activity in prickly pear, a non climacteric fruit, showed an increase in the activity at the mature green stage during storage (Armando *et al.*, 2002). However, PG activity that seems to mediate pectin degradation cannot alone account or not the sole determinant for the major changes in fruit texture during ripening (Gray *et al.*, 1992; Langley *et al.*, 1994).

2.4.4.2 Pectin methylesterase (PME)

Along with PG, another enzyme that also plays an important role in fruit ripening is known as pectin methylesterase (PME). PME is specific for galacturonide esters and its action is to remove methoxyl groups from methylated pectin by nucleophilic attack. Eventually this action results in the formation of carboxylate groups along pectin chain (Wong, 1995). A continuous spectrophotometric assay has been developed based on the reaction of PME on pectins in the presence of a pH indicator bromothymol blue (Hagerman and Austin, 1986) to determine the activity of PME enzymes. There are few studies that show in level of PME activity in peach decreased (Prabha *et al.*, 2000), or increased (Manganaris *et al.*, 2006) or remain constant (Hayama *et al.*, 2000) during ripening. Besides PME, other enzymes may also be involved in fruit softening. PME

activity was also detected in prickly pear in which the activity remained constant during storage of the mature green fruit (Armando *et al.*, 2002).

2.4.4.3 Pectate lyase (PL)

Originally thought as a microbial enzyme, pectate lyase has now been detected in plant species (Marin-Rodriguez *et al.*, 2002). This enzyme acts by depolymerising cell wall polysaccharides in the presence of calcium ions, thus destroying the integrity of the plant tissues (Barras *et al.*, 1994). Early studies to measure the presence of PL activity in tomato fruit proved unsuccessful (Besford and Hobson, 1972). However, more recently PL activity has been obtained directly from banana pulp with a substantial increase in activity during ripening (Marin-Rodriguez *et al.*, 2001; Imsabai *et al.*, 2005). Other reports have also shown that PL has an important role in fruit softening (Brummell & Harpster, 2001; Ishimaru and Kobayashi, 2002; Lohani *et al.*, 2004; Payasi and Sanwal, 2003). In strawberry the suppression of PL during ripening showed reduction of firmness loss (Bermudez-Jimenez *et al.*, 2002). PL activity was also found in non climacteric fruits such as grape berry (Nunan *et al.*, 2001) suggesting a role of this enzyme relevant to cell wall disassembly during ripening.

2.4.4.4 Cellulase

Plant cell walls contain a number of polysaccharides with 1, 4- β - D- glucosyl linkages, including cellulose. Plant enzymes that can hydrolyse such bonds at internal position in polysaccharides are referred as endo-1, 4- β -glucanases although they are also termed 'cellulase' by analogy from microbes that degrade cellulose. However, in most cases the endoglucanase activities that have been reported in ripening fruits should be referred to as CMCase, since the artificial substrate carboxymethylcellulase (CMC) has been

typically used (Ariel *et al.*, 2007). This enzyme may degrade cellulose and the β -1,4-glucan backbone of xyloglucan, a hemicellulosic polysaccharide prominent in walls of dicotyledons (Sethu *et al.*, 1996). An increase in cellulase activity has been reported in ripening strawberries (Woolley *et al.*, 2001), guavas (Abu-Goukh and Bashir, 2003) and mangoes (Roe and Bruemmer, 1981). However, there was no cellulase activity detected in pears (Ahmed and Labavitch., 1980). Activity of cellulase is also shown not correlated with an apparent cell wall modification thus, does not appear to be responsible for matrix glycan depolymerisation in either pepper or tomato (Harpster *et al.*, 2002a; 2002b). In prickly pear, the cellulase activity was reported to increase during storage of mature green fruits (Armando *et al.*, 2002). Nevertheless, the exact sequence of events and the contribution of each of these enzymes to softening in fruits are still not clear (Seymour *et al.*, 2002).

2.5 Postharvest consideration

In general, fruit which are allowed to ripen on the tree seems to have the best eating quality yet fruit are normally harvested mature but unripe in order to withstand postharvest handling and for long distance transportation purposes. The postharvest quality of a fruit is dependent to a large extent by its condition at harvest; hence harvest is the most critical starting point for postharvest management.

When fruit are harvested from the plant, they are still capable of respiring. Harvested fruit are self – sufficient with their own catalytic machine which means they will endure certain processes such as respiration, transpiration, biochemical changes, enzymatic reaction, senescence, microbial growth and also mechanical injury during handling of postharvest, eventually leading to postharvest losses (Burdon and Sexton, 1993). Thus,

proper postharvest managements need to be introduced in order to reduce postharvest losses and to maintain fruit quality during postharvest (Kader, 2002) and also prolong the shelf life of the fruit. Shelf life in this aspect means the time it takes for a produce or fruit to deteriorate under specific storage condition during postharvest management.

2.5.1 Storage temperature

There are many factors that can be regulated during postharvest management. In fact, processes such as respiration, enzymatic reactions, and microbial growth can be regulated by optimizing the environmental conditions; temperature, relative humidity (Jobling, 2001) and atmospheric composition (Yahia, 1998). However, temperature regulation seems to be the most important factor in maintaining fruit quality (Lee and Kader, 2000). This is because most of the biochemical functions are temperature dependent. To start with, temperature has a direct impact on shelf life due to high respiration rate. A 10°C increase in temperature will eventually double up respiration rate and therefore shorten the shelf life of fruit (Kader, 2002). Apart from that, temperature also plays a key role in preventing growth of microorganisms. At a high temperature, microorganisms grow at a faster rate and causes postharvest rots to the fruit meanwhile at a low temperature growth can be reduced and there after quality of the fruit can be assured (Brown, 1986). As a result low temperature has been introduced to maintain postharvest fruit quality. Despite the fact that low temperature storage being the most effective postharvest approach for extending shelf life, the negative impact of low temperatures and chilling injuries have been studied in many crops. This is because temperature beyond the appropriate range for the fruit will cause metabolic dysfunction that will result in uneven ripening, poor flavour, internal breakdown and sunken areas and discolouration (Perez Tello *et al.*, 2001).

However, optimum storage temperature varies according to fruit depending on the species or cultivar, preharvest cultural practices and also the ripening stage of the fruit. Fruit originated from a temperate climate can be stored at near 0°C meanwhile fruit of tropical and subtropical origin are recommended to be stored between 6°C and 13°C (Crisosto *et al.*, 1995; Thompson, 2003). Although temperature can maintain fruit quality but improper storage temperature will cause adverse effects on fruit quality such as discolouration, increased decay and ultimately leading to higher rate of deterioration.

Few studies have been done on dragon fruit in regulating storage temperatures. According to Nerd *et al.*, (1999), *H. polyrhizus* and *H. undatus* harvested close to full colour kept their visual acceptance and marketing values at 6°C for 3 weeks , 14°C for two weeks and 20°C for 1 week. As for yellow dragon fruit, *Selenicereus megalanthus* harvested at colour break and as well as advanced colour stage showed lower weight loss and better quality for those harvested at advanced colour stage. Although, fruit harvested at colour break achieved the physical properties as fruit ripen on tree, their sugar level and acidity were inferior as reported by Nerd and Mizrahi, (1999). Nevertheless, effect of harvesting fruit at earlier time period should be examined to have a better understanding of this new crop.

2.5.2 Modified atmosphere packaging

Besides temperature regulation, another important decisive factor of fruit postharvest storage is modified atmospheric (MA) composition which involves modification of oxygen and carbon dioxide concentration surrounding the fruit to a level different from the air (Kader and Watkins, 2000). The two main principles of creating modified

atmosphere are by introducing specified gas mixture actively or by simply using permeable films that creates the modified atmosphere passively or in other words attains gas mixture naturally (Kader *et al.*, 1989). Basically, modified atmosphere packaging (MAP) is introduced by sealing fruit in polymeric bags with relatively low permeability to gas exchanges (Reddy *et al.*, 1992) that impedes the diffusion of oxygen to the fruit.

Kader (1995), have reported that appropriate gas permeability of the film will ensure oxygen entering the package at rate offset by the consumption of oxygen by the fruit while expelling carbon dioxide from the package to compensate the production of carbon dioxide by the fruit. There are many readily available plastic bags; however polyethylene bags are the main film used for packaging fruit and vegetables (Kader, 2000). Polyethylene film are regarded the proper film due to certain characteristics such as low permeability to vapour, anti fogging properties and easy to seal (Batu, 1994).

The purpose of achieving this atmospheric condition is to ultimately reduce respiration rate and as direct effect, delay ripening-associated changes such as texture losses, chlorophyll degradation that leads to fruit senescence (Yahia, 1998). The suitable combination of commodity and film bag permeability will create MA within the package that can maintain postharvest fruit quality, although gas concentrations in the bags during storage cannot be controlled precisely. Furthermore, MAP is also capable of maintaining high relative humidity, and also inhibits spoilage microorganism growth (Babic and Watada, 1996). This is because the permeability of the film used will provide sufficient transfer of water vapour that accumulates as a part of respiration product or the anti fogging (Greengrass, 1993) characteristic of the film that prevents microbial growth.

As a type of MAP, film packaging has become a technique which has brought major changes in storage, distribution and marketing of products to meet consumer demands. However, exposure to oxygen or carbon dioxide levels outside the limits of tolerance may lead to anaerobic respiration with the production of undesirable metabolites and other physiological disorders (Soliva-Fortuny *et al.*, 2002). Too low oxygen atmospheres may trigger anaerobic metabolism in fresh fruits and result in an increase in fermentation (Kader and Ben Yehoshua, 2000).

Nevertheless, creation of MA alone in maintaining postharvest fruit quality is not sufficient enough but rather requires suitable storage temperature within the specified range because temperature is a factor that determines the respiration rate. Fundamentally, MAP is regarded as supplement and not as a replacement for proper storage temperature during postharvest management (Kader, 1986). Hence, film packaging with proper film permeability and storage temperature play roles together in maintaining postharvest fruit quality.

2.6 Scope of studies

The main objectives of this study are to study physiological and biochemical changes associated with different harvest maturities of *H. polyrhizus*. Apart from that the activity of cell wall degrading enzymes during ripening of *H. polyrhizus* will be monitored. In addition to that, investigation will be carried to determine the effect of storage temperatures on physiological and biochemical properties of *H. polyrhizus*. Finally, the effect of film packaging in extending the shelf life of *H. polyrhizus* will be conducted.