Chapter 1: Introduction

1.1 Introduction

Carica papaya L. cv. Eksotika II is one of the economically important fruits of Malaysia. It is a hybrid variety (Line 19 x Eksotika) and was developed by the Malaysian Agriculture Research and Development Institute (MARDI). Eksotika II papaya is produced not only for domestic trade but is also being actively exported to Singapore and Hongkong, the main importers of the Malaysian papayas.

However, to export, it is not only necessary to achieve quality standards, but also, phytosanitary standards. The marketing of the fresh Eksotika II papaya can be disrupted by the presence of pests. Fruit fly infestation is one of the major pest problems that limit the export of papaya fruit to other potential distant markets such as China, Japan, USA, Australia and New Zealand. The oriental fruit fly, *Bactrocera dorsalis* Hendel, is one of the most important pest species that contributed to the quarantine significance in the ASEAN region. In order to expand the Malaysian papaya market to those countries that require quarantine treatment, hot water treatment has been used as one of the disinfestation procedures. Nevertheless, the success of such treatment depends on the physiological and biochemical tolerance of the fruit to high temperatures and whether the treatment is effective in killing insect pests without causing heat damage to the fruit.

Other than that, maturity stage at harvest can influence fruit sensorial quality. For example, bananas harvested at more advanced maturity stages had better consumer acceptance (Ahmad *et al.*, 2001). In addition, papayas harvested at mature green stage do not achieve the full organoleptic properties of a ripe fruit harvested at more advance stage (Lam and Zaipun, 1987; Halimi *et al*, 1990). Maturity stages at harvest also affect

the biosynthesis of volatile compounds in mangoes, which are responsible for fruit flavour (Lalel *et al*., 2003). Therefore, knowledge on how the ripening physiology, biochemistry and quality of Eksotika II papaya are affected by maturity stages at harvest is essential to avoid poor quality fruit. Poor quality fruit is one of the limiting factors for expanding the papaya market.

1.2 Problem Statement

Quarantine restrictions exist for Malaysian exported produce by several importing countries. Currently, major export markets of Malaysian papayas are to countries which do not impose stringent Sanitary and Phytosanitary (SPS) measures. These countries include Hong Kong, Singapore, Brunei, Taiwan, Indonesia, Middle East (Saudi Arabia, Iran, United Arab Emirates) and European Union (EU) countries. In order to expand the market access of Malaysian papayas to China (one of the countries that require stringent quarantine requirement), the Department of Agriculture, Malaysia has made a bilateral negotiation with China to request for market access. Therefore, the export of Malaysian papayas to China is approved by the China quarantine authorities with the condition that the Malaysian papaya exporters should fulfil all the SPS requirements (DOA, 2011). In order to export to China, Malaysian papaya exporters should have the phytosanitary certificate and the papayas exported must be treated using hot water treatment (HWT) to ensure that the papayas are free from fruit flies infestation. However, the HWT procedure must be effective in disinfestation of fruit flies and must be audited and approved by the Chinese authorities. As for now, China has audited and approved three HWT facilities in Malaysia. Subsequently, the exported papayas must by pass the confirmation of disinfestation in Malaysia and inspection at the point of entry in China.

Consequently, the HWT procedure used in this study is the one which has been approved by China and was patented in China in September 2007 (Appendix 1). However, the effect of the approved HWT on the quality of the Malaysian papayas has not been determined. Hence, in this study, the effects of the HWT on fruit quality, biochemical changes and the cell wall degrading enzymes activity of the Eksotika II papaya were investigated, so that the Malaysian papayas will reach their intended markets with high eating quality.

1.3 Objectives of the Study

The objectives of this study are:

1. To investigate whether the hot water treatment applied has any effect on the quality of Eksotika II papaya by monitoring physiological and biochemical changes associated with ripening.
2. To investigate the effect of harvest maturity on the physiological and biochemical properties of Eksotika II papaya during ripening following hot water treatment.
3. To determine the effect of hot water treatment on fruit softening and cell wall degrading enzymes activities of Eksotika II papaya during ripening.

Chapter 2: Literature Review

2.1 Introduction

Papaya is an economically important fruit in many tropical and subtropical countries. Besides providing food to the people, the crop has potential to be exploited as an income generator in many countries. This can be accomplished by exporting and strategic marketing of the fruit. According to FAO (2010), the global production of papaya in the year 2008 was about 10.1 million tonnes. From this, 36 % was produced by India, which is the largest papaya producer with the total production of 3.6 million tonnes, followed by Brazil, Nigeria, Indonesia and Mexico. The top world papaya producers in 2008 are given in Table 2.1. The total world production of papaya has increased progressively from 8.9 million tonnes in 2006 to 10.1 million tonnes in 2008, underlining the economic importance of papaya in the international commodity market.

As shown in Table 2.2, the major exporters of papaya in 2008 are Mexico, Brazil, Belize, Malaysia and India. A lot of the harvest in some countries is not exported, particularly in Southeast Asian countries, and the fruit are consumed or traded locally. However, most of the papayas produced in Malaysia are exported and in 2008, only 47.5 % of the Malaysian papayas were traded for local market (FAO, 2010). In Malaysia, commercialization of Malaysian papayas especially Eksotika and Eksotika II has successfully reached the international market. There are fairly good markets for the fruit in Singapore, Hong Kong, China and the Middle East and imports of the fruit appear to be growing annually in these countries. In the year 2008, the export value of Malaysian papayas was about US$ 9.4 million, which is 11.9 % increase from the export value of the previous year which is about US$ 8.4 million (FAO, 2010).

Table 2.1: Top world papaya producing countries in 2008.

Country	Production (Tonnes)
India	3,629,000
Brazil	1,890,286
Nigeria	765,000
Indonesia	653,276
Mexico	638,237
Ethiopia	260,000
Congo	223,770
Colombia	207,698
Thailand	201,099
Guatemala	184,530
Philippines	182,907
Peru	167,387
Venezuela	128,020
China	120,359
Bangladesh	103,609

(Source: FAO, 2010)

Table 2.2: Top world papaya exporters in 2008.

Country	Exports (Tonnes)
Mexico	90,316
Brazil	29,986
Belize	28,967
Malaysia	24,168
India	13,834
Guatemala	9,794
United States of America	9,031
Netherlands	7,596
Côte d'Ivoire	6,382
Philippines	4,971

(Source: FAO, 2010)

2.2 Papaya (*Carica papaya* L.)

2.2.1 Botanical Description and Chemical Composition

Papaya or its scientific name, *Carica papaya* L., is believed to be native to tropical America, perhaps to southern Mexico and neighbouring Central America. However it is now cultivated in many tropical and subtropical countries around the world. Its classification is as follows: Division: Magnoliophyta, Class: Magnoliopside, Subclass: Dilleniidae, Order: Violales and Family: Caricaceae. A recent taxonomic revision proposed that family Caricaceae consists of six genera namely, *Carica,* with one species, *Cylicomorpha* with two species, *Jacaratia* with seven species, *Jarilla* with three species, *Horovitzia* with one species and *Vasconcellea* with 21 species. The genus *Carica* is characterized by a unilocular ovary and is represented by a single species, *Carica papaya* L., which is the most important economic species within the family Caricaceae (Badillo, 2000).

The papaya is a perennial herbaceous plant, bearing fruit continuously at the leaf axils, spirally arranged along the single erect trunk. It is a pantropical, small, soft-wooded, laticiferous, fast growing and short-lived tree (Wiart, 2000). Common names for papaya trees include betik (Malaysian and Indonesian), pawpaw (Australia and New Zealand), kepaya, papayer (French), pappali (Tamil), mugua (Chinese), melonenbaum (German) and lechosa (Spanish) (Tietze, 2001; Chan and Paull, 2008). In some areas, an unrelated plant, *Asiminia triloba* (Annonaceae), native to North America, is also called pawpaw (OGTR, 2003).

The papaya tree grows upright from 3 – 8 m high and under special circumstances even up to 10 m, terminating with a crown of large leaves. The stem is semi-woody, hollow and the bark is smooth but marked by prominent half-moon-shaped leaf scars. The tree normally grows without branching, unless the growing point is injured (Chan and Paull, 2008). The tree only has leaves on the top part of the trunk, similarly placed as palms in a spiral fashion. The stems of the leaves are from 30 cm up to 1 m long. The leaves themselves have mostly 5 to 9 main fingers with a diameter of between 30 and 70 cm (Tietze, 2001).

There are three primary groups of papaya flowers namely staminate (male), pistillate (female) and hermaphrodite, with many variants especially in the hermaphrodite group. Flowers are borne on modified cymose inflorescences that appear in the axils of leaves, pale yellow and slightly fragrant. The type of inflorescence depends upon the sex of the tree (Chan and Paull, 2008). The staminate trees produce long, pendulous, many flowered cymose inflorescence. The flower is unisexual and lacks a functional pistil. The pistillate trees have inflorescence with few flowers that have large pistils without stamens. Hermaphrodite trees normally produce bisexual flowers but are sexually variable since they are sensitive to changes in the environment which can switch on and off their reversion to either staminate or pistillate flowers. Warm night temperature and water stress induce the production of male flowers, while cool temperatures transform the stamens into fleshy carpel-like structures. Selection seems to have favoured hermaphroditism since perfect flowered types are common among cultivated papayas, but naturally occurring populations are uniformly dioecious with male and female flowers on separate individuals (Persley and Ploetz, 2003).

Papaya is a tropical plant and is very sensitive to cold. Optimum temperature for growth is between 21°C and 33°C. If the temperature falls below 12 – 14°C for several hours at night, growth and production are severely affected (Nakasone and Paull, 1998). Plantations should be situated in warm sheltered sites with windbreaks to maximize yield and fruit quality. Papaya can be grown on a variety of soil types with the most essential requirement being drainage, since poor drainage results in major water logging and root rot problems (Nakasone and Paull, 1998). The plant can tolerate soil pH between 5.0 and 7.0; however the range between 5.5 and 6.5 is optimum. Papayas grow well and produce substantial yields without supplementary irrigation if there is a minimum monthly rainfall of approximately 100 mm. Since most tropical areas have monsoon-type climates with well-defined wet and dry seasons, successful production depends upon the availability of supplemental irrigation during the dry period (Chan and Paull, 2008)

Fruit hang from the stalks attached to the upper trunk, below the old leaves, with the younger fruit above those more mature. The papaya fruit is a fleshy berry weighing from 200 g to well over 10 kg. Fruit shape is a sex-linked character and ranges from round to ovoid in female flowers, to long cylindrical or pyriform (pear-shaped) in hermaphrodite flowers. The skin of the fruit is thin, smooth and green when immature, turning yellow to strong orange when ripe. The fruit is normally composed of five carpels united to form a central ovarian cavity that is lined with the placenta carrying numerous black seeds. The seeds in the ripe fruit are easy to remove (Chan and Paull, 2008). The flesh is thick, succulent and easily bruised. Flesh colour is white in immature fruit, turning yellow to orange to red depending upon cultivar when ripe. Table 2.3 shows common papaya varieties in commerce and breeding worldwide.

Table 2.3: Common papaya varieties in commerce and breeding.

Variety	Origin	Average fruit size, Notable traits	Fruit characteristics (e.g. shape, colour)
Bettina	Australia (Florida Betty x Queensland var.)	1.36-2.27 kg	Round-ovoid. Well-coloured.
Cariflora	Florida, USA	0.8 kg. Tolerant to PRSV.	Round. Dark yellow to light orange flesh.
Coorg Honey Dew [H]	India	2-3.5 kg	Long to ovoid. Yellow.
Eksotika [H]	Malaysia (Sunrise Solo × Subang 6)	0.6-0.8 kg	Elongate (from hermaphrodite). Orange-red flesh.
Eksotika II [H]	Malaysia (Eksotika lines 19 × 20)	0.6-1.0 kg	Higher yield than Eksotika. Fewer freckles on skin, and sweeter than Eksotika.
Sekaki [H]	Malaysia	1.0-2.5 kg	Long, cylindrical, with smooth skin. Red, firm flesh.
Hortus Gold (selection: Honey Gold)	South Africa	1 kg Propagated by cuttings.	Round-ovoid. Golden yellow.
Known You 1 [H]	Taiwan	1.6-3 kg. Tolerant to PRSV.	Very long and slender. Yellow flesh.
Maradol	Cuba	2.6 kg	Elongate. Green or yellow skin.
Rainbow [H]	Hawaii, USA (SunUp × Kapoho Solo)	0.65 kg. Transgenic resistance to PRSV.	Pear-shaped to ellipsoid. Yellow-orange flesh.
Red Lady 786	Taiwan	1.5-2 kg. Tolerant to PRSV.	Elongate. Red flesh.
Red Maradol	Mexico	2.5-2.6 kg	Red flesh; yellow orange skin.
Solo [H]	Developed in Hawaii, USA; from Barbados originally.	0.5-1 kg. Bisexual flowers highly selfing.	Pear-shaped (from hermaphrodites). Orange-yellow skin; golden orange flesh.
Kapoho Solo [H]	Hawaii, USA	0.45 kg	Pear-shaped, but shorter neck than Sunrise Solo. Orange-yellow flesh.
Sunrise Solo [H]	Hawaii, USA	0.57 kg	Pear-shaped. Reddish pink flesh.
Tainung 1 [H]	Taiwan	1.1 kg	Pointed blossom-end (from hermaphrodite). Red flesh.

[H] Hermaphrodite variety (*i.e.*, gynodioecious)
(Source: OECD, 2005)

Ripe fruit are sweet and have delicate aroma. Flavour and odour can range from pleasantly aromatic as in the 'Solo' and 'Eksotika' varieties to undesirably musky as in the 'Maradol' variety. Muskiness is due to the homozygous recessive allele of a single gene (Storey, 1969). The papaya is cultivated mainly for its fruit and it is favoured by the people as breakfast, dessert and as ingredients in jellies or cooked in various ways. The juice also makes a popular beverage among the people of the tropics. However, other papaya plant parts such as the shoots (young leaves) and flowers are also eaten as vegetables (Ong, 2004).

Papaya plant parts contain many biologically active compounds, selections of which are listed in Table 2.4. Papaya contains potassium, calcium, vitamins A, C and carpaine. Carpaine is used to slow down the heart and thus reduces blood pressure. However, higher doses of carpaine can produce vasoconstriction. Papaya belongs to a group of plant species known as laticiferous plants. These plants contain specialised cells (laticifers), dispersed throughout most of the plant tissues and secrete a substance known as 'latex'. Latex is a complex mixture of chemical compounds with diverse chemical activities. Collectively, these compounds are thought to be involved in the defence of the plant against a wide range of pests and herbivores (El Moussaoui *et al.*, 2001).

The latex of papaya plants is rich in cysteine proteinases, which are used widely for protein digestion functions in the food and pharmaceutical industries. Commercially, papaya latex is harvested from mature but unripe fruit, which the skin contains numerous laticifers. Ripe papaya fruit contains no latex (Villegas, 1997), probably because the latex-producing cells stop functioning or breakdown with age.

Cysteine proteinases may constitute as much as 80% of the enzyme fraction in papaya latex (El Moussaoui *et al.*, 2001). The most well studied proteinases from papaya are papain, chymopapain, caricain and glycyl endopeptidase. Papain is a proteolytic enzyme which soothes the stomach and is used to relieve indigestion. Other known enzymes from papaya latex include glycosyl hydrolases such as β-1,3-glucanases, chitinases and lysozymes, protease inhibitors such as cystatin and glutaminyl cyclotransferases and lipases (El Moussaoui *et al.*, 2001).

Table 2.4: Some chemical compounds occurring in *Carica papaya* L.

Chemical	Main plant part in which chemical occurs	Recorded level/concentration
Alkaloids	leaves	1,300 – 1,500 ppm
Carpaine	leaves	150 - 4,000 ppm
Dehydrocarpaines	leaves	1,000 ppm
Flavonols	leaves	0 - 2,000 ppm
Tannins	leaves	5,000 - 8,000 ppm
Nicotine	leaves	102.8 ppm
Prunasin (cyanogenic glycoside)	leaves	No data
Benzylglucosinolate	latex	116,000 ppm
Caoutchouc	latex	45,000 ppm
Chymopapain a & b	latex	No data
Lysozyme	latex	No data
Malic acid	latex	4,400 ppm
Papain	latex	51,000 - 135,000 ppm
Butanoic acid	fruit	1.2 ppm
Carpaine	fruit	200 ppm
Cis-and trans- linalool oxide	fruit	250 - 2,238 ppm
α-phellandrene	fruit	No data
α-terpinine	fruit	No data
Caricin	seeds	No data
Benzyl-isothiocyanate	seeds	2,000 - 5,000 ppm
Carpasamine	seeds	3,500 ppm
Linoleic acid	seeds	5,389 ppm
Oleic acid	seeds	193,545 - 202,400 ppm
Palmitic acid	seeds	28,791 - 30,107 ppm

(Source: OGTR, 2008)

2.2.1.1 *Carica papaya* L. cv. Eksotika II

Eksotika II is a newer Malaysian hybrid variety than Eksotika (Eksotika is a hybrid of the local Subang 6 variety with the Sunrise Solo, and formerly known as Line 20). It was released by the Malaysian Agricultural Research and Development Institute (MARDI) on 15 October 1991. Eksotika II was developed after 7 years of breeding and selection. It is a result of hybridization between two purelines, Line 19 and Eksotika (Chan, 1993).

Trees of Eksotika II (Figure 2.1) show heterosis, more vigorous and taller than the Eksotika. They have the character of precocity or earliness to fruiting where fruit are borne 60 – 90 cm from ground level about 42 – 44 weeks after seed sowing. The ratio of female trees in Eksotika II may be higher than Eksotika because the seeds are usually obtained from hybridization of female flowers with hermaphrodite pollen as compared with Eksotika whose seeds are developed from self-pollination (hermaphrodite x hermaphrodite). Such seeds produced from hermaphrodite x hermaphrodite cross will cost more because of additional work in removing stamens. However, there is evidence that it is still economical to use hermaphrodite flowers for seed production because of the higher proportion of hermaphrodite fruit which bring higher prices (Chan *et al*., 1994).

Fruit of Eksotika II are similar in shape and appearance to the Eksotika, however, the fruit size is larger, weighing between 600g and 800g. The appearance of the Eksotika II fruit is more attractive, with smooth skin and less susceptible to freckles. The skin of Eksotika II is thicker with firmer flesh and deeper hue of orange-red than Eksotika. The Eksotika II papaya has high TSS value (12 – 14%), and it has longer postharvest shelf

life, making it more preferred than its predecessor for export. In several trials over different locations, Eksotika II consistently yielded better than both its inbred parents (Chan, 1992). The mean yield of the Eksotika II over a two-year crop cycle ranged from 40 – 100 tonnes per hectare which was about 25 % better than the Eksotika. The Eksotika II is a F_1 hybrid, therefore its pure seeds cannot be propagated by growers. Seeds of Eksotika II can only be obtained by crossing the two inbred parents, Line 19 and Eksotika. Presently, seed production for this hybrid is undertaken by MARDI and the Department of Agriculture, Malaysia.

Figure 2.1: Tree of *Carica papaya* cv. Eksotika II

2.2.2 Commercial Cultivation and Production

2.2.2.1 Economic and Commercial Value of Eksotika II papaya

The trade of Malaysian papaya was limited only to local market until the Malaysian Agriculture Research and Development Institute (MARDI) introduced the Eksotika and Eksotika II cultivars. Nowadays, the Eksotika II is successfully exported to the international market. Eksotika II produces good, uniform quality fruit which are readily accepted in many markets and this certainly has led to market expansion of the fruit in recent years. Currently, Eksotika II is being exported to Singapore, Hong Kong and China where the prices are very lucrative. This cultivar is also exported to Indonesia, Brunei, United Arab Emirates, Netherlands, Saudi Arabia and Taiwan (Table 2.5). Besides, Malaysian papayas are also accepted in United Kingdom (UK) market. However, in UK, papayas are mainly from Brazil, India and Pakistan. Malaysia's exports were minimal, around 1% of the total papayas imported by the UK in the year 2007 (FAO, 2010).

The advantage in papaya cultivation is the rapid return on investment and high yield. Fruit in the tropics can be harvested 8 or 9 months after seeds sowing and since papaya is non-seasonal, the plant continuously flowers and sets fruit. Therefore harvesting takes place all year-round. In Malaysia, papaya is cultivated mainly in Johor and in the central parts of Peninsular Malaysia. As shown in Figure 2.2, papaya production volume in Malaysia has increased from 32,800 tonnes in 2006 to a value of 45,813 tonnes in year 2009. The area for papaya cultivation also is increasing from 2117 hectares in year 2006 to 3648 hectares in 2009. The increase in production volume and planted areas of papayas shows that in Malaysia, papaya is among the most important tropical fruit crop which has high economic value that can contribute to the country's income.

Table 2.5: Top Malaysian's papaya importers in 2007

No.	Country	Quantity (Tonnes)	Value (RM '000)
1	Singapore	18, 636.50	12,100
2	Hong Kong	6,408.42	12,898
3	China	778.20	1,447
4	Indonesia	526.53	566
5	Brunei Darussalam	251.20	160
6	United Arab Emirates	247.46	1,350
7	Netherlands	45.03	143
8	Saudi Arabia	40.67	192
9	Taiwan	34.78	30
10	Others	3.64	8
	Total	**26,972.43**	**28,894**

(Source: DOA, 2010)

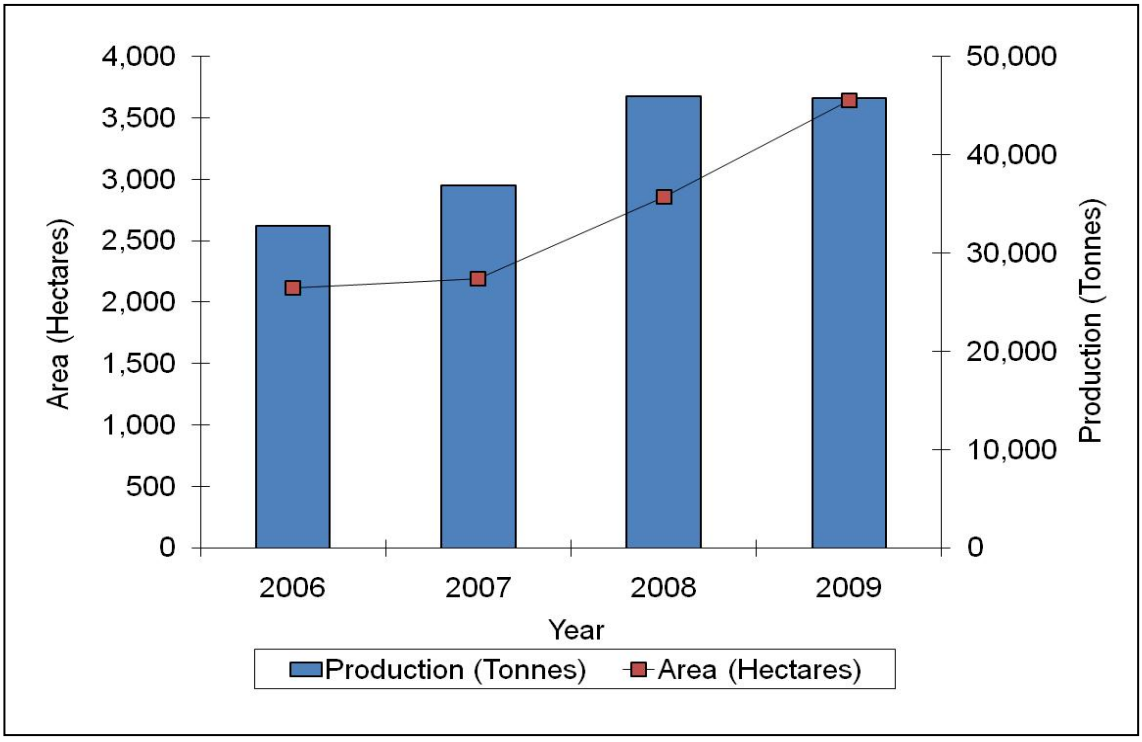


Figure 2.2: Planted area and production of papaya in Malaysia (2006 – 2009) (Source: DOA, 2010)

Most of the papaya is produced for fresh consumption. Only a small portion is used for processing of fruit cocktails, fillers for chilli sauces, cakes and pickles. Papaya has been planted in commercial farms ranging from 10 to 30 hectares involving the private sector, individual entrepreneurs and government agencies. The government gives incentives to the farmers as an encouragement to plant the papaya on a commercial basis. The farmers are given the Pioneer Status and the Investment Tax allowance to help enhance the development of the fruit industry.

However, there are many problems and constraints which prevent rapid development of the papaya industry in Malaysia. One of the major problems is the unavailability of suitable land, especially in Peninsular Malaysia. The existing crops are on marginal land, which is steep and small in area, and therefore, not suitable for commercial cultivation of papaya. Moreover, there is competition from other commercial crops such as rubber, oil palm and cocoa. Another problem is the structure of the papaya industry which is based on smallholder mixed orchards which is unorganized and scattered all over the country. This leads to lack of uniformity in the size and quality of fruit which enter the market. Diseases and pests of papaya fruit in Malaysia also is one of the major problems faced by the Malaysian papaya industry nowadays. Papayas require disinfestation procedures for export. This hinders exports to overseas markets where quarantine requirements are stringent. At present, Malaysian fresh papayas cannot be exported to Japan, United States of America, Australia and New Zealand because of the stringent postharvest import quarantine regulations required by the countries.

2.3 Fruit Ripening

2.3.1 Physiological and Biochemical Changes During Ripening

Fruit ripening involves major changes in physiology and biochemistry, which physically alter their colour, flavour, texture and aroma. It is where a fruit develops its maximum desired eating quality. Ripening marks the completion of the development of a fruit and the commencement of senescence, and it is normally an irreversible event. Fruits which are categorized as climacteric are evidently dependent on the rise of respiration and increased ethylene production for the initiation of the ripening process (Fischer and Bennet, 1991). However, fruits, which are immature or unprepared to commence with ripening, fail to ripen correctly (Malar and Nair, 1992). Therefore, the quality of a postharvest ripened fruit is determined by many factors, of which the stage at harvest is the most important. Papaya should be harvested at the proper stage of maturity so that the fruit will ripen normally and develop good flavour and taste. The index of commercial maturity usually involves some expression of the stage of development (or maturation) and requires a measurement of some known characteristic. This is done either objectively or subjectively or a combination of both. Several indices are used to determine fruit maturity such as change in skin colour, time from anthesis, change in texture of flesh, change in specific gravity and change in chemical composition (Mohd Salleh *et al.*, 1989).

A systematic method of describing colour indices for papaya has been described by Lam and Zaipun (1987). The colour index of the fruit is given a numerical value according to the maturity of the fruit (Figure 3.1). Change in skin colour is commonly used by commercial growers. The degree of yellowing is visually determined and harvesting will depend on the market destination. Fruit for distant markets should be

harvested at colour index 2. At this stage the fruit can be kept longer. Fruit with colour index 1 are not suitable for table consumption because they are still green and will not ripen properly (Lam and Zaipun, 1987; Malar and Nair, 1992). Fruit harvested at colour indices 4 and 5 are suitable only for local markets.

Ripening of papaya is also characterized by a loss of tissue firmness. From a horticultural perspective, tissue firmness is an important quality attribute and the rate of firmness loss during ripening may influence not only fruit quality but also its storage life. Loss of firmness is mainly due to the modification of structure and chemical composition of the cell wall carbohydrates of the fruit tissue (Huber, 1983a). Since a very small amount of starch was detected in papaya (Selvaraj *et al.*, 1982), the contribution of starch breakdown to fruit softening may be negligible.

The characteristic flavour of a fruit is contributed mainly by the type and level of volatile compound present in tissue. However, the concentrations of sugars, organic acids as well as phenolic compound also give a significant contribution to the sensory component of the fruit. Loss of moisture through transpiration results in fruit shrivelling, shrinking and decreasing in weight. Eksotika papaya loses about 12% of the original weight if allowed to ripen at ambient (25˚C) temperature at about 80% relative humidity (Lazan *et al.*, 1990).

Chlorophyll fluorescence is a non destructive measurement technique that can be performed relatively fast with great precision. It can be performed by minimally trained personnel, making it ideal for applied research (DeEll *et al.*, 1999). The primary event of photosynthesis in all green plant tissue is the absorption of light by chlorophyll molecules. This process will cause the energy level of chlorophyll to be raised and thus

electrons are transferred into higher energy orbital. Most of this excitation energy of chlorophyll (~85%) is transferred to the reaction centres of the photosystem and is used to drive the reactions of photosynthesis. However, some excitations energy is also lost as heat and as fluorescence, as the electron moves back to ground state (DeEll *et al*., 1999). The fluorescence of green plants, approximately 3 – 5% of total excitation energy (Walker, 1985), is almost exclusively emitted by chlorophyll *a.* The characteristic pattern of fluorescence emitted from chlorophyll *a* of a dark adapted tissue upon re-illumination is known as the 'Kautsky Effect', named after the researcher who first conducted detailed studies on the phenomena.

Chlorophyll fluorescence parameters are widely used as indicators for functional changes of photosynthesis apparatus under wide variety of stresses including temperature stress (Yamada *et al.*, 1996; Weng and Lai, 2005). A change in Fv/Fm is due to a change in the quantum efficiency of PSII and is used as a sensitive indicator of plant photosynthetic performance. The optimal value of Fv/Fm is around 0.83 measured for most plant species (Björkman and Demmig, 1987; Johnson *et al.*, 1993). The fruit ripening process may affect chlorophyll fluorescence yield by loss of photosynthetic activity and decrease in chlorophyll content which will reduce PSII activity and increase chlorophyllase activity, respectively (Smillie *et al.*, 1987; Tucker, 1993). A decline in chlorophyll fluorescence during the ripening of banana and mango is normally related to the loss of chlorophyll content and chloroplast competence (Smillie *et al*., 1987). Moreover, Blackbourn *et al.* (1990) reported that they observed a drop in the Fv/Fm parameters with advancing ripening and senescence in bananas. Similarly, Bron *et al.* (2004) reported that during papaya ripening, the Fv/Fm ratios decreased indicating that chlorophyll fluorescence which is closely associated with chloroplast function, declined in parallel with the decline of other quality characteristics during papaya fruit ripening.

2.3.2 Cell Wall Degrading Enzymes Activities during Ripening

Fruit ripening involves changes in the composition and organization of pectin, hemicelluloses and cellulose polysaccharides of the cell wall, which take place as a coordinated series of assembly and disassembly steps (Goulao *et al.*, 2007). The plant cell wall is a highly complex and dynamic structure composed of a network of hemicelluloses linked to cellulose microfibrils, embedded in a matrix of pectic polymers and other less abundant compounds, like phenols, structural proteins and enzymes (Brett and Waldron, 1996). Due to the nature of the polymers, a large number of linkages exist within the cell wall, maintaining and reinforcing its structure. Thus various families of enzymes and their different isoforms are suggested to affect these processes. Cell wall modifying enzymes that act on the linkages between cell wall polymers are usually classified as pectolytic and non-pectolytic, according to the specific class of polysaccharides used as substrate (Goulao and Oliveira, 2008). Among the pectolytic or pectin degrading enzymes are polygalacturonase (PG) (endo- and exo-type), pectin methylesterase (PME) and pectate lyase (PL). These enzymes are able to cleave or modify the nature of the polysaccharide backbone or to remove neutral sugars from branched side chains (Figure 2.3). Furthermore, among the non-pectolytic enzymes include the cellulases namely endo-1,4-β-glucanases, exo-1,4-β-glucanases and glucosidase. The non-pectolytic enzymes are responsible for cellulose and hemicellulose modification.

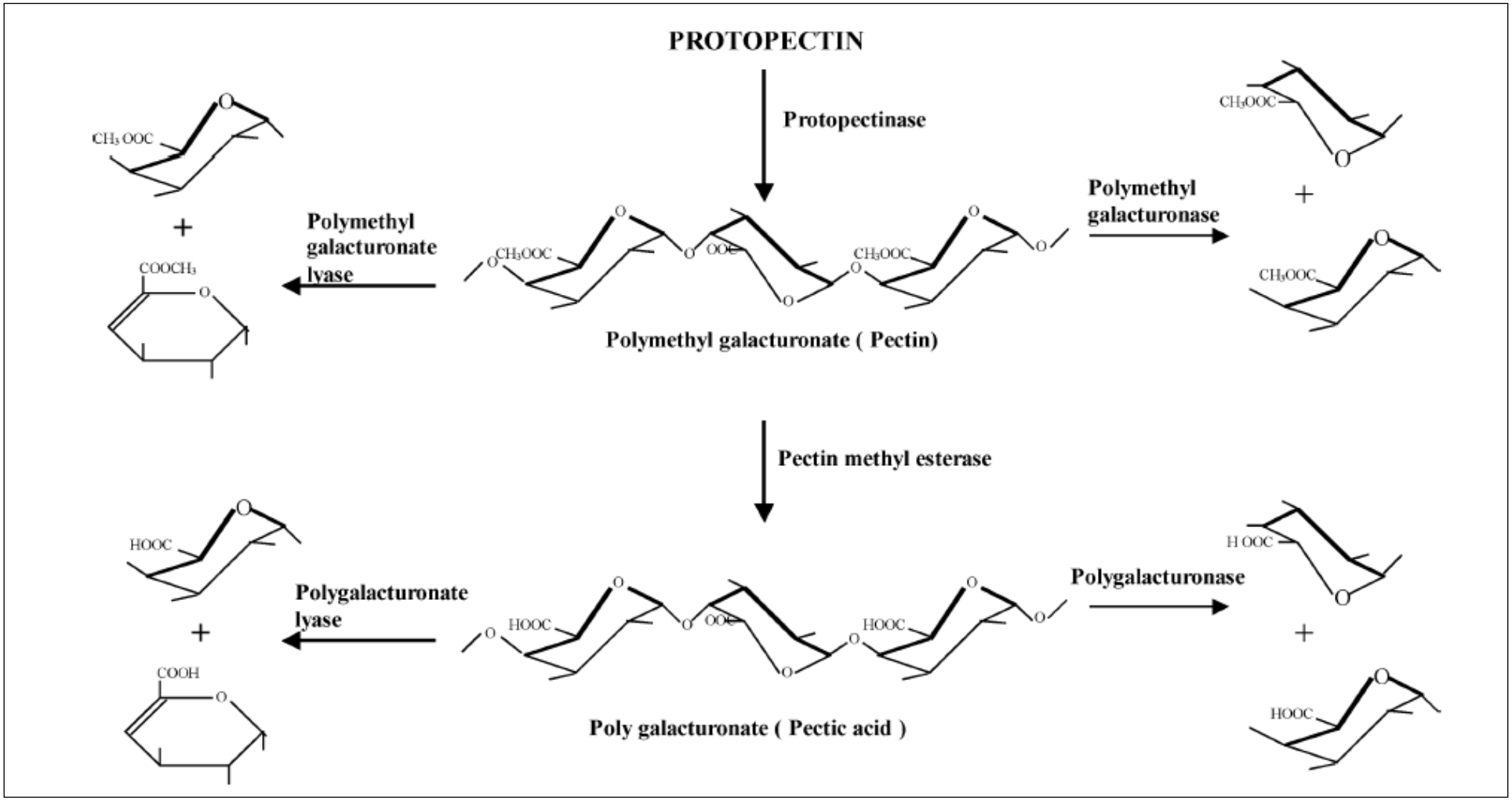


Figure 2.3: Mode of action of pectin degrading enzymes (Adapted from Prasanna *et al.*, 2007)

2.3.2.1 Polygalacturonase (PG)

Polygalacturonase is a hydrolytic enzyme which acts on pectic acid (polygalacturonic acid, PGA). PG catalyzes the hydrolytic cleavage of galacturonide linkages, and can be of the exo- or endo- acting types based on their mode of action. The exo- type (EC 3.2.1.67) remove single galacturonic acid unit from the non-reducing end of polygalacturonic acid, while the endo type (EC 3.2.1.15) cleaves such polymers at random. The substrate for PG in the cell wall is mainly homogalacturonans, which are secreted to the cell wall in a highly methyl-esterified form which must be de-esterified before they can become a substrate for PG (Brummel and Harpster, 2001). In climacteric fruits, whose texture alters considerably during ripening, a maximum loss of firmness was directly correlated with a rapid increase in PG (Roe and Bruemmer, 1981; Abu-Sarra and Abu-Goukh, 1992). PG activity also has been reported to increase during ripening of papaya fruit (Paull and Chen, 1983; Ali *et al*., 2004). Moreover, Payasi and Sanwal (2003) found that PG activity in bananas increased during fruit ripening and continued until the postclimacteric phase.

2.3.2.2 Pectin Methylesterase (PME)

Polygalaturonans (pectins) are secreted to the cell wall in a highly methyl-esterified form, and are de-esterified during cell development. This is accomplished by pectin methylesterase (EC 3.1.1.11), which de-esterifies polyuronides by removing methyl groups from the C6 position of galacturonic acid residues of high molecular weight pectin. Demethylation of pectin to their free carboxyl groups changes the pH and charge in the cell wall, allows the aggregation of polyuronides into a calcium-linked gel structure, and makes the polyuronides susceptible to degradation by PG (Brummel and Harpster, 2001). 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). PME activity has been shown to decrease in mangoes (Abu-Sarra and Abu-Goukh, 1992; Ketsa *et al*., 1998; Ali *et al*., 2004) or increase in papayas (Paull and Chen, 1983; Ali *et al*., 2004; Thumdee *et al*., 2010) during fruit ripening. PME activity was also detected in banana in which the activity was sustained at high levels during ripening (Ali *et al*., 2004).

2.3.2.3 Pectate Lyase (PL)

The lyases or *trans* eliminases cleave the glycosidic bond by *trans β*-elimination mechanisme, i.e., elimination of hydrogen from the C-4 and C-5 position of the aglycone portion of the substrate (Whitaker, 1984). Pectate lyases (EC 4.2.2.2) catalyses the cleavage of de-esterified or esterified galacturonate units by a trans β-elimination of hydrogen from the C-4 and C-5 positions of galacturonic acid. Exo-PL acts from the non-reducing end, whereas endo-PL acts randomly on de-esterified galacturonans (Prasanna *et al.*, 2007). Cleavage by PL requires the presence of calcium ions and generates oligosaccharides with unsaturated galacturonosyl residues at their non-reducing ends (Marín-Rodríguez *et al*., 2002). PL activity in bananas has been reported to increase during ripening (Marín-Rodríguez, 2001; Payasi and Sanwal, 2003; Lohani *et al*., 2004). The role of PL in ripening fruit has been addressed mainly based on the observation that there is mRNA accumulation of putative PL transcript in ripening strawberries (Medina-Escobar *et al.*, 1997) and bananas (Marín-Rodriguez *et al.*, 2003). Moreover, strawberry fruit suppressed in PL mRNA expression resulted in significantly firmer fruit than controls where the highest reduction in softening occurred during the transition from the white to the red stage. However, at the stage of full ripen, no differences were observed between the antisense strawberry and controls (Jiménez-Bermúdez *et al.*, 2002). These results become the reasons why this enzyme family became a strong candidate to be investigated in other species.

2.3.2.4 Cellulase

Cellulase is a multienzyme system composed of several enzymes namely endoglucanase (EC 3.2.1.4), exo-glucanase (EC 3.2.1.91) and glucosidase (EC 3.2.1.21). Endoglucanase hydrolases the β-1,4-link between adjacent glucose residues at random positions. Exo-glucanase breaks the bond at non-reducing ends of the chain, producing glucose or cellobiose (dimmers of β-1,4-linked glucose), whereas β-glucosidase splits cellobiose into glucose molecules (Prasanna *et al.*, 2007). Endo glucanase or Endo-β-1,4-glucanase (EC 3.2.1.4), *in vitro* are active against xyloglucan, cello-oligosaccharides, non-crystalline cellulose and the CMC (carboxymethyl cellulose). In most cases, the endoglucanase activities that have been reported in ripening fruits should be referred to as CMCase, since the artificial substrate CMC has been typically used (Vicente *et al.*, 2007). Cellulase activity increased during the ripening of avocado, peach, strawberry, tomato, and papaya (Hobson, 1981; Paull and Chen, 1983). The loss of firmness, climacteric rise of respiration and ethylene evolution in ripening fruit have been directly correlated with marked increase in cellulase activity (Roe and Bruemmer, 1981; Abu-Sarra and Abu-Goukh, 1992). However, in papaya, it has been reported that cellulase activity increases before the rise in ethylene production and therefore does not correlate with the climacteric increase in respiration (Paull and Chen, 1983). Cellulase activity also has been reported in several Indian mango cultivars, where its activity increased during ripening (Selvaraj and Kumar, 1989).

2.4 Postharvest Impediment: Fruit Fly Infestation

Fruit flies of the family Tephritidae are among the most economically damaging insect pests, with many causing severe phytosanitary problems in international trade (White and Elson-Harris, 1992). Worldwide, about 70 tephritid species are considered to be important pests of agricultural products, with many others ranked as minor or potential pests. The most economically important belong to five genera: *Bactrocera*, *Anastrepha*, *Ceratitis*, *Dacus* and *Rhagoletis*. The most significant genus, *Bactrocera*, contains about 40 pest species, including the Oriental fruit fly (*Bactrocera dorsalis*) and Asian papaya fruit fly (*Bactrocera papayae*). The oriental fruit fly, *Bactrocera dorsalis* Hendel, is one of the most important pest species of quarantine significance in the ASEAN region. The flies attack papaya, banana, mango, sapota, rambutan and other tropical fruits. Due to the fruit fly infestation, the Malaysian papayas and most of the other tropical fruits such as mangoes and bananas are banned from entering Japan, Australia, New Zealand and the USA. The female oriental fruit fly deposits its eggs in the ripe flesh of the fruit and the emerging larvae cause damage. Egg lay is a problem only when the fruit have 25% or more skin yellowing (Seo *et al*., 1982). The Asian papaya fruit fly, *Bactrocera papayae*, a major polyphagus species recorded from 193 species, is native to South-East Asia, invaded Queensland in 1995 (Hancock *et al*., 2000) and was subsequently eradicated. *B. papayae* is a very destructive pest where the females can lay eggs in green papayas, young bananas and citrus fruit. Female *B. papayae* has long ovipositor allowing it to penetrate past the sap layer of green fruits. Both of this fruit fly species, *B. dorsalis* and *B. papayae* are among the major cause of the quarantine significance in the ASEAN region.

2.4.1 Common Postharvest Heat Treatment to Overcome the Fruit Flies Problem

Fruit fly quarantine treatments usually involve killing eggs or larvae that are developing inside the living host. This system requires that the insect obtain a lethal treatment inducing very high (usually sufficient to kill 99.9968% of pests) mortality while the plant tissue is minimally affected (Shannon, 1994). Additionally, the treatment must have reasonable cost and minimally burden the marketing system. Various methods have been devised for applying heat to fresh commodities in order to kill quarantined pests which may be infesting the commodities. Commercially available heat treatments fall into two general categories depending on the carrier of the heat, air or water. Water is a more efficient and uniform carrier of heat compared to air, by having a high surface heat transfer coefficient (Stewart *et al*., 1990). Major heat treatments methods that have been used commercially are vapour heat treatment, forced hot air treatment and hot water treatment.

Vapour heat treatment is a method of heating fruit with air saturated with water vapour at temperatures of 40 - 50°C to kill insect eggs and larvae as a quarantine treatment. Heat is transferred from the air to the commodity by condensation of the water vapour (heat of condensation) on the relatively cooler surfaces of the fruit being treated. Fruit may be gradually heated over time to a target temperature, which may be at the end of the treatment (where all insects have been killed) or held for a specific time (holding time) required to kill all insects (Armstrong and Mangan, 2007). Gradual heating of fruit is more desirable than rapid heating in order to prevent damage to the fruit (Armstrong, 1994). Commercial facilities operate in many countries, mainly for use on tropical fruits such as mango and papaya. For example, vapour heat treatment for papayas from Thailand has been approved for the export of the fruit to Japan. Vapour heat to a pulp temperature of 46°C followed by a holding period of 20 minutes at 46°C

has been used as a quarantine treatment for papayas in Thailand prior to exportation to Japan (Armstrong and Mangan, 2007).

Forced hot air, also known as high-temperature forced air, is a modification of the vapour heat treatment. It was developed by Armstrong *et al*. (1989) to kill Mediterranean fruit fly, melon fly and oriental fruit fly eggs and larvae in papaya. Forced hot air and vapour heat are essentially the same treatment method, except that fruit surfaces are wet during vapour heat and dry during forced hot air. Laidlaw *et al*. (1996) observed that forced hot air treatment provide better fruit quality than either hot water immersion or vapour heat treatment methods. Forced hot air became the treatment of choice for the Hawaiian papaya industry for fruit exported to the US mainland and for papaya exported from Fiji to New Zealand (Armstrong and Mangan, 2007). Japan, USA and New Zealand have each developed commercial forced-air treatment systems primarily for the treatment of fruit against tephritid fruit flies (Heather and Hallman, 2007).

2.4.2 Hot Water Treatment

Hot water treatment has been used for insect disinfestation for fresh produce. It has been widely used since hot water is a more efficient heat transfer medium than hot air (Shellie and Mangan, 1994), when properly circulated through a load of fruit, a uniform temperature profile is established in the bath. Longer treatment time is needed for disinfestation procedure because not just the surface has to be brought to the proper temperature, but the core temperature of the fruit should also be raised to the same temperature.

Generally, the treatment consists of harvesting fruit less than one quarter ripe and then the fruit load is immersed in a hot water tank at a selected temperature and time. The temperature of the fruit is monitored during the treatment where thermometers are inserted in one or two fruit to different depths. The time of immersion can be one hour or more and temperatures are usually below 50°C. An example of a hot water treatment that was easily adopted for commercial use is the two-stage hot water immersion for the mature green papaya fruit. This hot water treatment was developed for disinfesting papaya of fruit fly eggs before export from Hawaii (Couey and Hayes, 1986). The hot water immersion phases of the treatment consisted of immersing papaya in 42°C water for 30 – 40 minutes, followed immediately by a second immersion in 49°C water for 20 minutes. The fruit were then hydro-cooled to ambient temperatures and dried before sorting and packing. Hot water immersion treatment equipment is relatively easy to engineer, whereas forced hot air and vapour heat treatment equipment requires complicated computer programmes to operate and monitor the treatment parameters and equipment. Besides, these treatment facilities are relatively expensive compared with hot water treatment equipment (about US$120,000 for a chamber that will treat 8 tonnes of fruit such as papaya at a time). Hot water treatment equipment only costs about one-third of that forced hot air or vapour heat treatment equipment to treat the same quantity of fruit (Armstrong and Mangan, 2007). Hot water treatment remains a viable treatment that should be investigated to be used as a quarantine treatment for most of fresh commodities because of its broad efficacy against pests, relative low cost and simple ease of application.

2.5 Effects of Heat Treatment on Fruit Ripening

Postharvest heat treatments of fruits are used for insect disinfestation, disease control, to modify fruit responses to other stresses and maintain fruit quality during storage (Lurie, 1998). Postharvest heat treatments lead to an alteration of gene expression and fruit ripening can sometimes be either delayed or disrupted. Heat treatments can cause a variety of physiological damage to the commodities, including surface lesions, pitting, scalding, loss of aroma, surface and internal discolouration, unusual softening, loss of shelf life and a predisposition to postharvest decay organisms (Paull and McDonald, 1994).

The transfer of plants to an elevated temperature produces stress. The severity of stress is primarily determined by the temperature differential and the duration of exposure (Lurie, 1998). The influence of heat on postharvest fruit ripening is dependent upon (i) level of field-induced thermo-tolerance, (ii) cultivar, (iii) fruit size and morphological characteristics, (iv) physiological state (stage of ripeness), (v) heat transfer rate and energy balance (thermal difference, heat capacity and relative humidity), (vi) final temperature, and (vii) the duration of exposure at different temperatures (Paull and Chen, 2000). The role of cultivar in heat responses has been less well studied, though differences are found for grapefruit (Miller and McDonald, 1991), and observations suggest ‘Kapoho’ papaya is more susceptible than ‘Sunrise’ (Paull, 1995), while no difference was found for two apples varieties (Klein and Lurie, 1990).

Fruit softening is often slowed down following exposure to heat treatments although disinfestation procedures for mangoes and papayas of forced hot air for 4 hours at 50°C led to faster softening than the controls after the treatment (Shellie and Mangan, 1994).

In tomato, heat stress interfered with PG accumulation (Yoshida *et al.*, 1984) and inhibited the normal increase in soluble polyuronides (Mitcham and McDonald, 1992). In papaya, PG activity was reduced after exposure to heat treatments (Chan *et al.*, 1981; Lazan *et al.*, 1989). Moreover, papaya does not recover the ability to soften after heat shock exposure (Paull and Chen, 1990). The softening disruption has been ascribed to the reduction of cell wall hydrolytic enzymes. Although heat disruption of cell wall breakdown has been proposed as the cause for delayed or poor softening, the actual enzyme having the central role in softening has not been determined (Lashbrook *et al.*, 1998; Rose *et al*., 1998).

Furthermore, flavour characteristics of fruits can be affected by a heat treatment. Titratable acidity declines in apples held for 3 or 4 days at 38°C while soluble solids concentration is not affected by the treatment (Klein and Lurie, 1990). In some commodities, the sugar content is favourably affected by heat treatment. For instance, 3 hours immersion in 45°C water before cool storage of muskmelons prevented the loss in sucrose which occurred to the non-heated fruit during storage (Lingle *et al.*, 1987). Other than that, heat treated buttercup squash at 30°C hot air was perceived as sweeter by a taste panel (Bycroft *et al.*, 1999). Moreover, heat treatment leads to an accelerated rate of degreening in apples (Klein *et al*., 1990). However, colour changes of papaya skin or flesh were not affected by hot water immersion treatment at 42°C for 30 minutes followed by 49°C for 90 minutes (Paull and Chen, 1990).

Researchers have empirically developed many procedures to reduce the injury caused by heat treatment, most of which seem to rely on the heat shock response (Paull and Mc Donald, 1994). Bacteria, plants and animals exposed to 34 – 42°C acquire transient thermotolerance within 30 minutes, a unique group of proteins is synthesized (Paull,

1990). It has been suggested that an immediate response of high temperature, generally temperature above 35°C, is disassociation of polyribosomes and then a reassocciation of some ribosomes into polyribosomes which preferentially translate the mRNA of heat shock proteins (HSP) (Ferguson *et al.*, 1994). This response both down-regulates normal protein synthesis, even without degradation of the mRNAs and upregulate HSP synthesis (Lurie, 1998). Although heat shock response is known to occur in many fresh commodities, researchers have spent several decades investigating the metabolic pathways for inducing thermotolerance. Induction of heat tolerance in papaya has been reported by Paull and Chen (1990). Holding the papaya fruit at 38 – 42°C for 1 hour reduces damage after hot water treatment at 49°C for 70 minutes. Conditioning the fresh commodities in 37°C or 39°C air before a 46°C or 47°C hot water treatment reduces damage on avocados and mangoes (Joyce and Shorter, 1994; Jacobi *et al*., 1995). Chan and Linse (1989) increased thermotolerance in cucumbers in order to apply a hot water treatment against fruit flies. They found that preconditioning cucumbers at 32.5 ± 0.5°C in air for 24 hour increased tolerance to hot water immersions of 30 – 60 minutes at 45°C and 30 – 50 minutes at 46°C. The response of plant tissues to elevated temperatures involves the accumulation of HSP, which can help protect against a further elevation in temperature that would generally be harmful. In some cases, the presence or persistence of HSP can indicate the resistance of the tissue to a further stress, but it is unclear whether HSP alone can be used as markers to determine whether an organism will be sensitive or resistant to a high-temperature stress. Further work in this area is needed, particularly on fresh commodities subjected to the quarantine treatments in order to minimize heat damage to the commodities.

Chapter 3: Physiological Properties of Hot Water Treated and Untreated Eksotika II Papaya

3.1. Introduction

Ripening is a complex process which occurs during the final phase of fruit development through senescence, when a fruit experiences a series of physiological and biochemical changes. The papaya fruit is classified as 'climacteric' by Biale *et al.* (1954) and has been demonstrated to exhibit the characteristic burst in CO_2 and ethylene production during ripening (Halimi *et al*., 1990; Fabi *et al.*, 2007). The postharvest physiology of the ripening papaya fruit can be affected by cultivar, environmental conditions and also by harvest time. Harvest time is essential to obtain high quality fruit with storage potential (Bron and Jacomino, 2006). The response of the fruit to the postharvest environment is affected by the stage of ripeness at detachment. The papaya harvest maturity index is often determined by visual assessment of the fruit's skin colour which is used commercially as a harvest index criterion to assure adequate ripening and maximum shelf life. Although some effort has been made to develop objective methods to determine papaya harvest maturity index (Forbus *et al*., 1987; Basulto *et al.*, 2009), it can vary for different cultivars. Therefore, one of the objectives of the present study is to develop objective maturity indices that can function as standard harvest indices for this cultivar.

The Eksotika papaya is the flagship variety of Malaysian's papaya industry which has been exported both by refrigerated sea reefers and air freight to its major market in Singapore, Hong Kong and China. The export trade to the Middle East countries and Europe is also increasing. However, disease and pests of the papaya fruit in Malaysia have become a major problem which prevents the rapid development of the industry in

Malaysia. Fruit flies (Diptera: Tephritidae) infestation is the major pest problem which hinders exports of fresh Eksotika papaya fruit to overseas market where quarantine requirements are stringent. Thus, effective disinfestation procedure must be developed to overcome this problem.

Heat treatment has been used in many fresh produce to delay softening, insect disinfestation, disease control as well as to maintain fruit quality during storage (Lurie, 1998; Paull, 1990). Postharvest heat disinfestation has emerged over the past decade as a viable non-chemical control method for fruit flies in papaya (Couey and Hayes, 1986; Couey, 1989). In Malaysia, hot water treatment is applied by the producers and exporters of papayas as a disinfestation procedure because it is cost effective compared to hot air treatment which is more expensive. However, the optimum condition for the commercial use of this procedure has not been standardized and there are several concerns regarding the quality and the appearance of the treated fruit.

Therefore, the objective of this study is to evaluate physicochemical parameters that would be important to determine the overall quality of Eksotika II papaya. The physiological changes of hot water treated and untreated Eksotika II papayas harvested at different maturity stages during ripening will also be evaluated in order to determine the optimal harvest maturity index of Eksotika II papayas for hot water treatment.

3.2. Materials and Method

3.2.1 Plant Material (Fruit)

Index 1, 2 and 3 papaya (*Carica papaya* L. cv. Eksotika II) fruit of uniform size and free from external defect were harvested from a private farm at Trosor, Sungai Siput, Perak. The papayas were harvested according to a standard colour chart described by Lam & Zaipun, (1987) based on visual assessment of the peel colour (Figure 3.1). For the Eksotika papaya, it is often difficult to distinguish Index 1 (mature green) fruit from green immature fruit that are similarly sized. Therefore, to avoid incorrect harvesting, Index 1 fruit in the present study is identified as fruit directly above an Index 2 fruit along the stem spiral (Malar and Nair, 1992). After harvest, fruit were packed in cartons and transported for 5 hours to the laboratory. Soon after arrival in the laboratory, the fruit were rinsed with tap water and air dried. Fruit of each ripening stage were divided into two groups and subsequently one group was treated with hot water at 47±1°C for 10 minutes in the laboratory following a commercial procedure applied by the fruit exportation industry in Malaysia (see 3.2.2). The other group of papayas was left untreated and acted as control. All the fruit were left to ripen at ambient temperature (25 ± 0.1 °C).

Five fruit were sampled at different ripening indices. Determination of the ripening index was done subjectively based on visual assessment according to standard colour chart (Lam and Zaipun, 1987). By referring to the colour chart, the progress of ripening can be assigned into six developmental stages or indices. However, fruit at colour Index 6 were too soft and prone to disease infection. Therefore, all the measurements were only carried out until the fruit reach colour Index 5. Visual characteristics of the fruit were assessed daily from the commencement to termination of experiment.

For subsequent biochemical and protein analyses, pulp tissues of the fruit were cut into block sized pieces, frozen under liquid nitrogen and stored at -20°C. The Eksotika II papayas used in this study were referred to as below:

H1 : Harvested at Index 1 (Mature green fruit)

H2 : Harvested at Index 2 (Green with trace of yellow)

H3 : Harvested at Index 3 (More green than yellow)

Index 1 of H1 fruit, Index 2 of H2 fruit and Index 3 of H3 fruit in these series of experiments are equivalent to fruit at day one after harvest.

Colour Index	Skin Colour
1	Full green
2	Green with trace of yellow
3	More green than yellow
4	More yellow than green
5	Yellow with trace of green
6	Fully yellow

Figure 3.1: Colour indices of *Carica papaya* L. cv. Eksotika (Lam and Zaipun, 1987)

3.2.2 Hot Water Treatment

Fruit were immersed in a room temperature (RT) water bath (25°C) and thermometers were inserted in one or two fruit to different depths, to monitor the core temperature of the fruit. The water bath was then set at 47°C and the temperature was constantly monitored by thermometers and never rose or fell 1°C above or below the set temperature during the treatment. When the bath temperature and the core temperature of the fruit reached 47°C (Appendix 2), the fruit were left for another 10 minutes to fully eliminate the fruit fly larvae. Subsequently, the fruit were cooled in a RT water tank for about 5 minutes and were air dried. The whole treatment procedure took approximately 60 – 90 minutes.

3.2.3 Measurement of Physiological Properties

3.2.3.1 Skin Colour Measurement (L*a*/b*)

Peel colour index of the fruit samples was objectively determined using a Chromameter (Minolta CR 200, Japan) in L*a*/b* co-ordinates in the Munsell Colour System. The colour was evaluated by measuring *L* (brightness, 100 = white, 0 = black), *a* (+, red; -, green) and *b* (+, yellow; -, blue) (McGuire, 1992). L*a*/b* values at nine different areas near the stalk end, middle and the blossom end of the papaya fruit were measured and the average recorded. Colour measurement was taken from day one after harvest until the fruit reached colour Index 5. The L*a*/b* values against days after harvest were plotted. Following this objective colour measurement, the fruit were re-classified into six indices according to the proposed maturity indices values (Table 3.1) to compare its effectiveness with the subjective visual observation method.

3.2.3.2 Visual Observation

Fruit were visually assessed for peel colour changes on a scale of 1-6 according to the colour chart described by Lam and Zaipun, (1987) (Figure 3.1) for the determination of maturity stages.

3.2.3.3 Weight Loss

Weight (water) loss was expressed as percentage of fresh weight against initial weight at harvest. Fruit weight was taken daily using an electronic balance (Mettler PJ3000, Highstown, NJ). The values of weight loss against days after harvest were plotted.

$$\text{Weight loss (\%)} = \frac{W_0 - W_1}{W_0} \times 100\%$$

W_0 = weight at day of harvest

W_1 = weight at day of sampling

3.2.3.4 Pulp Firmness

Fruit pulp firmness was determined using a hand penetrometer (Model KM-1, Fujiwara Factory, Japan). The fruit was cut longitudinally and the probe placed on the pulp at three different areas (stalk end, middle and blossom end) and the force required for the maximum penetration of the probe was recorded. Firmness was expressed as kilogram force (Kgf).

3.2.3.5 pH

The papaya fruit was homogenized using a Philips juicer and the juice was clarified using a metal sieve. The pH of the fruit juice was determined using a pH meter model Hanna pH211, Italy.

3.2.3.6 Total Soluble Solids

The papaya fruit juice or pulp extract was prepared the same way as for the pH measurement. Total soluble solids contents (TSS) were determined using a portable hand refractometer (Atago Model PR-1, 0 - 32°Brix, Japan). A drop of the pulp juice was mounted on the stage of the refractometer and its content expressed in standard °Brix units.

3.2.3.7 Chlorophyll Fluorescence

A portable Plant Efficiency Analyser (PEA) fluorometer (Handy PEA version 1.07, Hansatech, King's Lynn, UK) was used to determine the minimal and maximal chlorophyll fluorescence (Fo and Fm, respectively), variable chlorophyll fluorescence (Fv = Fm – Fo), and potential quantum yield of photosystem II (Fv/Fm). Before the chlorophyll fluorescence measurements were taken, the fruit were dark-adapted for 30 minutes using a dark cloth. The fiber optic was fitted with a fiber optic adapter to fix the distance between the fiber optic terminus and the fruit. Fo was measured with a measuring beam at a light intensity less than 0.05 µmol $m^{-2}s^{-1}$. Fm was obtained by measuring chlorophyll fluorescence during a 1.0 sec pulse of saturating light (3500µmol m^{-2} s^{-1}). Measurements were taken in two opposite positions of each fruit at the same location on the fruit surface and then averaged.

3.2.4 Statistical Analysis

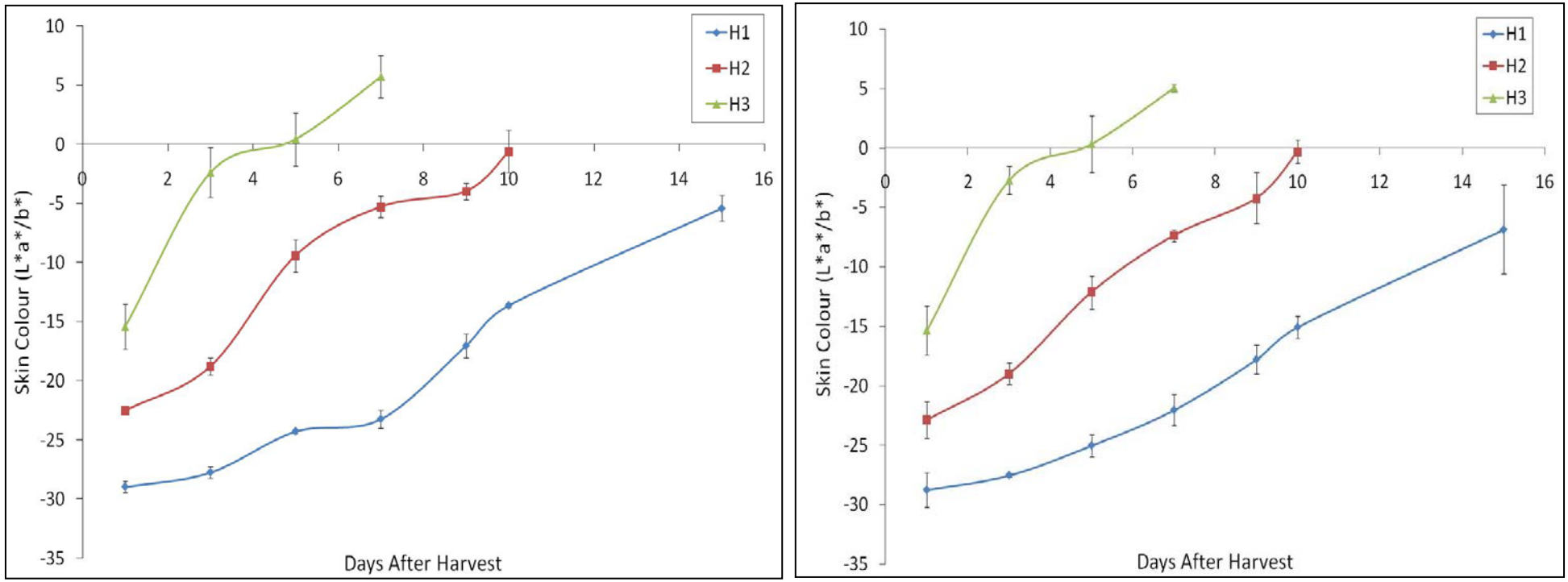
The experimental design was completely randomized. Data were analysed by analysis of variance (ANOVA). Where possible, mean comparisons were made using the Duncan Multiple Range test (DMRT) at $P < 0.05$. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) software (IBM Corporation, USA).

3.3. Results

3.3.1 Skin Colour Measurements (L*a*/b*)

The L*a*/b* values of untreated H1 fruit remained negative throughout the 15 days of storage and were unable to ripen to Index 5 since the fruit started to deteriorate although the period of storage was longer than that of H2 and H3 fruit. However, the L*a*/b* value of H1 fruit did increase gradually from -28.97 on day 1 to -5.41 on day 15, although they were unable to reach L*a*/b* values as high as the H2 and H3 fruit. Untreated H2 fruit ripened in a shorter time, with L*a*/b* value changed from -22.52 on day 1 to near zero (-0.61) on day 10. Meanwhile, untreated H3 fruit ripened within 7 days after harvest, with L*a*/b* value changed from negative on day 1 after harvest (-15.43) to positive value of 5.70 on day 7 [Figure 3.2 (a)]. Figure 3.2 (b) shows that the L*a*/b* values of treated H1, H2 and H3 fruit exhibited an almost identical pattern as the untreated H1, H2 and H3 fruit throughout the period after harvest. Furthermore, there were also no significant differences in L*a*/b* values of the treated and untreated Eksotika II papayas harvested at the three different maturity stages during the ripening period (Figure 3.3).

During ripening, the L*a*/b* values of treated and untreated Eksotika II papayas increased gradually from ripening stages Index 1 to Index 5 (Figure 3.4). There were no significant differences in L*a*/b* value between the H1, H2 and H3 fruit at all ripening stages, except for untreated Index 5 H2 and H3 fruit, where the Index 5 for the H3 fruit had a higher L*a*/b* value than the Index 5 H2 fruit at 7.78 and -0.52, respectively.



(a) (b)

Figure 3.2: L*a*/b* values of (a) untreated and (b) treated Eksotika II papaya fruit harvested at three different maturity stages. Vertical bars represent the standard error of the mean (n=5)

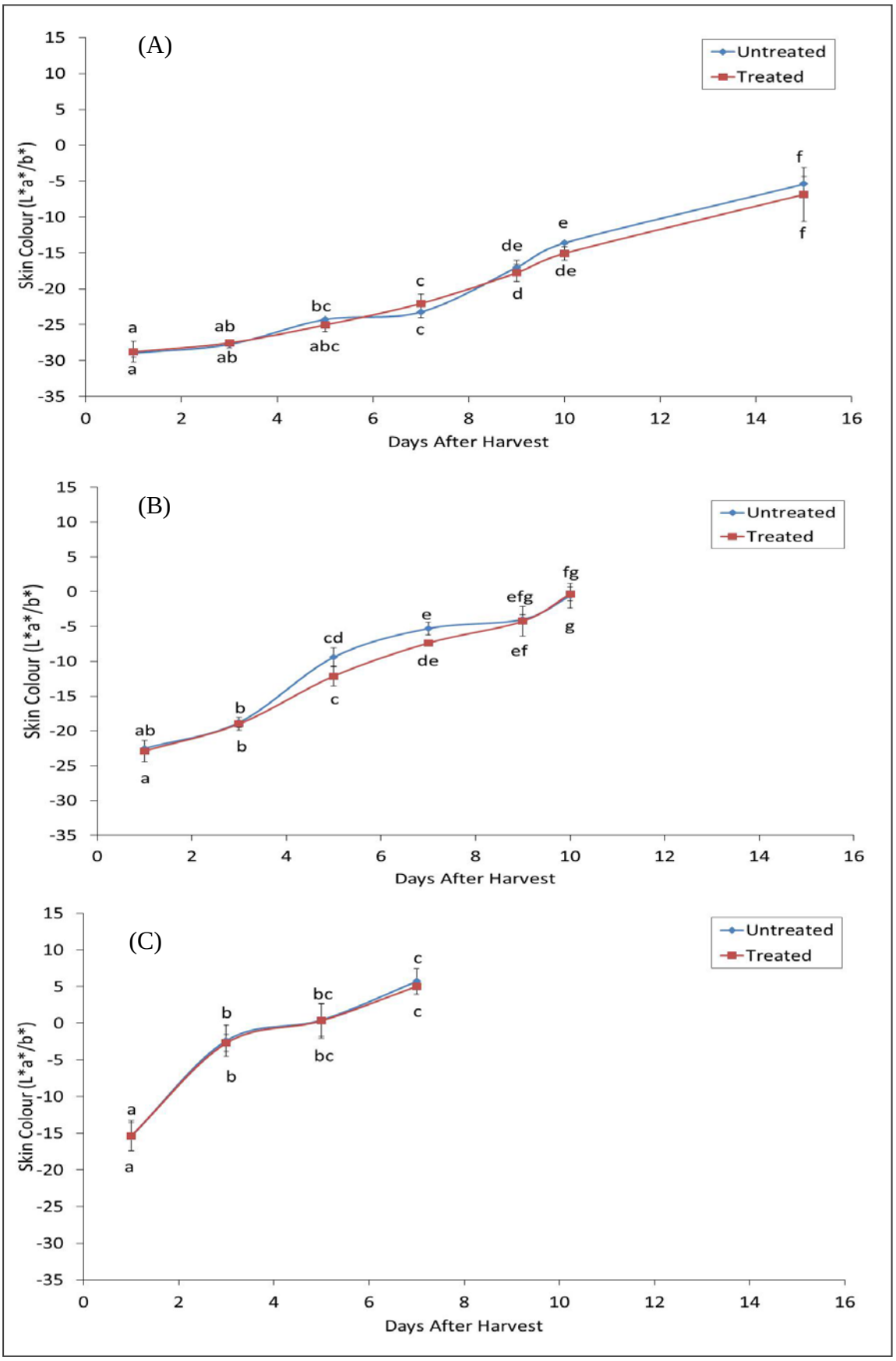


Figure 3.3: L*a*/b* values of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=5)

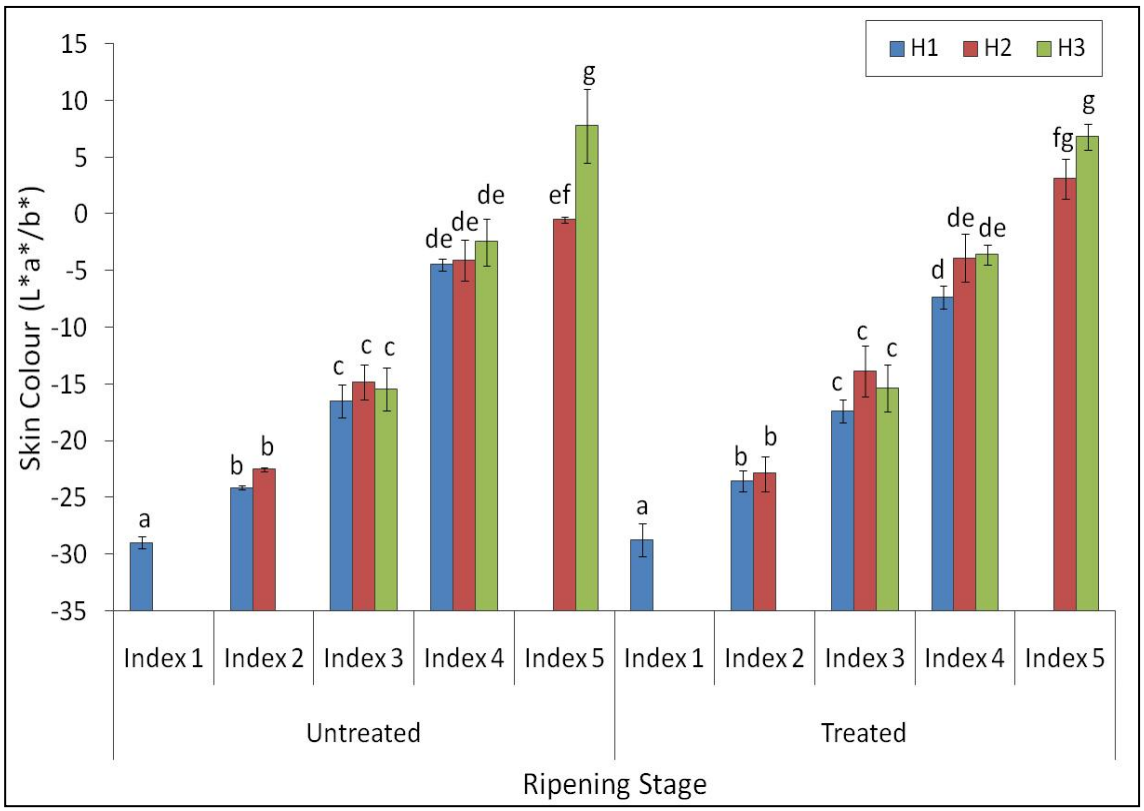


Figure 3.4: L*a*/b* values of treated and untreated Eksotika II papaya fruit at different ripening stages. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=5).

Table 3.1 shows a proposed chromatically assessed fruit maturity indices value of Eksotika II papaya which corresponded with the visual colour chart after Lam and Zaipun, (1987) that was used to describe the stages of ripening in untreated Eksotika papaya. The recorded L*a*/b* values were rounded to the highest and the lowest average in order to establish quality standards. It was observed that the chromatically determined L*a*/b* colour range became positive as the fruit reached Index 5. Figure 3.5 shows the relationship between the ripening index and the L*a*/b* values of Eksotika II papaya during ripening. The chromatically determined L*a*/b* values exhibited a good linear correlation with the ripening index with R^2 (Coefficient Correlation) value of 0.997. Therefore, the chromatically determined L*a*/b* values proposed in Table 3.1 can be considered as good maturity stage indicator (if not better) besides subjective determination of the maturity stage by visual observation.

Moreover, inexperienced eyes may not accurately stage the maturity of the fruit. Thus, this chromatically assessed L*a*/b* based colour value would provide a useful objective and more precise assessment of Eksotika II papaya maturity and allows standardization of quality control for this commercially significant papaya cultivar.

Table 3.1: Proposed Eksotika II papaya fruit maturity indices value (L*a*/b*) range for the six maturity stages.

L*a*/ b* range (Chromatically determined)	Ripening stage (visual assessment)
< -30	Immature fruit (green)*
-30 to -26	Index 1 (green)
> -26 to -19	Index 2 (green, trace of yellow)
> -19 to -9	Index 3 (more green than yellow)
> -9 to -2	Index 4 (more yellow than green)
> -2 to 7	Index 5 (yellow with green tip)
> 7	Index 6 (all yellow)

* Immature fruit also have green skin same as Index 1 fruit but the pulp is white in colour and the seeds are also white or slightly dark in colour (Appendix 3)

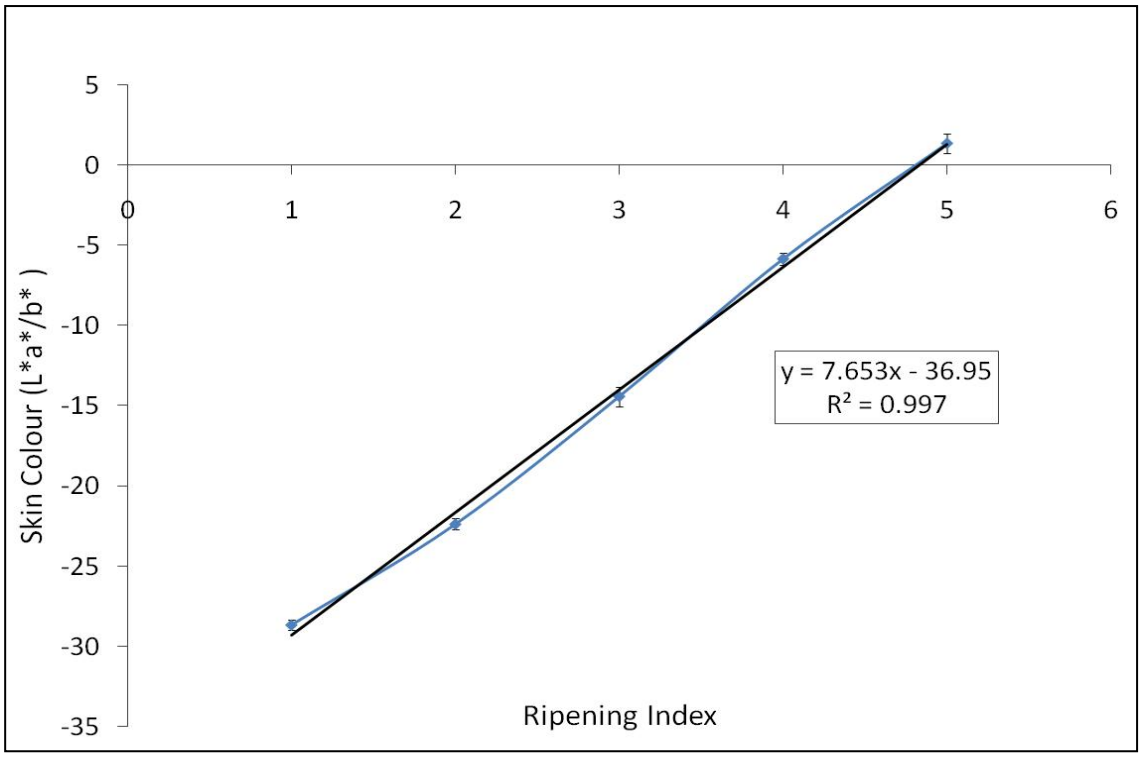


Figure 3.5: Relationship between the ripening index and the skin colour (L*a*/b*) values of Eksotika II papaya fruit during ripening. Each point is the mean of 30 replicates.

3.3.2 Visual Observation

The external physical appearance (whole fruit) and internal (longitudinally cut fruit) characteristics of the sampled treated and untreated H1, H2 and H3 fruit at each maturity stage are shown in Figures 3.7, 3.8 and 3.9, respectively. The yellow colour development was initiated with the appearance of light yellow longitudinally stripes from the blossom end of the fruit and turn progressively yellow towards the stalk end. However, the early appearance of yellow coloured stripes can also appear almost everywhere on the fruit skin as observed in some of the sampled fruit.

Untreated H1 fruit failed to develop the red-orange colour seen in untreated H2 and H3 fruit when it reached Index 3 and 4. The pulp colour was pale orange and less intense. Conversely, untreated H2 and H3 fruit ripened correctly, where the peel and pulp of the fruit developed full colour when it reached Index 4 and 5, with the colour more intense at Index 5.

Furthermore, there were no differences in the external and internal appearances between treated and untreated H1 fruit at Index 1 and Index 2. At Index 3 and Index 4, the pulp colour of treated fruit failed to develop the same red-orange colour of untreated fruit. The colour was less intense and pale orange. However, no differences were found between treated and untreated H2 and H3 fruit at each ripening stage.

Ripening Stage	*Carica papaya* L. cv. Eksotika II Harvested at Index 1	
	Untreated	Treated
Index 1		
Index 2		
Index 3		
Index 4		

Figure 3.6: Visual observation of representative hot water treated and untreated Eksotika II papaya fruit at each ripening stage. Fruit were harvested at maturity stage Index 1 (H1).

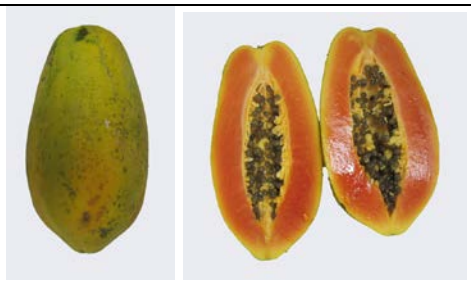
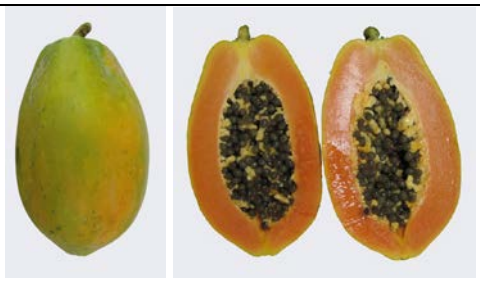
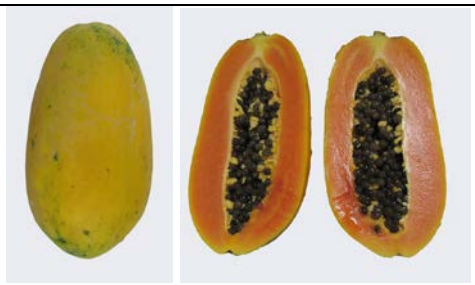
Ripening Stage	*Carica papaya* L. cv. Eksotika II Harvested at Index 2	
	Untreated	Treated
Index 2		
Index 3		
Index 4		
Index 5		

Figure 3.7: Visual observation of representative hot water treated and untreated Eksotika II papaya fruit at each ripening stage. Fruit were harvested at maturity stage Index 2 (H2).

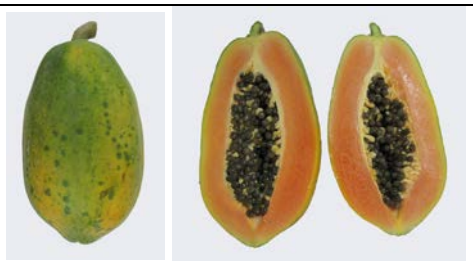
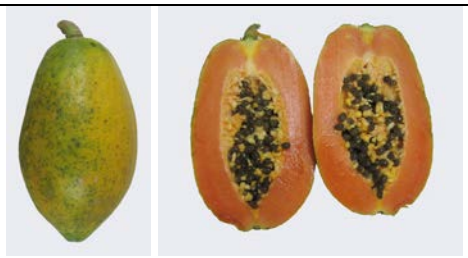
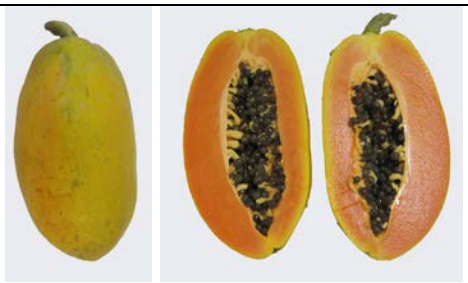
Ripening Stage	*Carica papaya* L. cv. Eksotika II Harvested at Index 3	
	Untreated	Treated
Index 3		
Index 4		
Index 5		

Figure 3.8: Visual observation of representative hot water treated and untreated Eksotika II papaya fruit at each ripening stage. Fruit were harvested at maturity stage Index 3 (H3).

3.3.3 Weight Loss

Figure 3.9 shows that H1 and H2 Eksotika II papaya fruit, left to ripen at ambient temperature, experienced about 12 % weight loss during the storage period. However, H3 fruit lost only about 5% water until day 5 after harvest. There were no significant differences in weight loss between the untreated and treated H1, H2 and H3 Eksotika II papaya fruit at each ripening stage (Figure 3.10).

3.3.4 Pulp Firmness

Figure 3.11 shows the initial pulp firmness values of treated and untreated Eksotika II papaya harvested at three different maturity stages. Eksotika II papaya harvested at maturity stages Index 1 (H1) and Index 2 (H2) did not show any significant difference in the pulp firmness value. However, Eksotika II papaya harvested at Index 3 (H3) showed significantly lower pulp firmness value than the H1 and H2 fruit. Moreover, there is no difference in pulp firmness between the hot water treated and untreated fruit harvested at different maturity stages. Figure 3.12 shows that treated Index 3 and Index 4 H1 fruit was significantly firmer than untreated Index 3 and Index 4 H1 fruit. Moreover, treated Index 3 H2 fruit had significantly higher firmness value than the untreated fruit at the same ripening stage. However, at the subsequent ripening stages, no significant differences were found between the treated and untreated H2 fruit. As for H3 fruit, treated Index 4 fruit was significantly firmer than untreated fruit. However, at Index 5, the softening process occured normally since treated Index 5 fruit showed similar firmness value as untreated fruit.

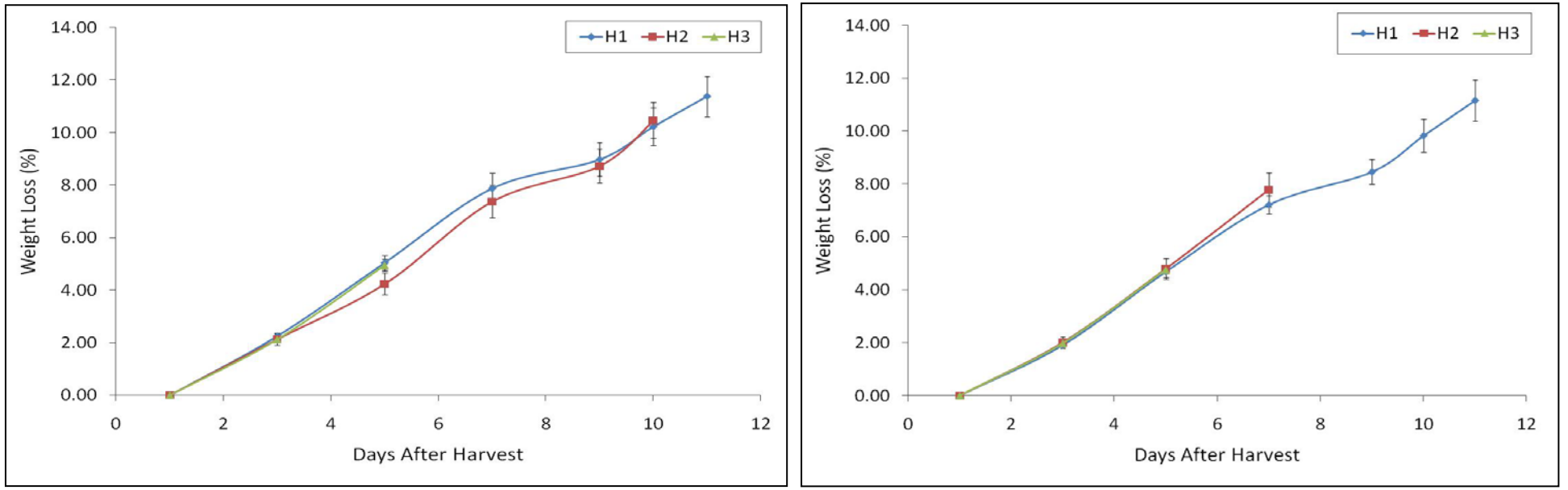


Figure 3.9: Percentage of weight loss of (a) untreated and (b) treated Eksotika II papaya fruit harvested at three different maturity stages. Vertical bars represent the standard error of the mean (n=5).

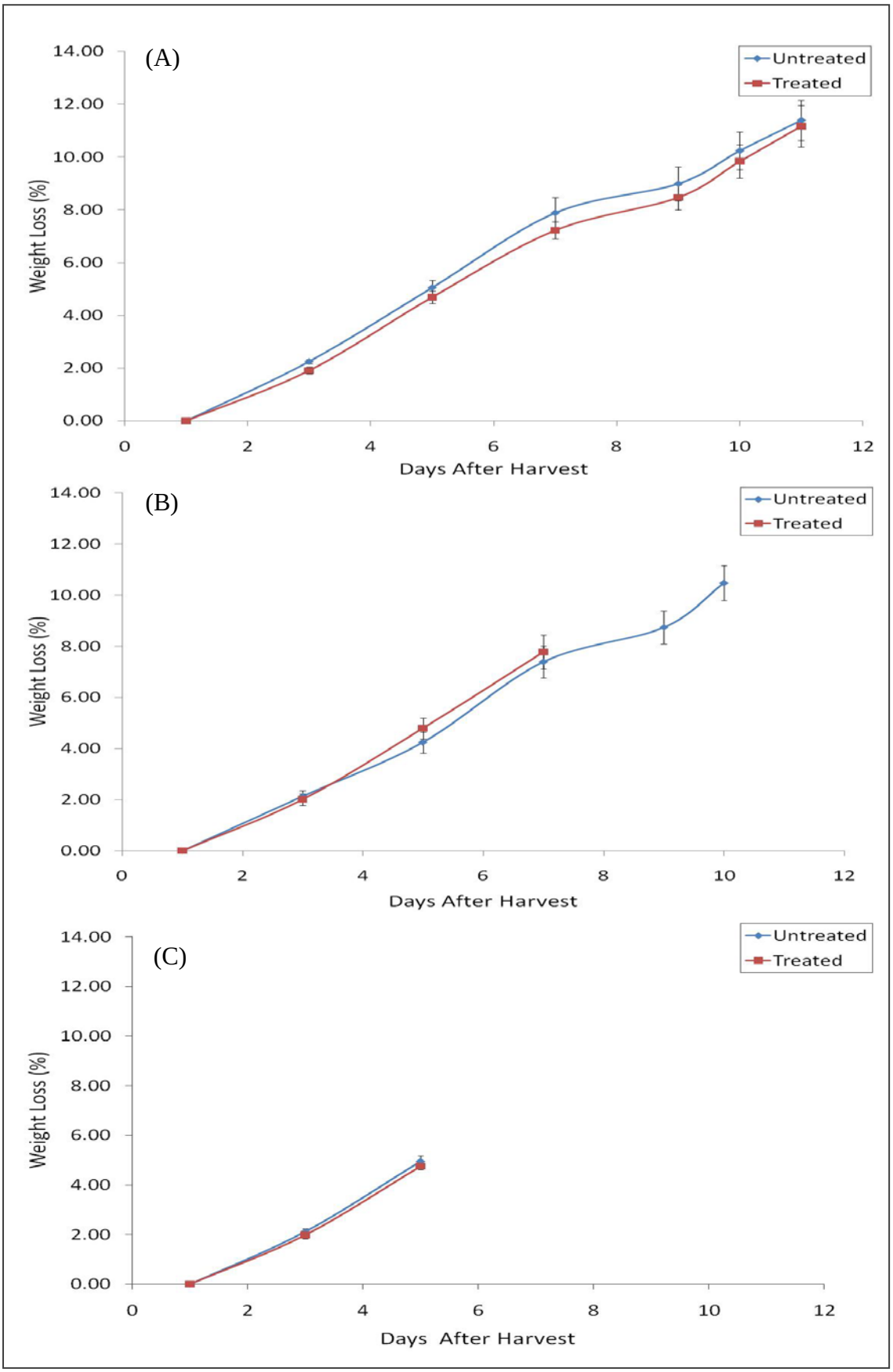


Figure 3.10: Percentage of weight loss of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Vertical bars represent the standard error of the mean (n=5)

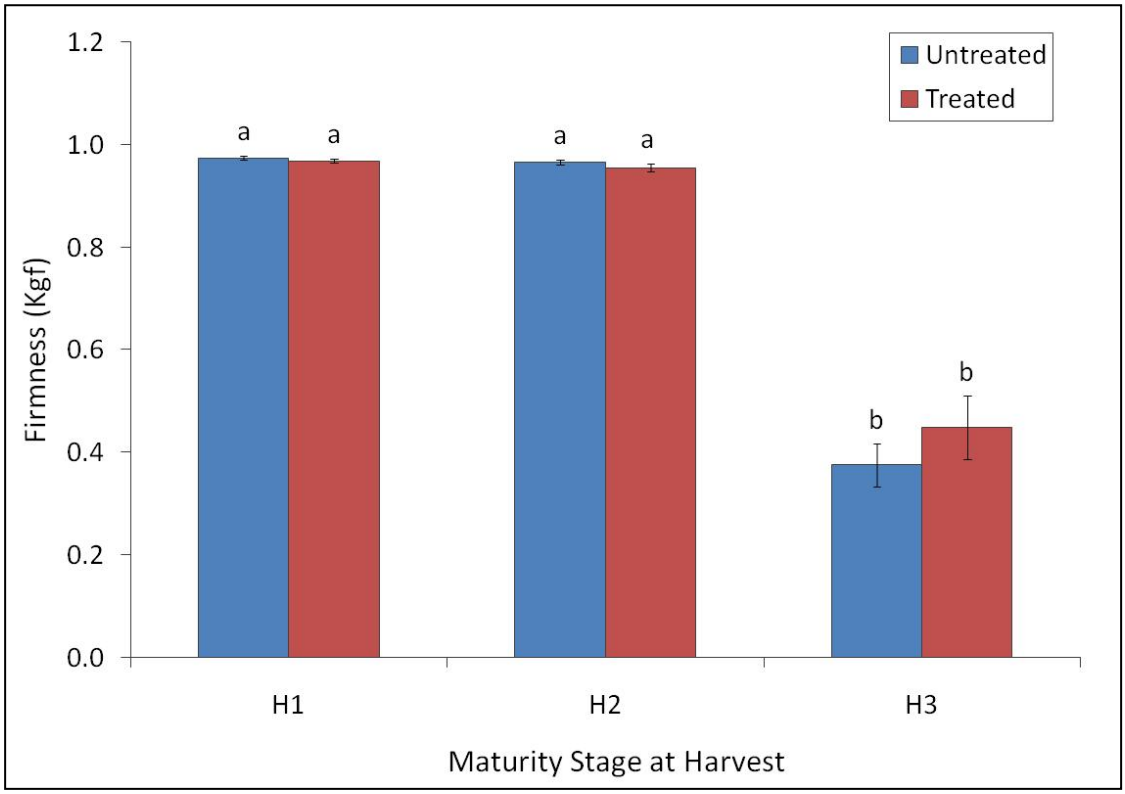


Figure 3.11: Initial pulp firmness values of treated and untreated Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=5)

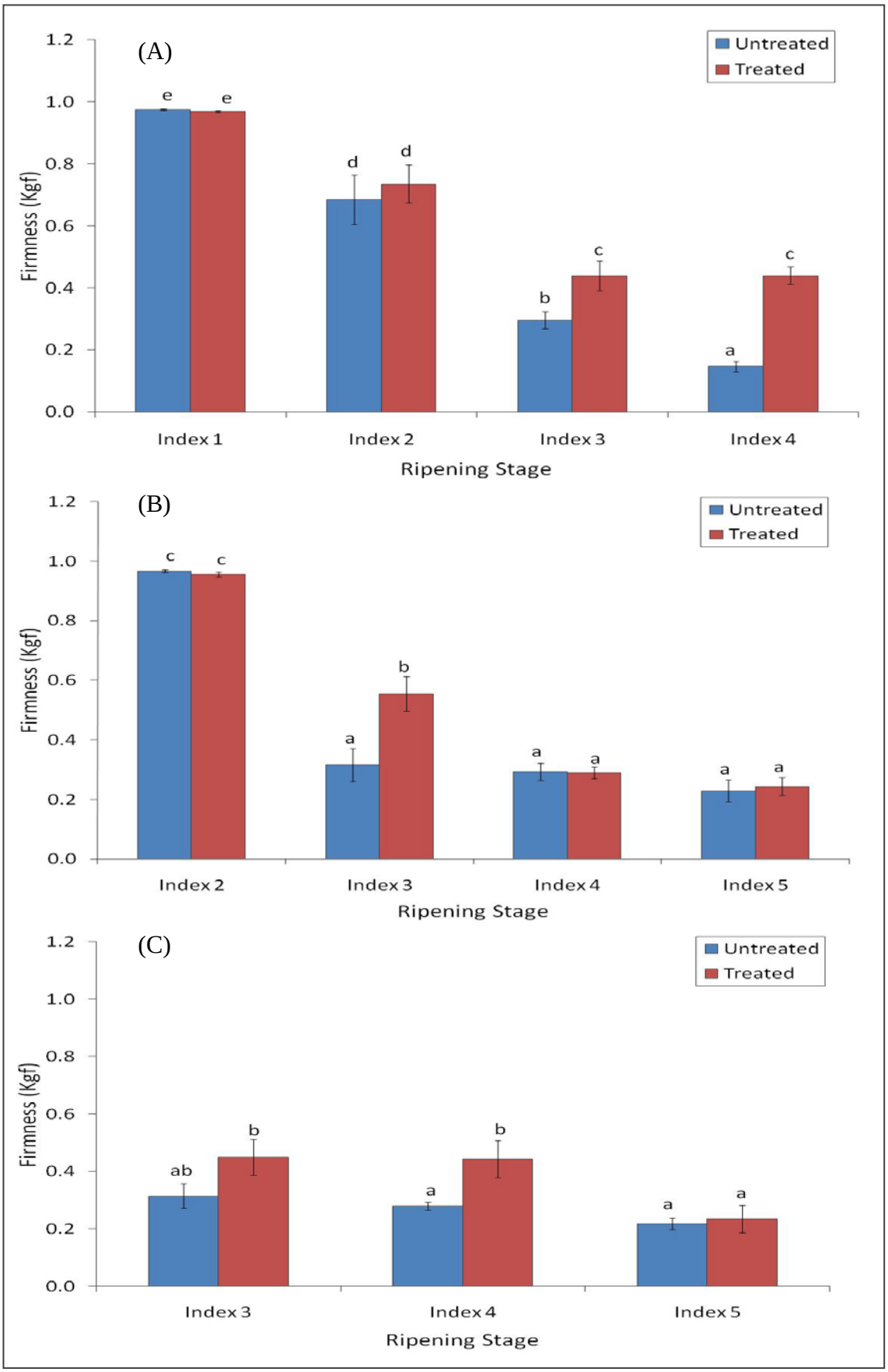


Figure 3.12: Pulp firmness values of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=5)

3.3.5 pH

The pH of the fruit juice showed that it was weakly acidic. Figure 3.13 shows that while H1 and H2 fruit showed no difference in pH value, H3 fruit had significantly lower pH value. However, there was no significant difference found for treated and untreated fruit harvested at different maturity stages. Figure 3.14 shows that the pH value did not change throughout the ripening process for all the treated and untreated fruit harvested at the different maturity stages. A pH value of about 5.3 – 5.7 was obtained in treated and untreated fruit at all ripening stages.

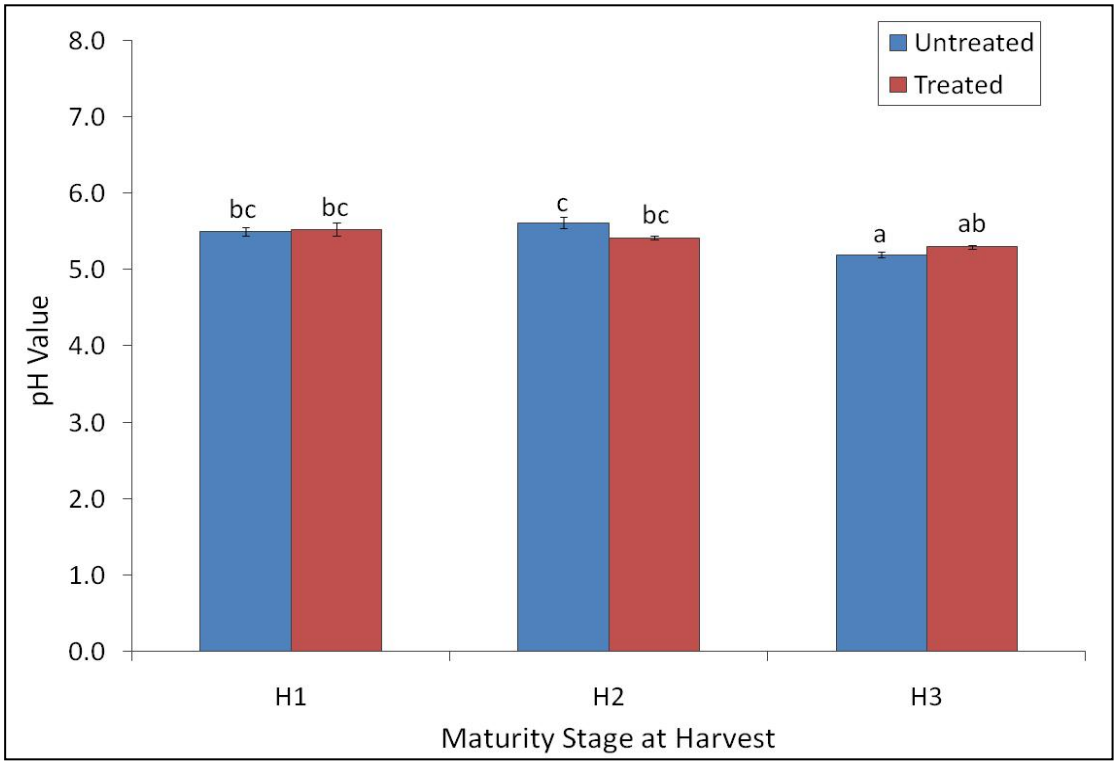


Figure 3.13: Initial pH of treated and untreated Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=5)

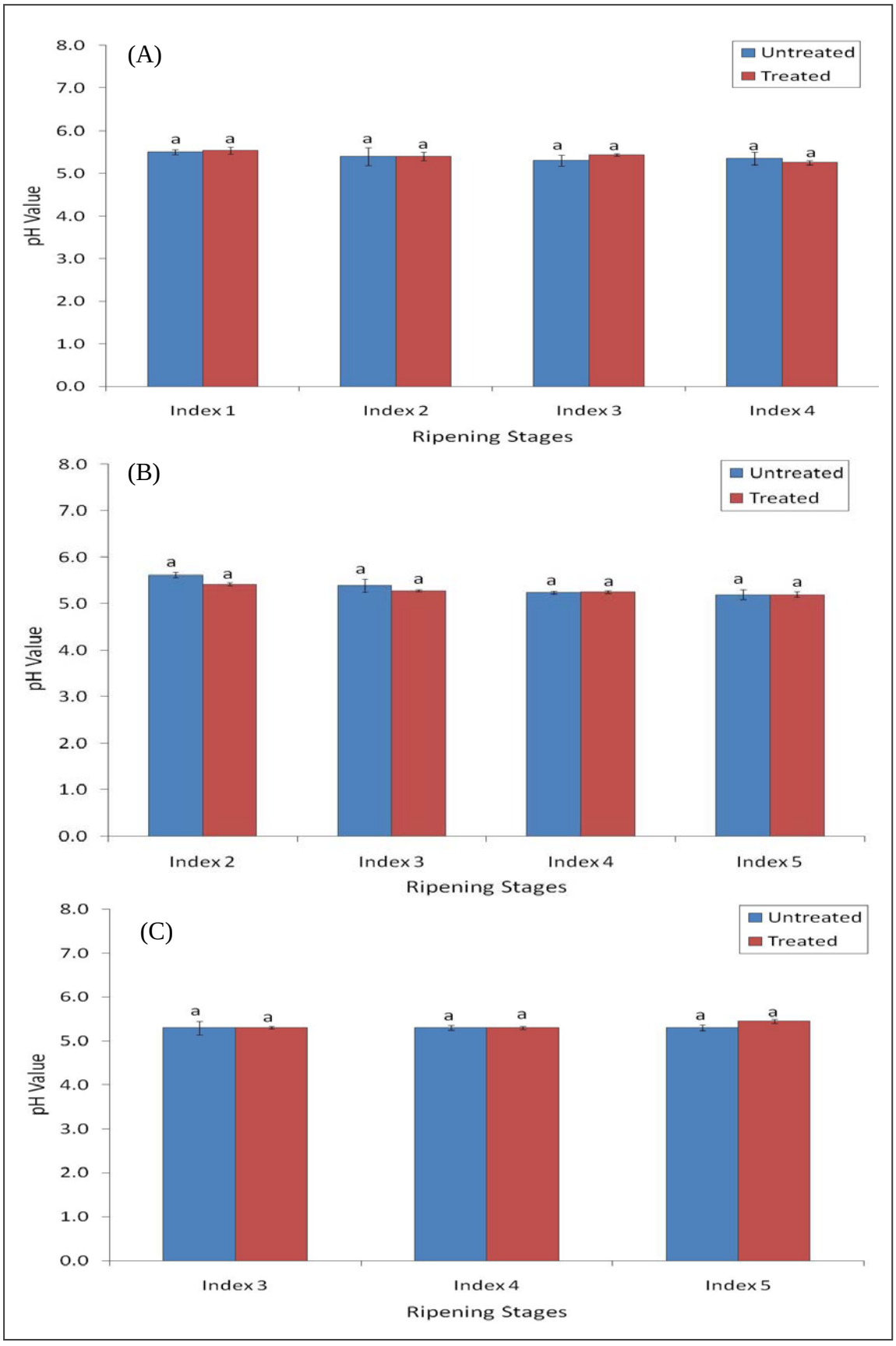


Figure 3.14: The pH of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=5).

3.3.6 Total Soluble Solids (TSS)

As shown in Figure 3.15, H2 and H3 fruit showed higher TSS value compared to H1 fruit. However, no difference was found in TSS value between treated and untreated H1, H2 and H3 fruit at harvest. Furthermore, the total soluble solids value remained unchanged as ripening progressed for H1, H2 and H3 fruit (Figure 3.16). Besides, hot water treatment also did not give any effect to the TSS value of Eksotika II papaya during ripening since no difference was found in the TSS between treated and untreated fruit at all ripening stages.

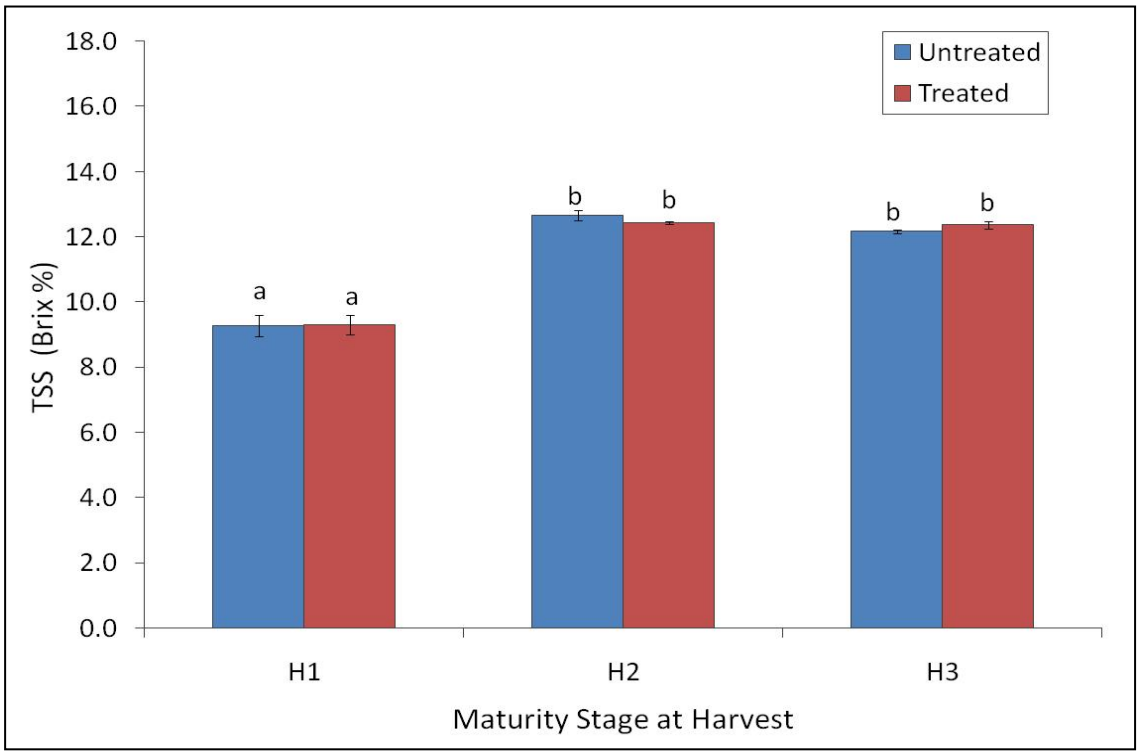


Figure 3.15: Initial total soluble solids value of hot water treated and untreated Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=5)

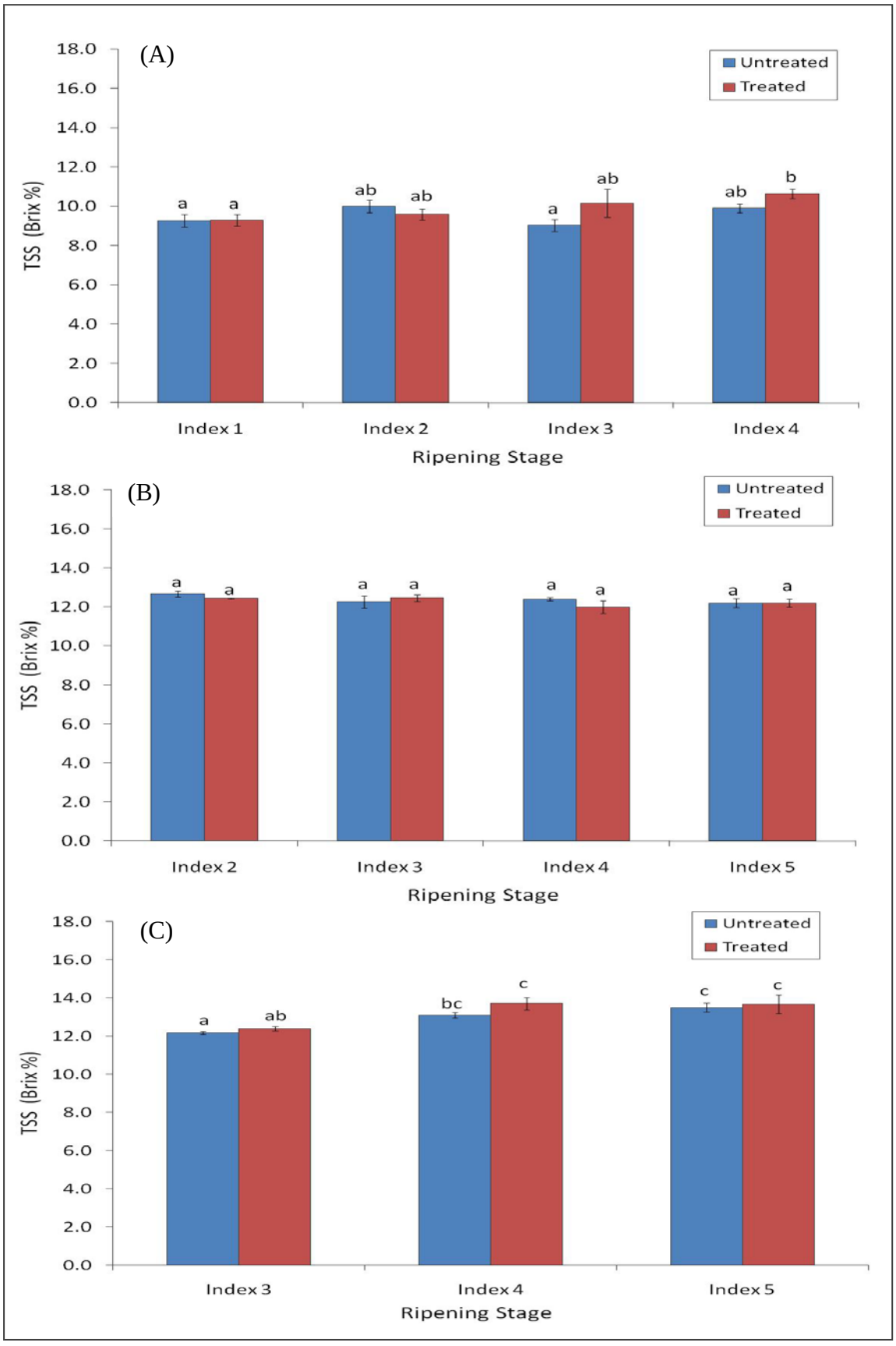


Figure 3.16: Total soluble solids values of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=5).

3.3.7 Chlorophyll Fluorescence

Figure 3.17 shows that the Fv/Fm values of untreated H1, H2 and H3 fruit were not affected by the maturity stages at harvest. However, for the treated fruit, H3 fruit exhibited the lowest Fv/Fm value followed by H1 and H2 fruit with the values of 0.51, 0.59 and 0.68, respectively. As shown in Figure 3.18, the Fv/Fm value of untreated H1 fruit decreased slightly but not significantly during ripening. Untreated H2 fruit exhibited a significant decrease in Fv/Fm between Index 3 (0.82) and 4 (0.73) and then decreased further when it reached Index 5 (0.72). In addition, the Fv/Fm value of untreated H3 fruit decreased significantly when it reached Index 4 and then slightly at Index 5.

Figures 3.17 and 3.18 also show that within one hour after treatment, treated H1, H2 and H3 fruit showed significantly lower Fv/Fm values than the untreated fruit. The same effect was observed after the fruit reach the subsequent stages of ripening except for H3 fruit where there was no significant difference between treated and untreated fruit at ripening stages Index 4 and 5. Nevertheless, Fv/Fm values in treated H1 and H3 fruit recovered when it reached the subsequent ripening stage with a sharp increase in Fv/Fm values of 0.76 and 0.68, respectively. On the other hand, the Fv/Fm value for the treated H2 fruit remained constant until Index 3 and then decreased slightly at Index 4 and Index 5. However, the recovery of the Fv/Fm values of treated H1, H2 and H3 fruit at the subsequent ripening stages still remained significantly lower than the Fv/Fm value of the untreated fruit at the same ripening stage.

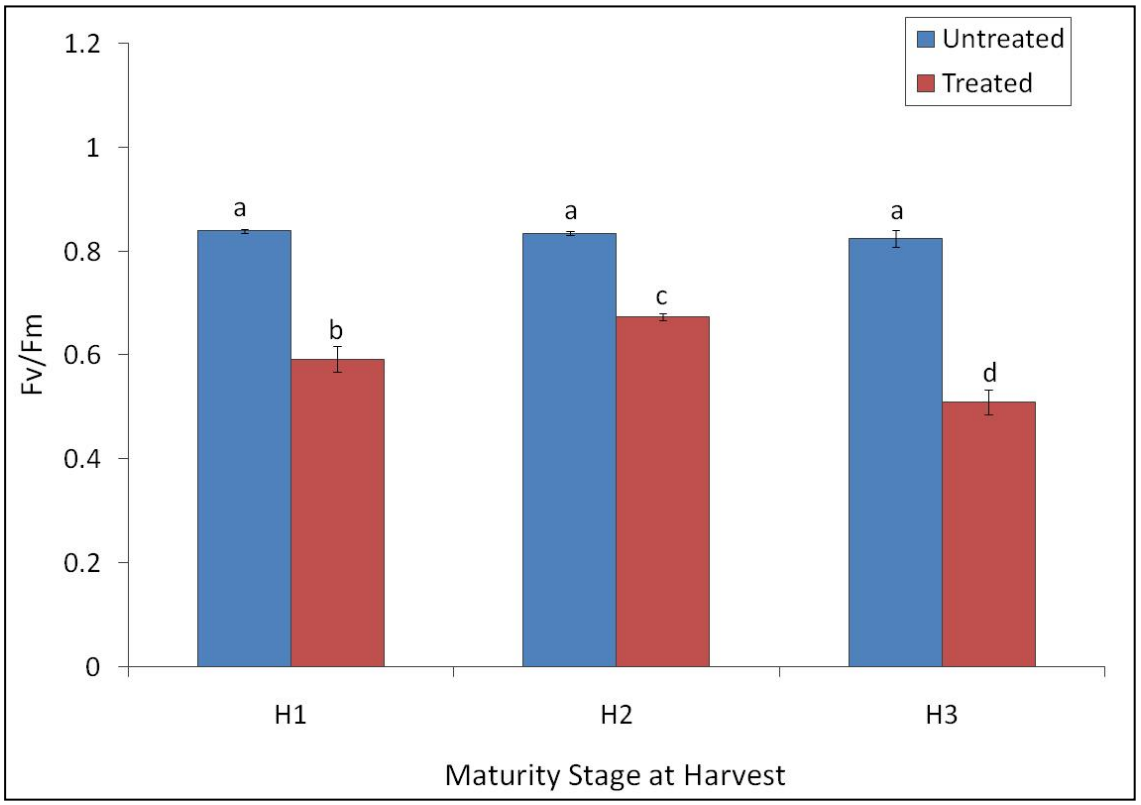


Figure 3.17: Initial photosynthetic yield (Quantum efficiency of PSII, Fv / Fm) of treated and untreated Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=10)

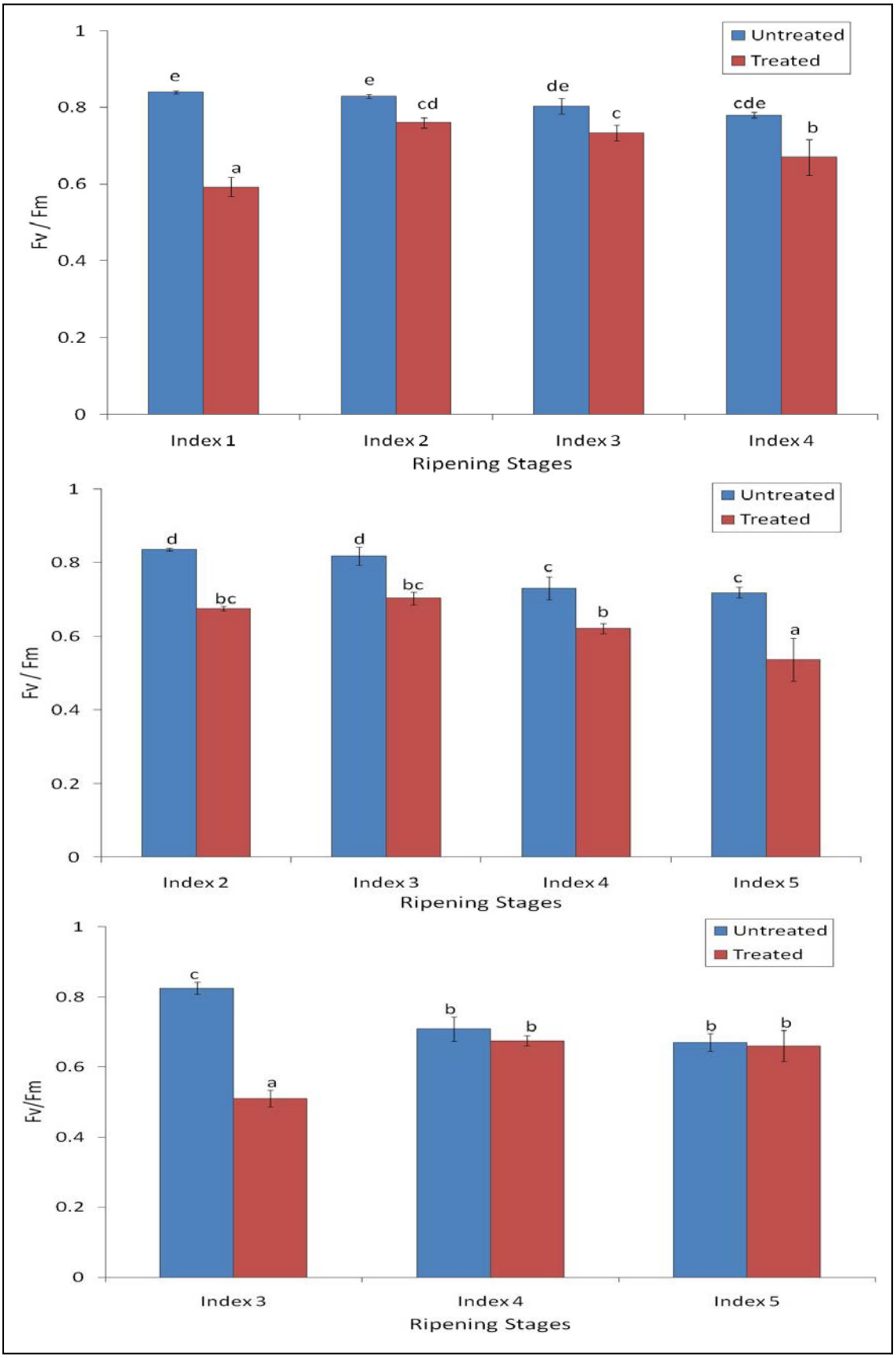


Figure 3.18: Photosynthetic yield (Quantum efficiency of PSII, Fv / Fm) of hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=10)

Figure 3.19 shows the values of chlorophyll fluorescence parameters, Fo, Fm and Fv of treated and untreated H1 fruit at different ripening stages. The Fo values of treated H1 fruit were higher significantly than untreated fruit at Index 1, Index 3 and Index 4. The Fo values fluctuated during ripening. The Fm values of treated Index 1 fruit were lower than the untreated fruit. However at the subsequent ripening stages, there were no differences in the Fm values between treated and untreated H1 fruit. The Fm values decreased during the ripening of untreated fruit and fluctuated during the ripening of treated fruit. The Fv values of treated Index 1 fruit were lower by 2 fold than the untreated fruit at the same stage. However, the Fv value in treated fruit increased to 1.5 fold from the previous value, but still remained lower than the Fv value for untreated fruit. The Fv values of both treated and untreated fruit then decreased sharply towards Index 4.

The values of chlorophyll fluorescence parameters, Fo, Fm and Fv of treated and untreated H2 fruit at the different ripening stages are shown in Figure 3.20. The Fo values of treated Index 2 fruit were significantly higher than untreated fruit at the same stage. However, at Index 3, 4 and 5, there were no significant differences between the Fo values of treated and untreated fruit. The Fm values of treated fruit were lower than untreated fruit at Index 2, but no differences were observed between treated and untreated fruit at the subsequent ripening stages until at Index 5 where the Fm values of treated fruit was significantly lower than the value in untreated fruit. The Fv values of the treated fruit remained significantly lower than that in untreated fruit at all ripening stages, except at Index 4, where no significant difference was found between treated and untreated fruit. The Fv values of both treated and untreated fruit declined gradually during ripening.

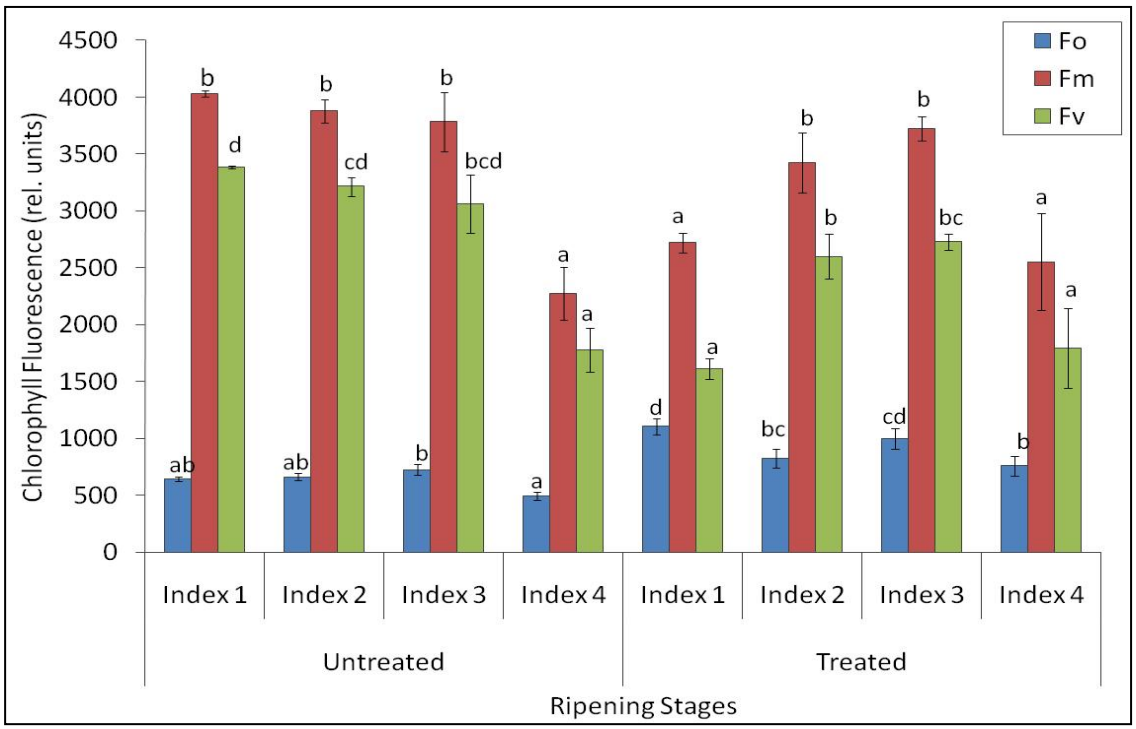


Figure 3.19: Chlorophyll fluorescence parameters (Fo, Fm and Fv) of hot water treated and untreated Eksotika II papaya fruit at different ripening stages. Fruit were harvested at maturity stage Index 1 (H1). Different letters represent significant statistical differences by DMRT ($P < 0.05$) between ripening stages. Vertical bars represent the standard error of the mean (n=10)

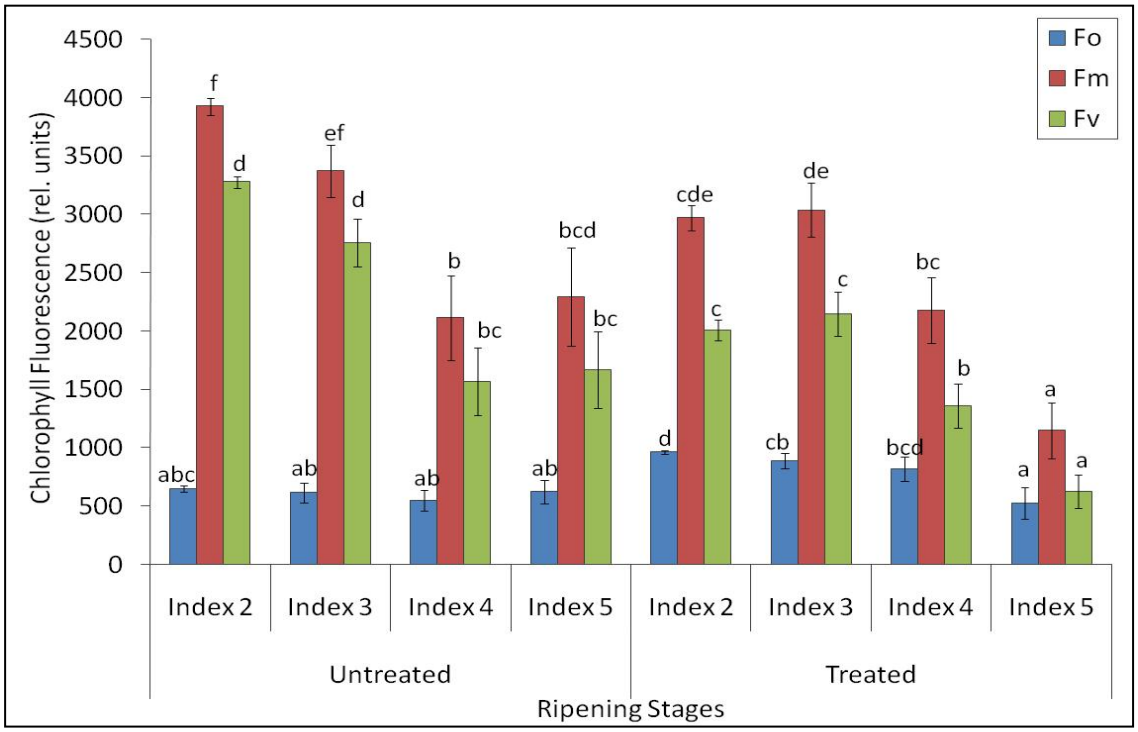


Figure 3.20: Chlorophyll fluorescence parameters (Fo, Fm and Fv) of hot water treated and untreated Eksotika II papaya fruit at different ripening stages. Fruit were harvested at maturity stage Index 2 (H2). Different letters represent significant statistical differences by DMRT ($P < 0.05$) between ripening stages. Vertical bars represent the standard error of the mean (n=10)

Figure 3.21 shows the values of chlorophyll fluorescence parameters, Fo, Fm and Fv in treated and untreated H3 fruit at different ripening stages. Within one hour after treatment, treated Index 3 fruit exhibited a significantly higher Fo value compared to untreated fruit of the same stage. However, the value decreased following storage at ambient temperature. Thus, at subsequent ripening stages, there were no differences in Fo values between treated and untreated fruit. The Fm values of treated Index 3 fruit were lower than the untreated fruit but not significantly difference by DMRT. Similarly as in Index 3 fruit, Index 4 and 5 fruit also showed no significant differences in Fm values between treated and untreated fruit. The Fv values of treated Index 3 fruit was significantly lower than that in the untreated fruit. However, at the subsequent ripening stages, no significant difference was observed in Fv values between both treated and untreated fruit.

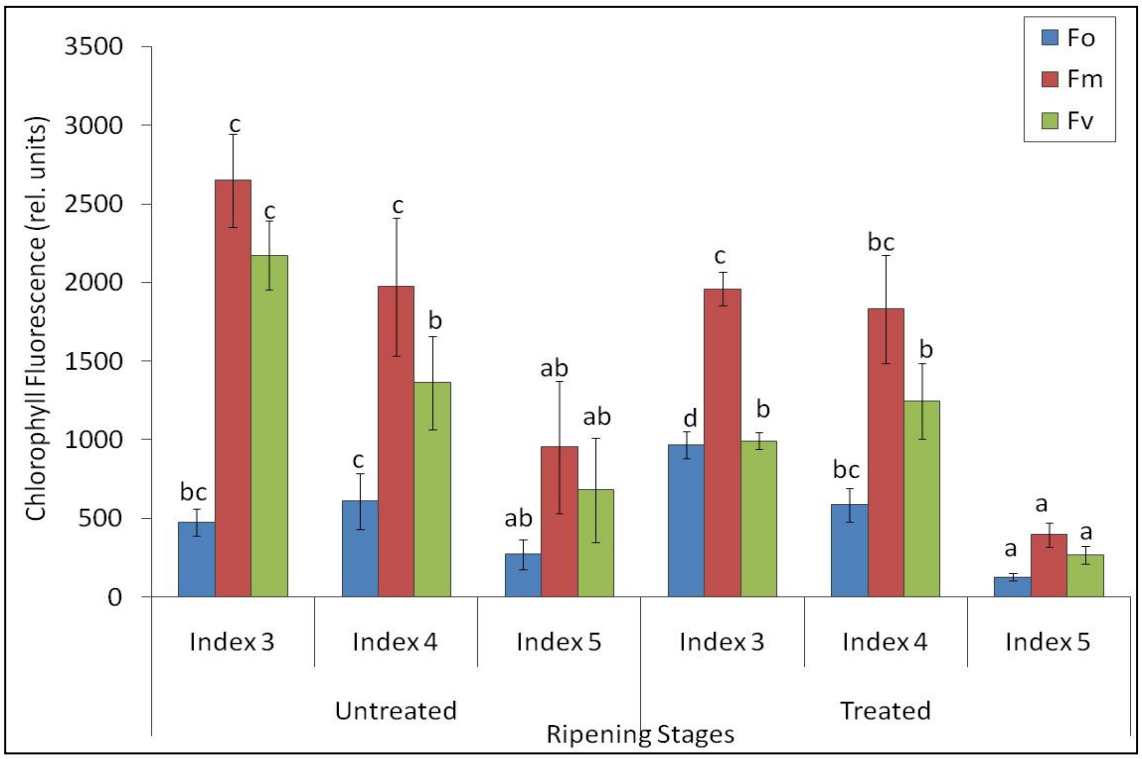


Figure 3.21: Chlorophyll fluorescence parameters (Fo, Fm and Fv) of hot water treated and untreated Eksotika II papaya fruit at different ripening stages. Fruit were harvested at maturity stage Index 3 (H3). Different letters represent significant statistical differences by DMRT ($P < 0.05$) between ripening stages. Vertical bars represent the standard error of the mean (n=10)

3.4 Discussion

In many fruits, peel colour change is widely used as a visual maturity index (Reid, 2002). Peel colour change during fruit ripening in many fruits is due to the unmasking of other pigments within the fruit, which was previously formed during fruit development. It includes the degradation of chlorophyll and the dismantling of the photosynthetic apparatus, the synthesis of different types of anthocyanins and their accumulation in the vacuoles and accumulation of carotenoids such as β-carotene, xanthophyll esters, xanthophylls and lycopene (Aked, 2000; Ferrer *et al.*, 2005; Prasanna *et al.*, 2007). Eksotika II papaya fruit begins ripening with the appearance of light longitudinal stripes that turn progressively yellow, although the yellow colouration pattern is not necessarily restricted to longitudinal stripes, and the yellow coloured sites can appear almost anywhere on the fruit skin (Peleg and Gómez- Brito, 1975).

The subjective evaluation made in this study indicated that the treated and untreated H1 fruit failed to develop full colour compared to H2 and H3 fruit. As an outcome, the L*a*/b* value of treated and untreated H1 fruit remained negative throughout the 15 days of storage. The objective L*a*/b* colour measurement method was used to enhance accuracy and precision of the subjective evaluation of the fruit peel colour (Chandran, 1998). The results observed in the present study of Eksotika II papaya fruit harvested at the mature green stage (H1) are consistent with results reported in earlier studies (Lam and Zaipun, 1987; Halimi *et al.*, 1990; Malar and Nair, 1992) for Eksotika papaya fruit, where the fruit failed to develope full flavour and colour, while the pulp colour was often pale orange. In contrast, H2 fruit ripened adequately and was able to develop full colour when it reached Index 5. This suggest that Index 2 can be considered the physiological maturity stage with average L*a*/b* value > -26. Based on colour values and storage shelf life, Index 2 appears to be the proper time to harvest for long

distance shipment, while Eksotika II papaya can be harvested at Index 3 for local markets.

In this study, a simple objective method to determine harvest maturity indices of Eksotika II papaya by colour indexing was deployed (Table 3.1). As such, by just reading the colour of a fruit at harvest, one can predict the maturity stage of the fruit by referring to the proposed maturity indices value (L*a*/b*) which are chromatically determined (Table 3.1). This should be useful in order to reduce the incidence of harvesting physiologically immature Eksotika II papaya fruit which will result in poor quality papayas for marketing.

As for the response of Eksotika II papaya fruit to the hot water treatment, this study shows that the visual appearance of treated H2 and H3 fruit were not affected by the heat treatment. However, for treated H1 fruit, hot water treatment did affect the visual appearance of the fruit where at Index 3 and Index 4, the pulp colour was pale orange and less intense, although the skin colour was not affected. Similar findings were documented by Paull and Chen (1990) who reported that papaya fruit heated for 70 minutes at 49°C was slower to develop internal carotenoid colour than untreated fruit. This is apparently due to the inhibition of carotenoid synthesis similar to that reported in tomatoes where carotenoid synthesis is inhibited when tomatoes are exposed to high temperatures (> 30°C), although the process recovers after removal of the heat (Lurie *et al.*, 1996; Sozzi *et al.*, 1996).

Loss of moisture through transpiration results in fruit shriveling, shrinking and a decrease in fruit weight. In this study, H1 and H2 papaya fruit, left to ripen at ambient temperature experienced about 12 % weight loss during the storage period. A similar

trend was also observed by Lazan *et al*. (1990) where Eksotika papaya weight loss was about 12% of the original weight when allowed to ripen at 25˚C at about 80% relative humidity. In addition, H3 fruit lost their weight at the same rate as that observed for H1 and H2 fruit, but only until day 5 after harvest because of their short shelf life. As for the heat treated fruit, there were no significant differences in weight loss between the untreated and treated H1, H2 and H3 papaya fruit at each ripening stage. This suggests that the hot water treatment at 47°C did not affect fruit weight loss, and thus probably can maintain fruit marketability since high water loss affects visual appearance of a fruit (Kader, 1992).

Texture is a major fruit attribute which has a great effect on consumer perception of fruit quality. Changes in fruit texture that result in fruit softening are responsible in turning a hard fruit into a soft edible fruit during ripening (Prasanna *et al*., 2007). Loss of tissue firmness is mainly due to the modification of the structure and chemical composition of the cell wall carbohydrates of the fruit tissue (Huber, 1983a). It is also brought about by a reduction in cell turgor, due to increasing concentrations of solutes in the cell wall space and to wall loosening (Shackel *et al.,* 1991). In this study, pulp firmness of untreated H1 fruit at Index 4 was lower than that in the untreated H2 and H3 fruit. Possibly the loss of firmness in untreated H1 fruit is due to an altered cell wall carbohydrate metabolism during storage which has been associated with increased susceptibility to infection by fungal pathogens (Ali *et. al*., 1993). This was supported by the data in the present study that showed H1 fruit failed to ripen properly and exhibited inferior fruit quality during ripening. Since a very small amount of starch has been detected in papaya (Selvaraj *et al.*, 1982), the contribution of starch breakdown to fruit softening during ripening is probably negligible. On the other hand, the softening of H1 fruit can possibly be disrupted following hot water treatment as observed in Index 3 and

Index 4 treated H1 fruit which had higher pulp firmness than untreated fruit. This might be due to a reduction in cell wall hydrolytic enzymes brought about by the heat treatment (Qiu *et al.,* 1995). Nevertheless, even treated H2 and H3 fruit were firmer than the untreated fruit in the middle of the ripening process, the pulp firmness value of the treated H2 and H3 fruit reached the same value as the untreated fruit at the later stages of ripening and softened normally, which suggest that H2 and H3 fruit can tolerate the hot water treatment at the selected temperature.

Independent of the maturity stages at which the papaya fruit were harvested, the pH value of treated and untreated Eksotika II papayas in this study showed that it has a very low acidity content with pH value ranging from 5.3 to 5.7. These observations are in agreement with that of Bron *et al*. (2006) who reported that papaya had very low acidity content compared to other fruits. However, fruit harvested at more advanced maturity stage, H3, had a lower pH value than H1 and H2 fruit. This result supported the findings by Lazan *et al*. (1989) who found that the pH for papaya cv. Backcross Solo decrease as the mature fruit ripens to the value of 5.2, which is similar to the pH value of H3 fruit in the present study. The pH value also did not differ very much during ripening for all the fruit harvested at the different maturity stages. The pH of the Eksotika II papaya fruit harvested at the three different maturity stages were not affected by the hot water treatment. Similar results have been reported in tomatoes, where the acidity of the fruit was not affected by heat treatment, either in water or hot air at 38 – 48°C for 1 hour to 3 days (Lurie and Klein, 1991; McDonald *et al.,* 1999).

The total soluble solids (TSS) for H1 fruit were significantly lower than the TSS values for H2 and H3 fruit. According to Selvaraj *et al.* (1982), the sucrose content increases when fruit begins to change its colour (colour break), so that fruit harvested at an

advanced stage of ripening exhibited higher total soluble solids. Akamine and Goo (1971) also reported that Solo papaya need to have at least 6% surface yellow colouration to meet the minimum TSS of 11.5%, required by the Hawaiian grade standards for marketable papayas. Furthermore, the TSS values of the H1, H2 and H3 fruit did not differ significantly during ripening. Selvaraj *et al.* (1982) had also reported that the papaya fruit do not accumulate starch during development and therefore, does not have significant amounts of starch to be hydrolyzed during ripening, which results in little, if any, change in soluble solid contents during the postharvest period. Soluble solids in fruits comprise not only sugars but organic acids, salts and pigments. However, since papaya has low acidity, soluble solids is attributed mainly to sugar content (Bron and Jacomino, 2006). As for the heat treatment, there was no significant effect on the TSS value in the H1, H2 and H3 Eksotika II papaya fruit. Similar results have also been reported for mangoes where the soluble solids were not affected by an insect disinfestation vapour heat treatment (Jacobi *et al.*, 1995; Jacobi and Giles, 1997). These results also are comparable to that reported for tomatoes by Lurie and Klein, (1991) who observed that heat treatments, either water or hot air at 38 – 48°C for 1 hour to 3 days had no effect on tomato soluble solids.

With regard to chlorophyll fluorescence, which is indicative of the tissues' photosynthetic capacity, the Fv/Fm ratios in untreated H1, H2 and H3 fruit at day 1 after harvest were at 0.84, 0.83 and 0.83, respectively, representing healthy plant tissue. As the fruit ripens, the Fv/Fm ratio in the untreated H1, H2 and H3 fruit decreased, although some of the decreases were not statistically different. It is reasonable to expect that chlorophyll fluorescence yield declines during ripening, with the fact that chlorophyll content decreases and there is a general breakdown of the photosynthetic units during fruit ripening (Sanxter *et al.*, 1992), which will affect the fluorescence

measurement. Similarly, Bron *et al.* (2004) reported that during papaya ripening, the Fv/Fm ratios decreased indicating that chlorophyll fluorescence which is closely associated with chloroplast function, declined in parallel with the decline of other quality characteristics during papaya fruit ripening. This phenomenon was also reported by Blackbourn *et al.* (1990) who worked with bananas. They observed a drop in the Fv/Fm parameters with advancing ripening and senescence in bananas.

It was also observed that the Fo increased while the Fv/Fm, Fm and Fv parameters decreased rapidly in treated H1, H2 and H3 fruit within one hour after hot water treatment. This phenomenon is probably due to the temporary disassociation of the light harvesting complexes from the reaction centres of PSII in the thylakoid membrane in the heated tissues. This disassociation would decrease the probability of energy transfer and thus light energy absorbed in the light harvesting complex would be given off as stray fluorescence from the pigment bed, increasing the Fo value and decreasing the Fv/Fm value (DeEll *et al.*, 1999). Furthermore, Calvin cycle activity or the dark reactions of photosynthesis is generally more sensitive to inactivation by heat than either photosynthetic electron transport or photophosporylation (Bilger *et al.*, 1987; Weis, 1981). Schreiber and Bilger (1987) found that the reduction of Calvin cycle activity preceded PSII damage in *Arbutus unedo* L., expressed by an increase in Fo and a decrease in Fm.

However, at subsequent ripening stages, Fo decreased and Fv/Fm increased in the treated H1, H2 and H3 fruit, although the value still lower compared to the untreated fruit, suggesting that the treated H1, H2 and H3 fruit were able to recover from the heat treatment and were not permanently damaged by hot water treatment. These results are similar to that reported on avocado fruit, where Woolf and Laing (1996) found that the

Fv/Fm rapidly decreased to near minimal level within one hour after hot water treatment at 50°C for 1 to 10 min, whilst only small changes in Fv/Fm ratios was observed during the following 8 days of storage. In addition, Tian *et al.* (1996) found that the Fv/Fm ratios in broccoli (*Brassica Oleracea* L.) decreased immediately after hot water treatment, but subsequently recovered during storage at 20°C. These results suggest that in broccoli and avocado, there was PSII recovery or repair following hot water treatment and our results indicate that this probably occurred in the hot water treated Eksotika II papayas in the present study.

Physiological and organoleptic observations from this study indicate that Eksotika II papaya should be harvested at Index 2 for hot water treatment. When harvested at this stage, the papaya fruit ripened correctly and was tolerant to the hot water treatment applied and has acceptable shelf life prior to exportation. The H3 fruit only will be suitable for the local market, which does not need the disinfestation hot water treatment. Harvesting of H1 fruit is not recommended as the ripened fruit (whether hot water treated or untreated) does not exhibit satisfactory organoleptic properties. In other words, the present study suggests that, while the Index 2 papaya fruit may already be predisposed to ripen independently, the papaya fruit harvested at Index 1 is not ready to ripen “off the tree”.

Chapter 4: Postharvest Changes in Sugar Accumulation of Hot Water Treated and Untreated Eksotika II Papaya during Ripening

4.1 Introduction

Sugars, either in its free state or as derivatives, basically influence the attractive flavour of fruits. Postharvest changes in sugar composition have been widely documented especially in climacteric fruits such as papaya (Chan Jr. *et al.*, 1979; Selvaraj *et al.*, 1982) and banana (Cordenunsi and Lajolo, 1995) and therefore it is very important for research on postharvest ripening to include the changes in sugar composition. The main sugars that are usually associated to the sweetness of a fruit are sucrose (non-reducing sugar), glucose and fructose (reducing sugars) with the predominate sugar varying in different fruits when they are ripe. For instance, the predominant sugar in a climacteric fruit such as bananas is sucrose, where at the green stage, bananas have a high starch content which is metabolized to sucrose after harvesting, leading to the fruit sweetness (Cordenunsi and Lajolo, 1995).

However, other climacteric fruit, such as papayas, do not accumulate starch during development; since only about 0.1 % starch was found in harvested mature green papayas (Selvaraj *et al.*, 1982) and about 0.06 % starch was found in postharvest ripened papaya fruit (Gomez *et al.*, 2002). Because papayas have a low starch content at harvest time, it would not be a sufficient carbon source for the increase in sucrose content and for postharvest sweetening. According to Zhou and Paull (2001), the papaya sugar content remains constant during postharvest ripening, suggesting that sugar accumulation in ripening papaya fruit is related to continued sugar translocation from the parent plant to the fruit. This indicates that papaya fruit that ripened attached to the

tree, will receive a constant supply of sucrose that originates from photosynthesis in the leaf. This is supported by the findings which reported for the Solo papayas cultivar where sucrose levels varying from 18% of the total sugars on the 110th day after anthesis to 80% on the 135th day after anthesis in the attached fruit (Chan *et al*., 1979). However, in detached fruit, a variation of about only 2% in the total level of sugars between the 2nd and 22nd day after harvest was reported by Hubbard *et al.* (1991). In the present study, experiments were conducted to improve our understanding of the sweetness process in papayas ripened after harvest.

In some commodities, the sugar content is favourably affected by heat treatment. For instance, 3 hours immersion in 45°C water before cool storage of muskmelons prevented the loss in sucrose which occurred to the non-heated fruit during storage (Lingle *et al.*, 1987). Other than that, heat treated buttercup squash at 30°C hot air was perceived as sweeter by a taste panel (Bycroft *et al.*, 1999). Thus, it was necessary to investigate whether the hot water treatment applied for insect disinfestation in the present study, had any impact on the sugar contents of Eksotika II papaya.

This chapter documents the postharvest changes of sugar content in hot water treated and untreated Eksotika II papayas which were harvested at different harvesting maturities and how the reducing sugar and non-reducing sugar change throughout ripening.

4.2 Materials and Methods

4.2.1 Plant Material and Extraction of Pulp Tissue Sample

Processing and handling of papaya fruit have been described earlier (3.2.1). Three fruit from each ripening stage of treated and untreated fruit were used for this sugar assays. Frozen pulp tissue was collected at various time intervals and kept at -20°C. They were then ground in liquid nitrogen into fine powder using mortar and pestle prior to sugar extraction (4.2.3.).

4.2.2 Preparation of Solutions

Standard Glucose Solution

100 mg of glucose was added to 25.0 ml of perchloric acid (60%) and the volume made up to 1000 ml with dH_20.

Copper Reagent

Reagent A

(i) 25.0 g $C_4H_4O_6KNa.4H_2O$
(ii) 25.0 g Na_2CO_3
(iii) 20.0 g $NaHCO_3$
(iv) 200.0 g Na_2SO_4

(i) - (iv) were dissolved in 800 ml of dH_2O and then made up to 1000 ml

Reagent B

15.0 g $CuSO_4.5H_2O$ was dissolved in 100 ml of dH_20

Copper Reagent was obtained by adding 1.0 ml of *Reagent B* to 25.0 ml of *Reagent A.*

Arsenomolybdate Solution

(i) 25.0 g $(NH_4)_6Mo_7.4H_2O$
(ii) 21.0 ml of concentrated H_2SO_4
(iii) 3.0 g Na_2HAsO_4

(i) was dissolved in 450 ml of dH_20 and (ii) was added slowly to (i) while mixing the solution and this was followed by the addition of (iii). The mixture was incubated at 37°C for 24 hours

4.2.3. Extraction of Sugars

Approximately 1g of papaya pulp powder was added to 4 ml of 0.5 *N* NaOH and the mixture was centrifuged at 3500 x g (5500 rpm) for 20 minutes at 4 °C. The supernatant was neutralized with 0.5 *N* acetic acid and the volume made up to 20 ml ddH_2O. This solution was used in the determination of sugars (Areas and Lajolo, 1981).

4.2.3.1 Determination of Total Sugars

Total sugar content was determined using the Phenol-sulphuric method (Dubois *et al.*, 1956). In this method, 1 ml of the sample solution (4.2.3.) was added to 0.1 ml of phenol, followed by the addition of 5 ml of concentrated sulphuric acid. Then test tubes were immediately placed on ice for 5 minutes to prevent the temperature rising above 30˚C. This step was extremely critical as the reaction of the acid causes rapid increase in temperature which could then caramelize the sugars, thus producing a darker colour which would interfere with sugar estimation. The mixture was then allowed to stand at room temperature for 10 minutes and then incubated in a 25 – 30°C water bath (Pharmacia Biotech Multi Temp III) for 15 minutes. The absorbance at 490 nm was recorded using spectrophotometer 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 glucose concentrations between 0-100 µg/ml. Total sugar was expressed in mg/g fresh weight.

4.2.3.2 Determination of Total Reducing Sugars

Reducing sugars were assayed using the Somogyi-Nelson method (Nelson, 1944; Somogyi, 1945; Somogyi, 1952). One ml of the sample solution (4.2.3.) was added to 1 ml of copper reagent in a test tube and vortexed. The mouth of the test tubes used were closed with marble and covered with aluminium foil and subsequently heated in boiling water bath for 20 minutes. After that, the mixture was cooled on ice and 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 a spectrophotometer 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 glucose concentrations between 0-100 μg/ml. Total reducing sugar was expressed in mg/g fresh weight.

4.2.4 Statistical Analysis

The experimental design was completely randomized. Data were analysed by analysis of variance (ANOVA). Where possible, mean comparisons were made using the Duncan Multiple Range test (DMRT) at $P < 0.05$. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) software (IBM Corporation, USA).

4.3 Results

4.3.1 Total Sugar Accumulation in Treated and Untreated Eksotika II Papaya during Ripening

Figure 4.1 shows that maturity stages at harvest had a significant effect on total sugar content of Eksotika II papaya. Untreated H3 fruit had the highest total sugar content followed by H2 and H1 fruit with the values of 69.98, 59.06, 46.14 mg/g, respectively. Besides, within one hour after treatment, the total sugar content of hot water treated H1, H2 and H3 fruit also were not affected by the heat treatment. Moreover, as shown in Figure 4.2, the total sugar content in untreated H1 fruit did not change throughout ripening until at Index 3 to Index 4, the total sugar content increased from 43.88 to 52.40 mg/g. For untreated H2 fruit, the total sugar content increased from 59.06 mg/g at Index 2 to 67.39 mg/g at Index 3. Then, the total sugar content remained constant until Index 5. For untreated H3 fruit, the total sugar content remained unchanged from Index 3 to Index 5. Throughout ripening, H1 fruit had lower total sugar content than H2 and H3 fruit. In H2 fruit, the total sugar content increased during ripening and reached almost the same amount as the total sugar content in H3 fruit even though initially at harvest, H2 fruit has significantly lower amount of total sugar than H3 fruit. As for the hot water treated fruit, there were no significant differences in the total sugar content between treated and untreated H1, H2 and H3 fruit at all ripening stages.

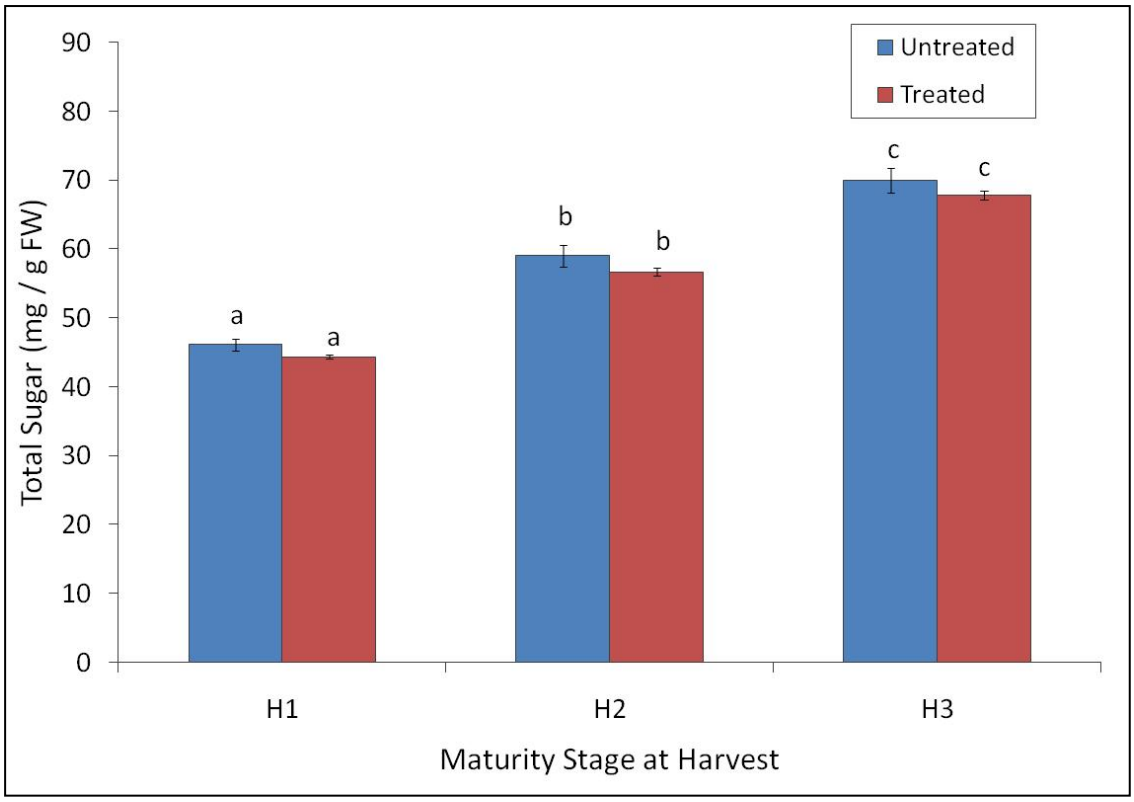


Figure 4.1: Initial total sugar content in Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9)

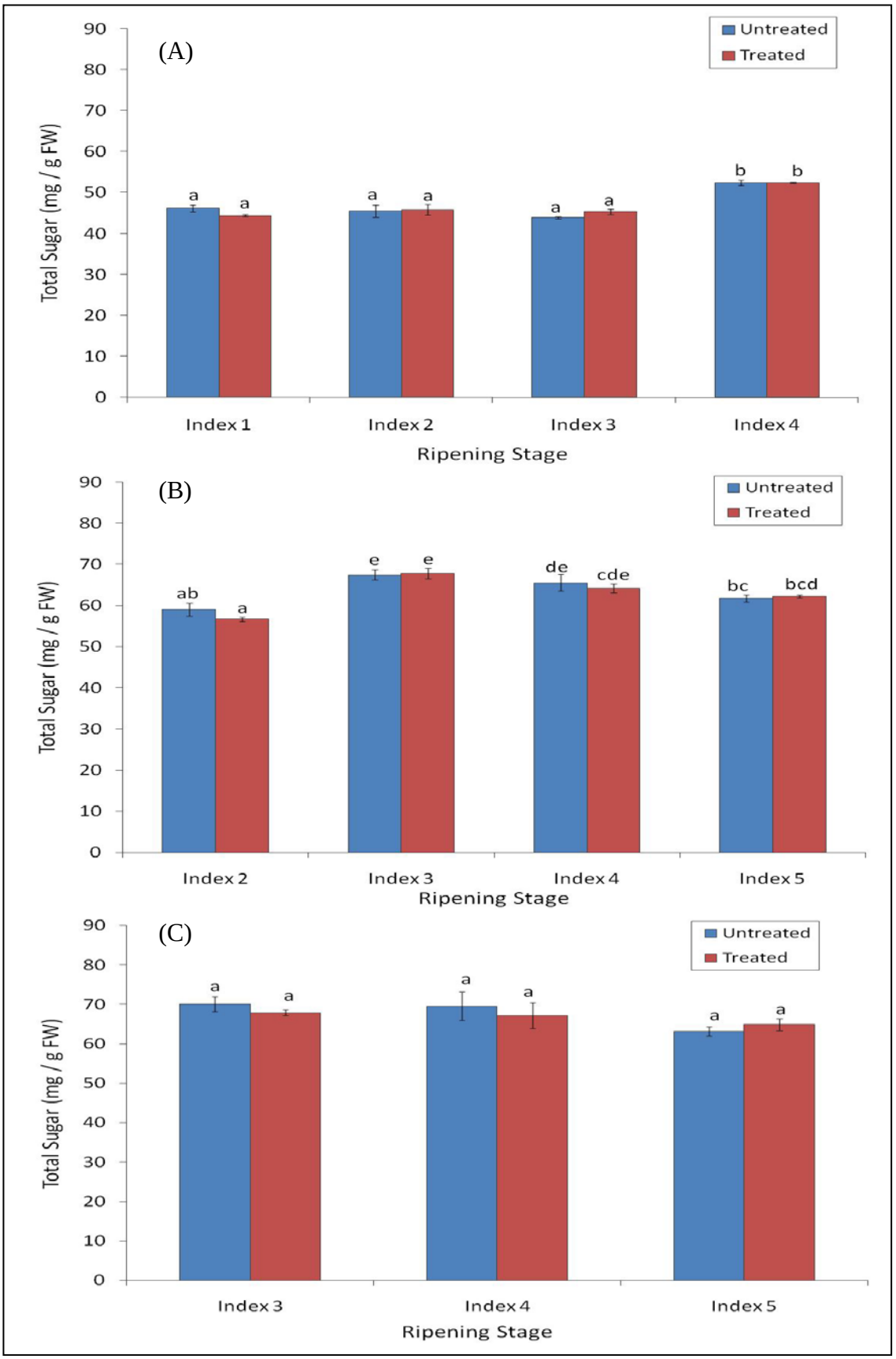


Figure 4.2: Total sugar content in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9).

4.3.2 Total Reducing Sugar Accumulation in Treated and Untreated Eksotika II Papaya during Ripening

Figure 4.3 shows that even though were harvested at different maturity stages, the total reducing sugar content in untreated H1, H2 and H3 fruit were not significantly different. Moreover, within one hour after treatment, the total sugar content of hot water treated H1, H2 and H3 fruit also were not affected by the heat treatment. The total reducing sugar content in untreated H1 fruit increased from 14.29 mg/g at Index 1 to 21.53 mg/g at Index 3. Subsequently, the value decreased to 18.19 mg/g at Index 4. In untreated H2 fruit, the total reducing sugar content increased slightly from 14.16 mg/g at Index 2 to 18.26 mg/g at Index 5. As for H3 fruit, the total reducing sugar content also increased significantly from 16.47 mg/g at Index 3 to 21.60 mg/g at Index 4 but then remained constant until Index 5. As for the heat treated fruit, treated H1 fruit were found to have a higher total reducing sugar content that untreated fruit at Index 2. Other than that, the hot water treatment did not have any effect on the total reducing sugar content in treated Eksotika II papaya fruit (Figure 4.4).

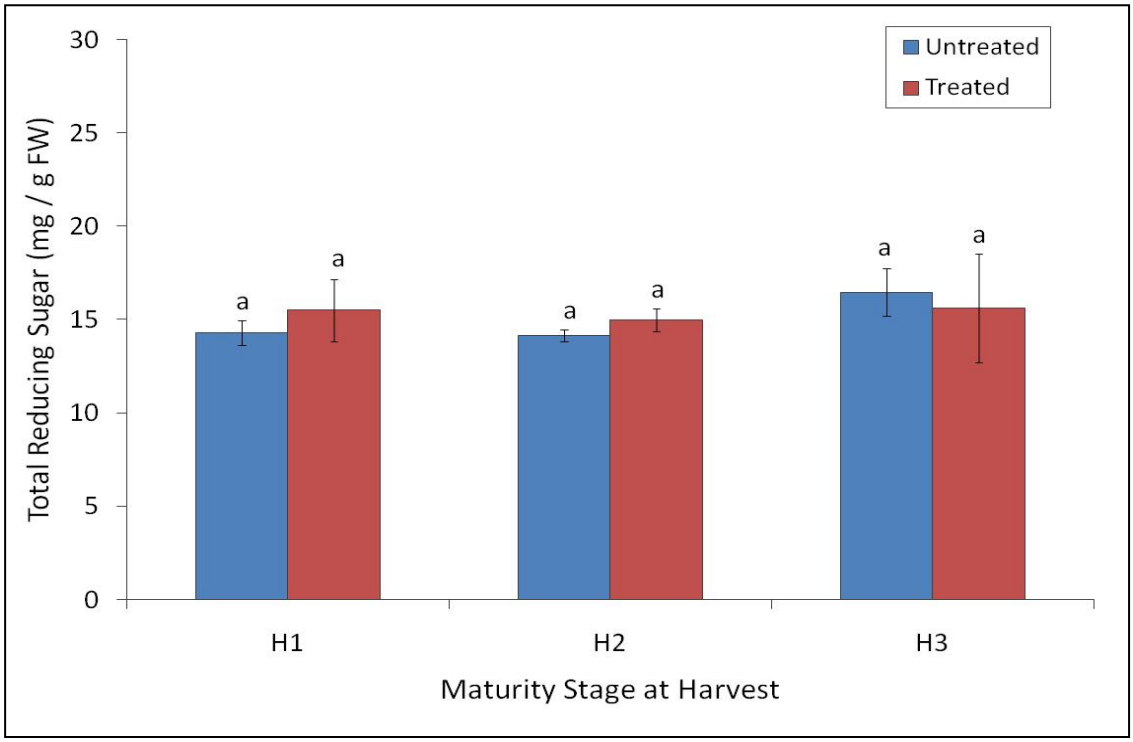


Figure 4.3: Initial total reducing sugar content of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9).

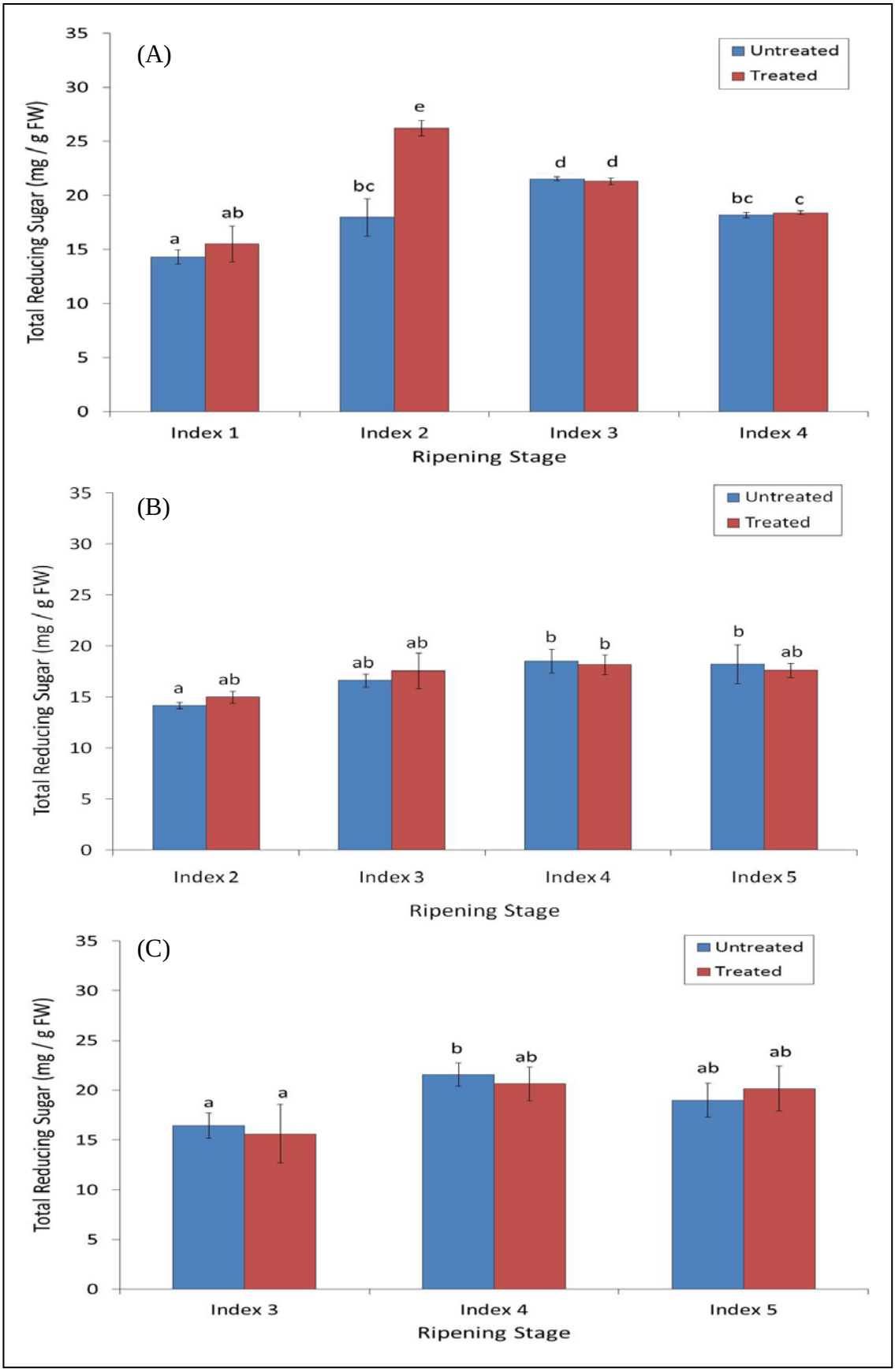


Figure 4.4: Total reducing sugar content in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9)

4.3.3 Total Non-Reducing Sugar Accumulation in Treated and Untreated Eksotika II Papaya during Ripening

The values of the total non-reducing sugar content of Eksotika II papaya were obtained by subtracting the amount of total reducing sugar content from the amount of total sugar content. The non-reducing sugar content was found to increase with the increase of maturity stages at harvest (Figure 4.5). At harvest, H3 fruit exhibited the highest total non-reducing sugar content, followed by H2 and H1 fruit and this trend continued until the end of the experiment. As shown in Figure 4.6, the total non-reducing sugar content in H1 fruit decreased at the early stages of ripening and then increased when it reached Index 4. In contrast, for untreated H2 fruit, the non-reducing sugar content increased from 44.88 mg/g at harvest to 50.75 at Index 3. Subsequently, the total non-reducing sugar content decreased slightly to 47.04 mg/g and 43.42 mg/g at Index 4 and 5, respectively. For untreated H3 fruit, the non-reducing sugar content decreased gradually throughout ripening but was not significantly different. As for the heat treated fruit, treated Index 1 and Index 2 H1 fruit had lower non-reducing sugar content than untreated fruit, but then recover to the same value as untreated fruit at the later ripening stages. As for treated H2 and H3 fruit, the non-reducing sugar content was not affected by the hot water treatment.

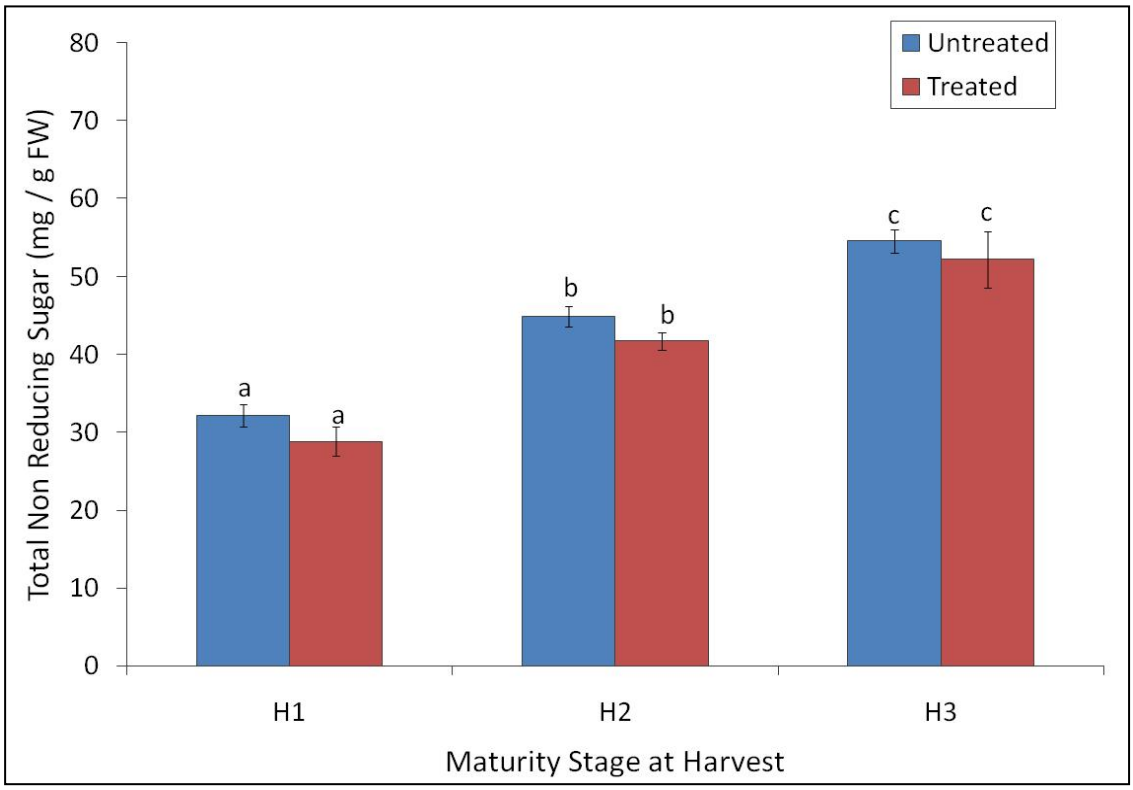


Figure 4.5: Initial total non-reducing sugar content of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9)

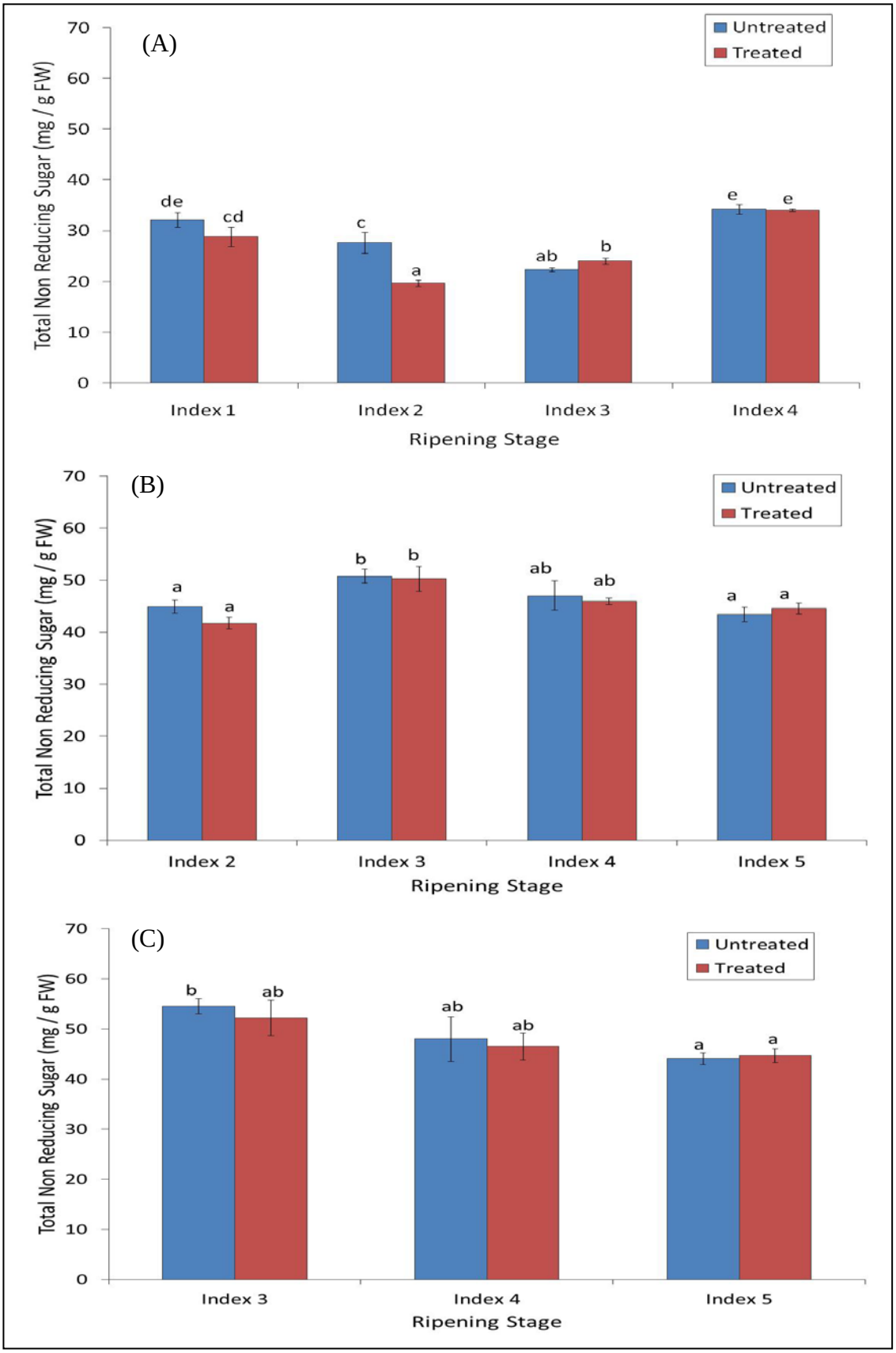


Figure 4.6: Total non-reducing sugar content in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9).

4.4 Discussion

Using a typical high performance liquid chromatography with pulse amperometric detection (HPLC-PAD), Gomez *et al.* (2002) reported that in Solo papaya cultivar, there are three primary soluble sugars found in the ripening fruit and these are glucose, fructose and sucrose. Therefore, it is suggested that the total sugars in papaya consist mainly of glucose and fructose as the reducing sugars, and sucrose as the primary non-reducing sugars. The total sugar content in H1 and H2 Eksotika II papaya fruit in the present study showed a significant increase throughout ripening, although the total sugar content in H3 fruit remained constant. A similar trend was also observed by Fabi *et al.* (2007) which found that the total soluble sugars in Golden papaya increased 2.5 times during ripening. As for the H3 fruit, the result was similar to those obtained by Zhou and Paull (2001), who found that the papaya sugar content remains constant during postharvest ripening.

It is highly unlikely that starch is the source of the sugars mainly because it has been well documented there is only trace amounts of the polysaccharide in the papaya fruit (Selvaraj *et al.*, 1982; Gomez *et al.*, 2002). Therefore, it is possible to speculate that sucrose synthesis is operative during ripening. However, if sucrose synthesis is operative during ripening, it can only be possible if the green tissues photosynthesize to provide the triose phosphates (carbon skeletons) for fructose-6-phosphate and glucose-1-phosphate formation. Both these latter sugar phosphates are precursors for sucrose phosphate and sucrose synthesis. If this does not occur, then the other possible source of sugars during ripening of detached papaya is from the disassembly of the cell wall as suggested by Lazan *et al*. (1995) and Paull *et al*. (1999). It is now more evident that, not only are the cell wall polysaccharides consist of mainly glucose, but its synthesis comes

from sucrose (glucose and fructose) via a membrane bound sucrose synthase which provides UDP-glucose for cellulose formation (Bowsher *et al*., 2008). Since the sucrose synthase reaction is reversible, it is possible, albeit speculative, that glucose released from cell wall degradation can link up with UTP to form UDP-glucose and subsequently sucrose.

Moreover, according to Gomez *et al.* (2002), water-soluble polysaccharides from papaya cell wall such as galactose could be a source of carbon for sugar synthesis during papaya ripening. This is due to the findings by their research group which showed a decrease in galactose and an increase in glucose contents in ripening papaya fruit which indicate a degalactosylation of the main polysaccharide chain, an event has also been observed in apples, tomatoes, strawberries and germinating seeds (Pressey, 1983; Brett and Waldron, 1996). Apart from that, sucrose synthesis during detached papaya ripening may be related to the sucrose phosphate synthase activity in the papaya pulp which has been reported by Gomez *et al.* (2002).

The stage of ripeness when harvested greatly influences the total sugar content of the papaya fruit. As shown in the results section, H1 fruit contained significantly lower total sugars amount compared to H2 and H3 fruit during ripening. This result correlated well with the TSS value of H1 fruit which was also found to be lower than that of the H2 and H3 fruit during ripening. Selvaraj *et al.* (1982) reported that the sucrose content increases up to five fold from 110 days to 130 days after anthesis in papayas attached to the tree, when skin colour begins to change. This probably explains why fruit harvested at Index 1 (mature green), did not have sufficient time to accumulate soluble sugars before harvest, which resulted in low soluble sugars content. Moreover, harvesting at Index 2 is sufficient to obtain good quality papaya fruit with high soluble sugar content.

This was evident in this study where although H2 fruit had significantly lower total sugar content compared to the H3 fruit when harvested, the total sugar content increased and reached the same value as fruit harvested at more advanced stage, when it ripened.

The total reducing sugars (mainly glucose and fructose) in H1, H2 and H3 fruit increased throughout ripening. This result is similar to the findings reported by Fabi *et al.* (2007) who found that glucose and fructose content increased during ripening in the Golden papaya fruit. This could be an indication that these reducing sugars come from the accumulated sucrose, especially by the action of the invertase enzyme which hydrolyzes sucrose to glucose and fructose (Chan and Kwok, 1975; Zhou *et al.*, 2003). Besides, an increase in the hexose content was concomitant with a decrease in non-reducing sugars (mainly sucrose) content.

The results in the present study showed that in the H2 and H3 fruit, the amount of total non-reducing sugar, which is mainly sucrose, accounts for around 65% - 76% of the overall total sugar content at all ripening stages. However, in H1 fruit, the non-reducing sugar content varied around 50% to 69% of the total sugar during ripening. This showed that the non-reducing sugar is still the predominant sugar at all ripening stages in H1 fruit. These results indicate that the predominant sugar of Eksotika II papaya found in the present study is the non-reducing sugar which is primarily sucrose. These results also support the findings of Gomez *et al.* (2002) who reported that in half ripe and ripe Solo papaya, sucrose account for about 60% of the total soluble sugars. However, this is in contrast to the findings of Fabi *et al.* (2007) who found that glucose and fructose content increased during ripening in the Golden papaya fruit and accounts for 70 - 80% of the overall total soluble sugar at the later ripening stages. Possibly, these differences are due to the different papaya cultivars used in the studies.

The results also showed that the hot water treatment applied in this study had a minimal effect on the sugar content of the Eksotika II papaya fruit. Treated H1 fruit seems to have significantly higher reducing sugar content at Index 2, although the overall total sugar content of the fruit was not affected. This increase in reducing sugar was followed by a decrease in non-reducing sugar at Index 1 and 2 of H1 fruit. Besides, it is also possible to speculate that sucrose synthesis might be affected by the hot water treatment because of reduced sucrose phosphate synthase activity which is involved in the continuous synthesis of sucrose (Gomez *et al.*, 2002) or reduced sucrose synthase activity, as these enzymes can also determine sucrose levels in the tissues. The activity of these enzymes in heat treated papaya might be reduced because of the heat stress which inactivates the enzyme activity. However, the total sugar, reducing sugar and non-reducing sugar content of treated H2 and H3 fruit at all ripening stages were not affected by the hot water treatment applied.

Chapter 5: Cell Wall Degrading Enzymes Activity of Treated and Untreated Eksotika II Papaya during Ripening

5.1 Introduction

Fruit ripening is accompanied by the softening of the flesh tissue. This textural change is a major event in fruit softening, and is the integral part of ripening, which involves structural as well as compositional changes in the various components of the wall carbohydrates, partly as a result of action of cell wall degrading enzymes (Fischer and Bennet, 1991). The major classes of cell wall polysaccharides that undergo modifications during ripening are pectins, cellulose and hemicelluloses (Prasanna *et al.*, 2007). Biochemical studies of cell wall changes during ripening indicate that the structural changes in pectin, hemicelluloses and cellulose together are responsible for the alteration of cell wall structure (Huber, 1983b; Fishman *et al.*, 1989; Paull *et al.*, 1999). The cell walls are thoroughly modified by solubilisation, deesterification and depolimerization, accompanied by an extensive loss of neutral sugars and galacturonic acid, followed by solubilization of the remaining sugar residues and oligosaccharides (Voragen *et al.*, 1995). The main enzymes that are responsible for the disassembly of the cell wall during ripening are the cell wall hydrolases such as pectin methylesterase (PME), polygalacturonase (PG), pectate lyase (PL) and cellulase (Vicente *et al.*, 2007).

PME, a ubiquitous enzyme in plant tissues, is plentiful in many fruits such as tomato (Gaffe *et al*., 1994) and peach (Glover and Brady, 1994). PME (EC 3.1.1.11) catalyzes the hydrolysis of galactosyluronate methyl esters that results in the deesterification of pectins (Sozzi, 2004). PME activity has been shown to decrease (Abu-Sarra and Abu-Goukh, 1992), increase (Paull and Chen, 1983; Selvaraj and Kumar, 1989) and remain constant (Ashraf *et al.*, 1981) during fruit ripening. Another pectolytic enzyme, exo-PG

(EC 3.2.1.67), acts on pectic acid (polygalacturonic acid, PGA). It hydrolyzes the α-1,4-glycosidic bonds between the galacturonic acid residues in galacturonans, which results in the release of galacturonic acid as the major reaction product (Prasanna *et al.*, 2007). It is generally accepted that PG is primarily responsible for dissolution of the middle lamella during fruit ripening (Jackman and Stanley, 1995). Pectate lyases (EC 4.2.2.2) (PL) are also enzymes involved in pectin degradation. They catalyse the cleavage of α-1,4-galacturonoside linkages in the polymer backbone, causing depolymerisation. However, the mechanism of reaction differs from the hydrolysis catalyzed by PG. PL causes a β-elimination that introduces an unsaturated C4-C5 bond at the non reducing terminus of the pectin polymer reaction product (Sozzi, 2004).

Another important cell wall degrading enzyme is cellulase or also known as endo-β-1,4-glucanase (EC 3.2.1.4). It hydrolyzes internal linkages of (1→4)*β*-D-linked glucan chains adjacent to unsubstituted residues, and *in vitro* are active against xyloglucan, cello-oligosaccharides, non-crystalline cellulose and CMC (carboxymethyl cellulose). In the cell wall, *in vivo*, their substrates probably include xyloglucan, integral and pheripheral regions of non-crystalline cellulose, and possibly glucomannan where sufficient consecutive (1→4)*β*-D linked glucan residues occur for substrate binding (Brummell and Harpster, 2001). Cellulase activity increased during the ripening of avocado, peach, strawberry, tomato, and papaya (Hobson, 1981; Paull and Chen, 1983). The loss of firmness, climacteric rise of respiration and ethylene evolution in ripening fruit have been directly correlated with marked increase in cellulase activity (Roe and Bruemmer, 1981; Abu-Sarra and Abu-Goukh, 1992). However, in papaya, it has been reported that cellulase activity increases before the rise in ethylene production and therefore does not correlate with the climacteric increase in respiration (Paull and Chen,

1983). Cellulase activity also has been reported in several Indian mango cultivars, where its activity increased during ripening (Selvaraj and Kumar, 1989).

Economically, softening is an extremely important postharvest event because physical injury that occurs during handling of fruits and their susceptibility to diseases increase proportionately with softening (Manrique and Lajolo, 2004). Besides, the textural properties of fruits in general play a very significant role in consumer acceptability. Therefore, in order to identify proper fruit handling to avoid mechanical damage during harvesting and transportation and also optimization of fruit quality, it is important to understand the process of softening that occurs during papaya fruit ripening.

Fruit softening is often slowed down following exposure to heat treatments. In tomato, heat stress interfered with PG accumulation (Yoshida *et al.*, 1984) and inhibited the normal increase in soluble polyuronides (Mitcham and McDonald, 1992). In papaya, PG activity was reduced after exposure to heat treatments (Chan *et al.*, 1981; Lazan *et al.*, 1989). Moreover, papaya does not recover the ability to soften after heat shock exposure (Paull and Chen, 1990). The softening disruption has been ascribed to reduction of cell wall hydrolytic enzymes. Although heat disruption of cell wall breakdown has been proposed as the cause for delayed or poor softening, the actual enzyme having the central role in softening has not been determined (Lashbrook *et al.*, 1998; Rose *et al.*, 1998). Moreover, the response of commodity to heat stress is highly dependent on the temperature and the exposure time. Therefore, the objective of the present study is to investigate the effect of hot water disinfestation treatment on the activities of cell wall degrading enzymes in Eksotika II papaya fruit during ripening, and at the same time attempt to understand the role of these enzymes in ripening Eksotika II papaya.

5.2 Material and Methods

5.2.1 Plant Material

Processing and handling of papaya has been described earlier (3.2.1). Three fruit from each ripening stage of treated and untreated fruit were used for these assays. Pulp tissues was collected at various time intervals and kept at -20°C. They were then ground in liquid nitrogen into fine powder using mortar and pestle prior to protein extraction (5.2.2).

5.2.2 Extraction and Estimation of Protein

5.2.2.1 Preparation of Protein Extraction Buffer

The protein extraction buffer was prepared according to Kanellis *et al.* (1989) and modified by Chandran (1998). The extraction buffer contained 50mM Tris-HCl, 0.5M NaCl, 10mM 2-mercaptoethanol, 10μM leupeptin, 1mM DTT, 1mM EDTA, 10% glycerol and 0.5% Triton X-100.

5.2.2.2 Protein Extraction

Total proteins were extracted by adding 6 g of papaya pulp powder (5.2.1) to 8 ml of the protein extraction buffer. The mixture was then vortexed thoroughly, left on ice for 10 minutes and then centrifuged at 25,000 x g for 30 minutes at 4 °C in a Sorvall RC5C refrigerated centrifuge. Upon centrifugation, the pellet was discarded and the supernatant was collected by filtering through one layer of miracloth. Extracted samples were used immediately for analysis.

5.2.2.3 Preparation of Reagents for Protein Estimation

Bradford Reagent

20 mg of Coomasie Blue G250 was dissolved in 10 ml of 95% ethanol. 20 ml of 85% phosphoric acid was added into the solution. The final volume was made up to 200 ml with distilled water. The solution was filtered using Whatman filter paper and stored at 4°C (Bradford, 1976).

Standard Protein Solution, Bovine Serum Albumin (BSA) – 1mg/ml

100 mg of BSA was dissolved in 80 ml of distilled water. The final volume was made up to 100 ml with distilled water.

Standard Curve for Protein Estimation

The various concentrations of BSA standard solutions are shown in the table below. Colour development was initiated with the addition of 5 ml Bradford reagent into each BSA standard solution.

Table 5.1: Preparation of standard BSA solution for protein estimation assay

Tubes	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
ddH_2O (µl)	100	90	80	70	60	50	30	20	0
BSA (µl)	0	10	20	30	40	50	70	80	100
BSA (µg)	0	10	20	30	40	50	70	80	100

5.2.2.4 Protein Estimation

The protein content in the pulp extracts was estimated using the Bradford (1976) method. 25 µl of extracted samples and 75 µl of distilled water were added into a test tube. Then 5 ml of Bradford solution was added into the solution. The mixture was vortexed and the absorbance at 595 nm was read and recorded using a spectrophotometer. Protein content was estimated using BSA as a standard.

5.2.3 Pectin Methylesterase (PME) Assay

The following method was adapted from Lohani *et al*. (2004) and the activity was calculated using a standard curve of galacturonic acid drawn as described by Hagerman and Austin (1986).

5.2.3.1 Preparation of Reagents

Sodium Hydroxide (NaOH) 2M

8 g of NaOH pellet was dissolved in 50 ml of ddH_2O. The final volume was made up to 100 ml with ddH_2O.

1% (w/v) Pectin Solution (pH 7.5)

One gram of pectin was dissolved in 80 ml of ddH_2O. The solution was heated to 40 °C while continually stirring. Then the solution was allowed to cool and the pH was adjusted to 7.5 using NaOH. The final volume was made up to 100 ml with ddH_2O and the solution was stored at 4 °C.

Potassium Hydrogen Phosphate (KH_2PO_4) (0.003M) pH7.5

i. 0.04 g of KH_2PO_4 was dissolved in 100 ml of ddH_2O

ii. 0.05 g of K_2HPO_4 (DiPotassium Hydrogen Phosphate) was dissolved in 100 ml of ddH_2O

Finally, 16.6 ml of (i) and 83.4 ml of (ii) were mixed together before use (Sambrook *et al*., 1989).

0.01% (w/v) Bromothymol Blue

1 ml of 0.1% Bromothymol blue was added into 9 ml of phosphate buffer [KH_2PO_4 (0.003M) pH7.5]

0.15M Sodium Chloride (NaCl)

0.17 g of NaCl was dissolved in 15 ml of ddH_2O. Final volume was made up to 20 ml with ddH_2O.

1mM Galacturonic Acid ($C_6H_{10}O_7$)

0.02 g of galacturonic acid was dissolved in 100 ml of ddH_2O.

5.2.3.2 Standard Curve

One ml of pectin solution and 0.1 ml of bromothymol blue were added into a test tube. Then galacturonic acid and ddH_2O were added as shown in Table 5.2 below. Absorbance at 620 nm was read using a spectrophotometer and readings were recorded immediately and at 3 minutes interval. Distilled water was used as blank in this assay.

Table 5.2: Preparation of standard galacturonic acid (GA) solution for PME assay

Tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
GA (ml)	0	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
ddH_2O (ml)	0.5	0.4	0.35	0.3	0.25	0.2	0.15	0.1	0.05	0
GA (µmol)	0	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5

5.2.3.3 Preparation of Samples and PME Assay

The pH of the extracted sample was adjusted to 7.5 with NaOH. Next, 1 ml of pectin solution, 0.2 ml of NaCl, 0.1 ml of bromothymol blue, 0.2 ml of ddH_2O and 0.1 ml of extracted sample were added into a test tube. The test tube was shaken gently and the absorbance at 620 nm was read immediately and after 3 minutes using a spectrophotometer. Distilled water was used as a blank for this assay. The difference in absorbance between 0 and 3 minutes was the measure of PME activity. Activities of enzyme were expressed as specific activity (per mg protein). One unit is defined as the amount of the enzyme required for liberating 1µmol of methyl ester per minute.

5.2.4 Polygalacturonase (PG) Assay

Polygalacturonase activity was assayed as describe by Pathak and Sanwal (1998) with modification by Lohani *et al.* (2004).

5.2.4.1 Preparation of Reagents

Sodium Acetate (NaAc) 0.2M pH 4.5

0.16 g of NaAc was dissolved in 8 ml of ddH_2O and the pH was adjusted to 4.5 with acetic acid. Final volume was made up to 10 ml.

Sodium Chloride (NaCl) 0.2M pH 4.5

0.4 ml of 5M NaCl was added to 9.6 ml of ddH_2O.

1% Polygalacturonic acid (PGA) pH 4.5

0.3g of PGA was dissolved in 25 ml of ddH_2O and pH was adjusted to 4.5 with acetic acid. Final volume was made up to 30 ml.

Dinitrosalicyclic Acid (DNS) stock solution (70 ml)

The following were added together and then the final volume was made up to 70 ml with ddH_2O.

i) 0.63% Dinitrosalicyclic acid (0.441g)

ii) 0.5% Phenol (0.35g)

iii) 0.5% Sodium bisulfite (0.35g)

iv) 2.14% Sodium hydroxide (1.498g)

40% Rochelle salt

20g of Potassium Sodium Tartarate (Rochelle salt) was dissolved in 40 ml of ddH_2O. Final volume was made up to 50 ml.

10mM Galacturonic Acid (Stock Solution 100 ml)

0.212 g of galacturonic acid was dissolved in 100 ml of ddH_2O.

5.2.4.2 Standard Curve

One ml of DNS was added into a test tube followed by galacturonic acid (GA) and ddH_2O as shown in Table 5.3 below. The mixture was placed in a boiling water bath for 5 minutes. Then, 0.4 ml of Rochelle salt was added into the mixture and cooled under running water. The absorbance was read at 540 nm using a spectrophotometer and the readings recorded. A mixture containing 0 mg of GA was used as blank for PG standard curve assay.

Table 5.3: Preparation of standard GA solution for PG assay

Tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
ddH_2O (ml)	1	0.976	0.952	0.928	0.904	0.880	0.856	0.832
GA (ml)	0	0.024	0.048	0.072	0.096	0.120	0.144	0.168
GA (µmol)	0	0.24	0.48	0.72	0.96	1.20	1.44	1.68

5.2.4.3 Preparation of Samples and PG Assay

In a test tube, 0.4 ml of extracted sample was mixed with 0.2 ml of NaAc and 0.1 ml of NaCl. Then 0.3 ml of 1% PGA was added into the solution. The solution was incubated at 37°C oven for one hour. After the one hour incubation period, 1 ml of DNS was added to stop enzyme reaction. The solution was placed in a boiling water bath for 5 minutes. Subsequently, the solution was taken out from the boiling water bath and 0.4 ml Rochelle salt was added and the solution was cooled under running water. The absorbance at 540 nm was read using a spectrophotometer and the readings recorded. A solution containing boiled extracted samples (boiled / denatured enzymes) was used as the control in this assay. Enzyme activity was expressed as specific activity (per mg protein). One unit of enzyme is defined as the amount of the enzyme required to liberate 1µmol of galacturonic acid per minute under the conditions of the enzyme assay.

5.2.5 Pectate Lyase (PL) Assay

Pectate lyase activity was assayed as described by Moran *et al.* (1968) with modification by Lohani *et al.* (2004).

5.2.51 Preparation of Reagents

4mM Sodium Acetate (NaAc) (Stock Solution 50 ml)

0.016 g of NaAc was dissolved in 45 ml of ddH_2O and the pH was adjusted to 4.5 with acetic acid. Final volume was made up to 50 ml.

1% Polygalacturonic acid (PGA) pH 4.5

0.3 g of PGA was dissolved in 25 ml of ddH_2O and pH was adjusted to 4.5 with acetic acid. The final volume was made up to 30 ml with ddH_2O.

4mM Calcium Chloride ($CaCl_2$)

0.059 g of $CaCl_2$ was dissolved in 10 ml of ddH_2O.

5.2.5.2 Preparation of Samples and PL Assay

In a test tube, 0.3 ml of extracted sample was mixed with 1.8 ml of 4mM NaAc, 0.8 ml of 1% PGA and 0.1 ml 4mM $CaCl_2$. Then, the test tube was incubated at 37 °C for 30 minutes and then placed in a boiling water bath for 2 minutes to stop enzyme reaction. The absorbance at 235 nm was read using a spectrophotometer in UV region and the readings recorded. A mixture containing boiled extracted samples (boiled / denatured enzymes) was used as blank in this assay. Enzyme activity was expressed as specific activity (per mg protein). One unit of pectate lyase activity was expressed as the amount of enzyme required to liberate 1µmol of aldehyde groups from PGA per minute under the conditions of the enzyme assay. Units of PL activity were calculated using the following equation:

PL activity (unit / ml) = $\frac{\text{OD (sample) x V (reaction)}}{\text{[Coefficient factor x t x V (sample)]}}$

V (sample)	=	Volume of sample
t	=	Incubation time
Coefficient factor	=	4.6
1 unit	=	1µmol / min

5.2.6 Cellulase Assay

Cellulase enzyme activity was assayed according to Kim *et al.* (1992) with some modifications.

5.2.6.1 Preparation of Reagents

Dinitrosalicyclic Acid (DNS) stock solution (70 ml)

The following were added together and then the final volume was made up to 70 ml with ddH_2O.

i) 0.63% Dinitrosalicyclic acid (0.441g)

ii) 0.5% Phenol (0.35g)

iii) 0.5% Sodium bisulfite (0.35g)

iv) 2.14% Sodium hydroxide (1.498g)

40% Rochelle salt

20g of Potassium Sodium Tartarate (Rochelle salt) was dissolved in 40 ml of ddH_2O. Final volume was made up to 50 ml.

Glucose standard solution (1mg/ml)

0.1g of glucose was dissolved in 100 ml of ddH_2O.

5.2.6.2 Standard Curve

To 1 ml of DNS in a test tube, glucose and ddH_2O was added as shown in Table 5.4 below. The mixture was placed in a boiling water bath for 5 minutes. Then, 0.4 ml of Rochelle salt was added into the mixture and cooled under running water. The absorbance was read at 540 nm using a spectrophotometer and the readings recorded. A mixture containing 0 µg of glucose was used as blank for cellulase standard curve assay.

Table 5.4: Preparation of standard glucose solution for cellulase assay

Tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Glucose (µl)	0	50	100	200	300	400	500	600	800	1000
ddH_2O (µl)	1000	950	900	800	700	600	500	400	200	0
Glucose (µg)	0	25	50	100	150	200	250	300	400	500

5.2.6.3 Preparation of Samples and Cellulase Assay

To 50 mg of water soluble carboxymethyl cellulose (CMC) in a test tube, 4 ml of extracted sample was added and incubated at 35°C, 390 rpm for 7 hours in a shaking incubator. After incubation, the solution was placed in a boiling water bath for 10 minutes. The DNS assay (Miller, 1959) was carried out for determination of reducing sugar content using glucose as standard. To 1 ml of DNS in a test tube, 1 ml of extracted sample was added and mixed thoroughly. The test tube was placed in a boiling water bath for 5 minutes. Subsequently, 0.4 ml of Rochelle salt was added to the solution and the mixture cooled under running water. The absorbance at 540 nm was read using a spectrophotometer and the readings recorded. A solution containing boiled extracted samples (boiled / denatured enzymes) was used as blank in this assay. Enzyme activity was expressed as µg glucose equivalent released / mg protein / hour.

5.2.7 Statistical Analysis

The experimental design was completely randomized. Data were analysed by analysis of variance (ANOVA). Where possible, mean comparisons were made using the Duncan Multiple Range test (DMRT) at $P < 0.05$. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) software (IBM Corporation, USA).

5.3 Results

5.3.1 Protein Estimation

The soluble protein content of untreated and treated H1, H2 and H3 Eksotika II papaya fruit at harvest is are shown in Figure 5.1. Harvest maturity had a significant effect on the total extractable protein of Eksotika II papaya. Untreated H3 fruit exhibited the highest total protein content followed by untreated H2 and H1 fruit with the value of 2.75, 2.17 and 1.90 mg/g, respectively. As for the treated fruit, within one hour after treatment, hot water treatment significantly reduced the protein content in H1 and H3 fruit, whereas total protein content in treated H2 fruit was higher than the untreated fruit. As shown is Figure 5.2, during ripening, the level of total protein in the untreated H1 fruit increased from 1.90 mg/g at Index 1 to 2.08 mg/g at Index 4. For H2 fruit, the level of total protein increased from 2.17mg/g at Index 2 to 2.99 mg/g at Index 5. As for H3 fruit, the protein content increased from 2.75 mg/g at Index 3 to 3.49 mg/g at Index 5. Initially, hot water treated H1 fruit showed lower protein content than untreated fruit, but recovered when the fruit reached Index 2 and continued to increase until Index 3 but decreased at Index 4. The soluble protein content of the water-heated H2 fruit appears to be slightly higher than the untreated fruit. However at the later stages of ripening, the treated fruit showed significantly lower level of protein than the untreated fruit. In H3 fruit, the protein content of treated fruit was significantly lower than that in the untreated fruit at all ripening stages.

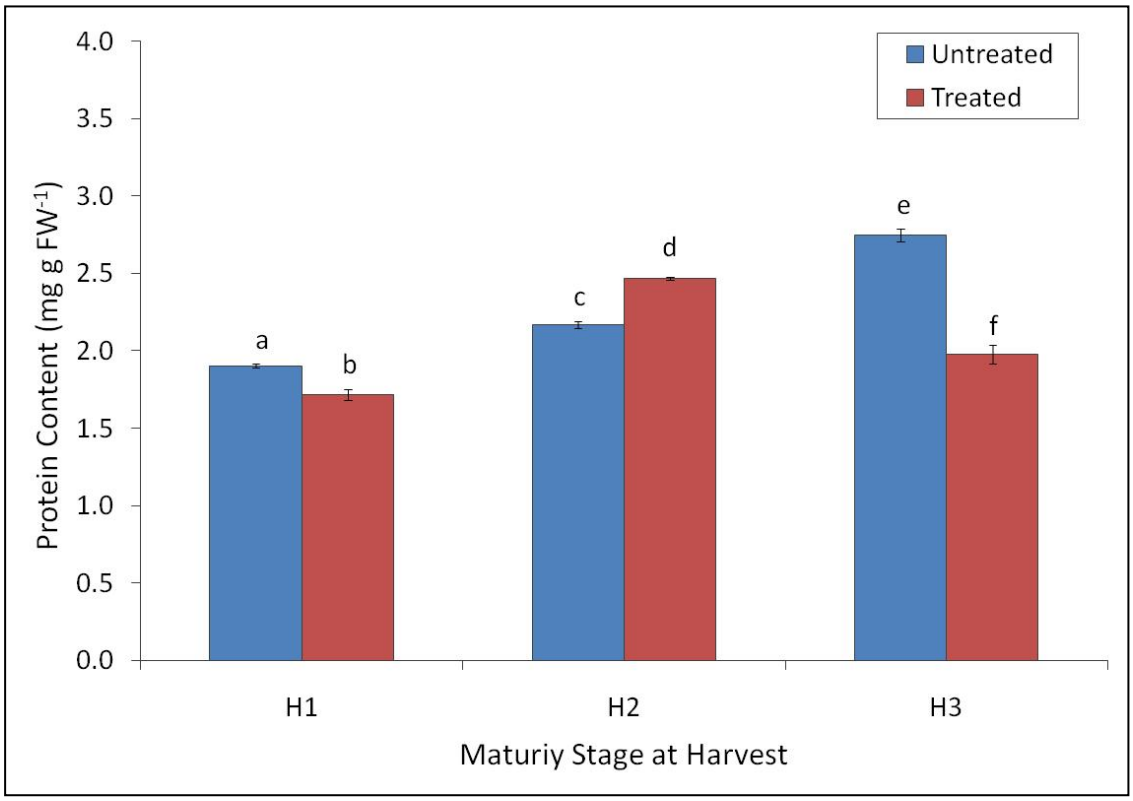


Figure 5.1: Initial protein content of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9)

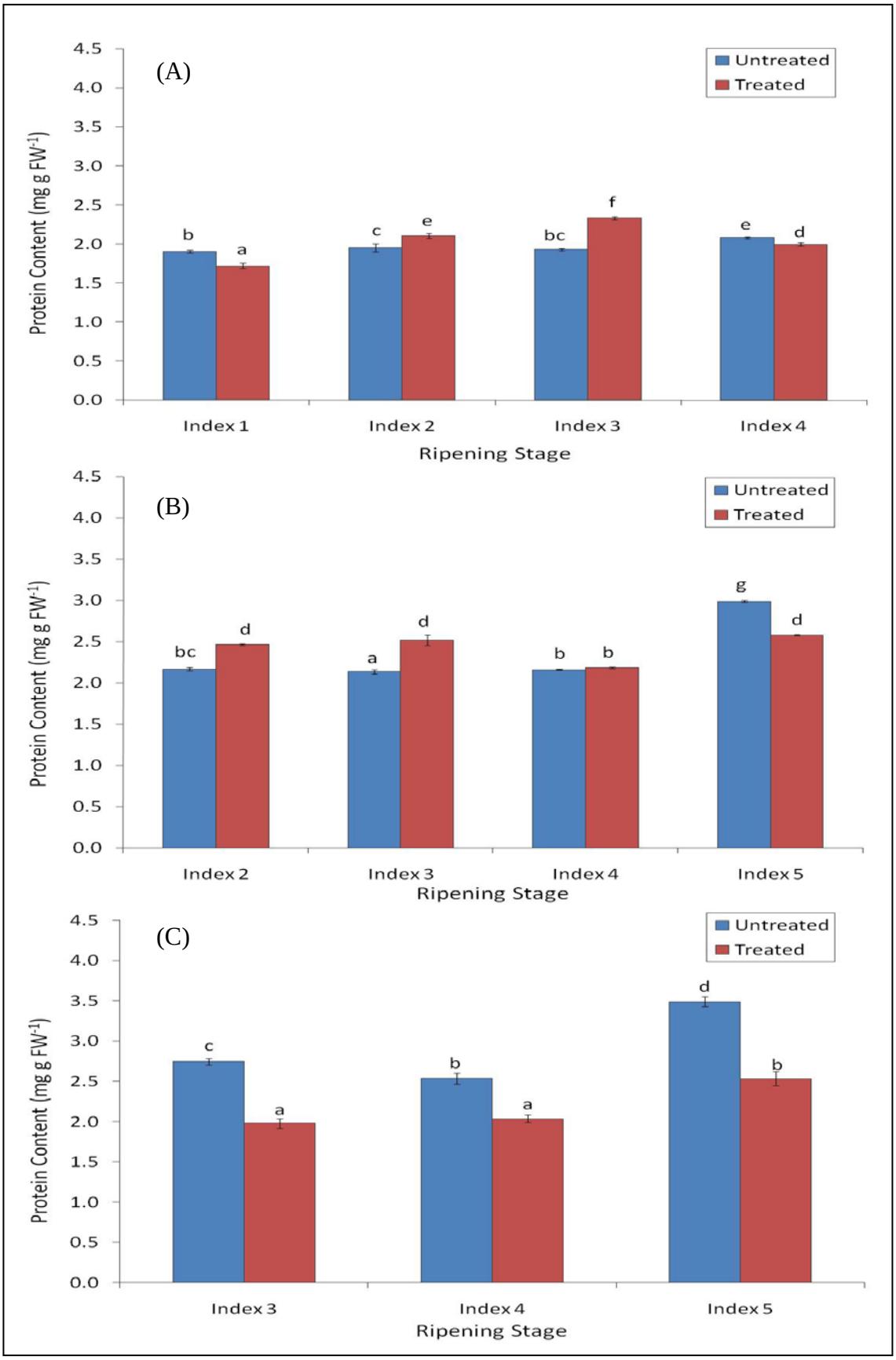


Figure 5.2: Protein content in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9).

5.3.2 Pectin Methylesterase (PME) Activity

Figure 5.3 shows that untreated H3 fruit had the lowest PME activity compared to the untreated H2 and H1 fruit which had a similar PME activity value. However, for treated fruit, maturity stages at harvest give a variable effect to the Eksotika II papaya PME activity. Treated H1 and H3 fruit had higher PME activity than the untreated fruit, while treated H2 fruit had a lower PME activity than the untreated fruit. From Figure 5.4, it is observed that PME activity in treated and untreated Eksotika II papaya fruit decreased during ripening. For H1 and H3 fruit, PME activity was significantly higher in treated than in untreated fruit throughout ripening. However for treated H2 fruit, initially within an hour after treatment, PME activity was slightly lower than in the untreated fruit but then recovered to the same level as untreated fruit at Index 3 and remained constant until Index 5. At Index 5, treated H2 fruit showed significantly higher PME activity than the untreated fruit.

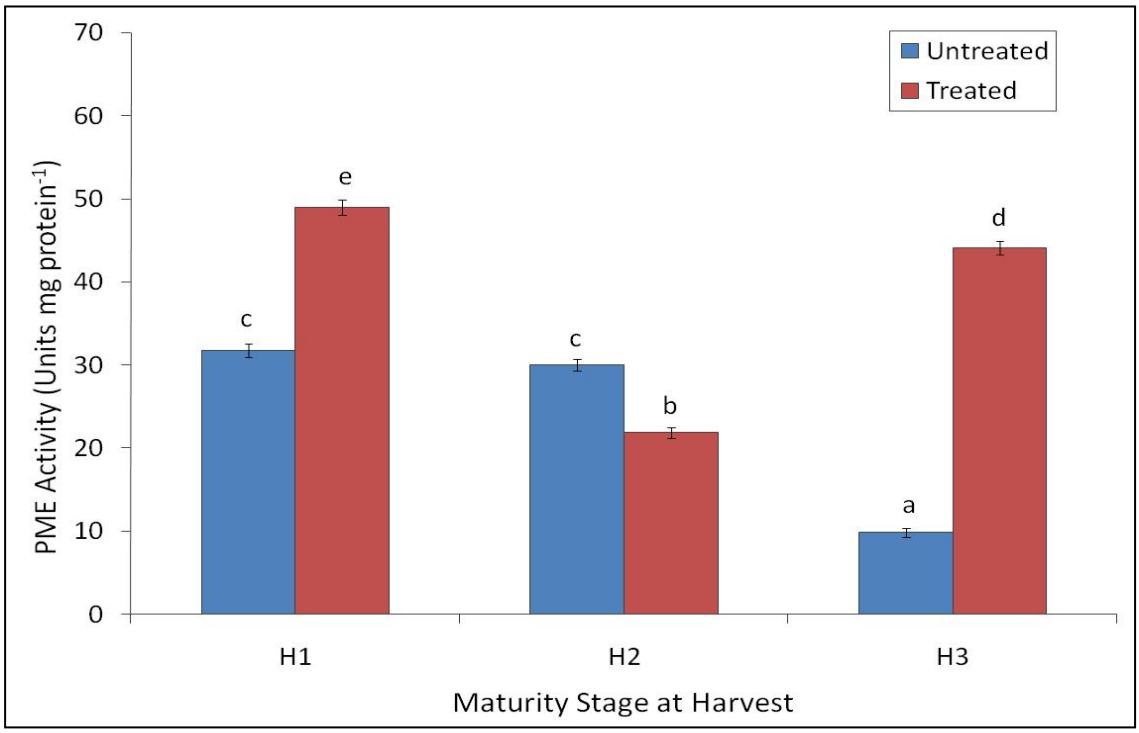


Figure 5.3: Initial pectin methylesterase activity of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9).

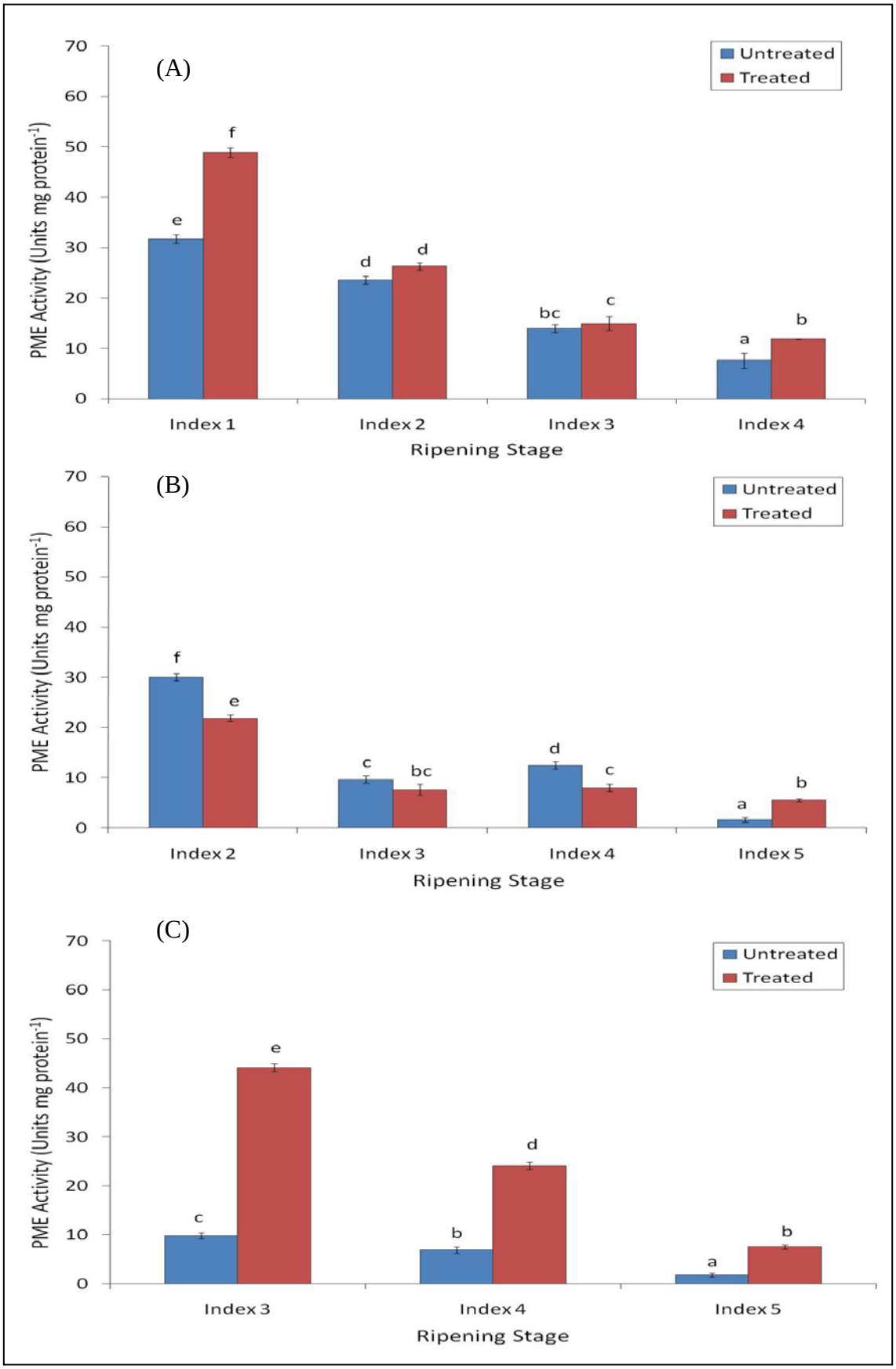


Figure 5.4: PME activity in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9).

5.3.3 Polygalacturonase (PG) Activity

Figure 5.5 shows that at harvest, PG activity in untreated H3 fruit was higher compared to H1 and H2 fruit, both of which showed similar level of PG activity. As for the treated fruit, within one hour after the treatment, PG activity in treated H2 fruit was unaffected by the treatment, whereas treated H1 and H3 fruit showed significantly lower PG activity than the untreated fruit. During ripening, PG activity in untreated H1 fruit remained low at Index 1 and 2 but increased dramatically at Index 3 and then decreased slightly at Index 4 (Figure 5.6). A similar trend was also observed for untreated H2 and H3 fruit, where at harvest, PG activity was low, but for the subsequent ripening stages, PG activity increased significantly and then decreased at the later stages of ripening. As for the heat treated fruit, PG activity of heated H1 fruit was significantly lower than untreated fruit at all ripening stages. PG activity in the treated H2 fruit seems unaffected by the heat treatment at the early stages of ripening, however at Index 4, PG activity showed a dramatic decrease and was significantly lower than that in untreated fruit. Nevertheless, PG activity in H2 fruit continued to decline but more slowly to reach the same level of activity as the untreated fruit at Index 5. For treated H3 fruit, PG activity was lower than untreated fruit at Index 3 and Index 4, but then recovered when it reached Index 5 and showed an activity that was not significantly different to that in the untreated fruit.

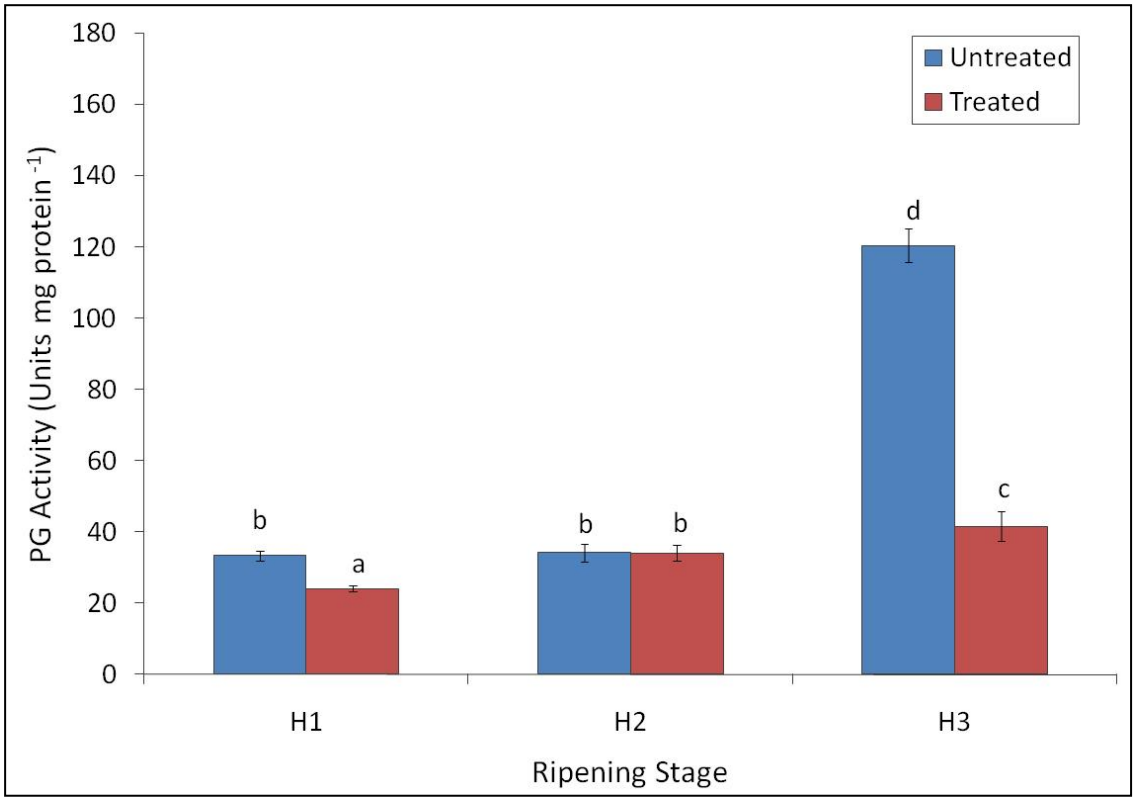


Figure 5.5: Initial polygalacturonase activity of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9)

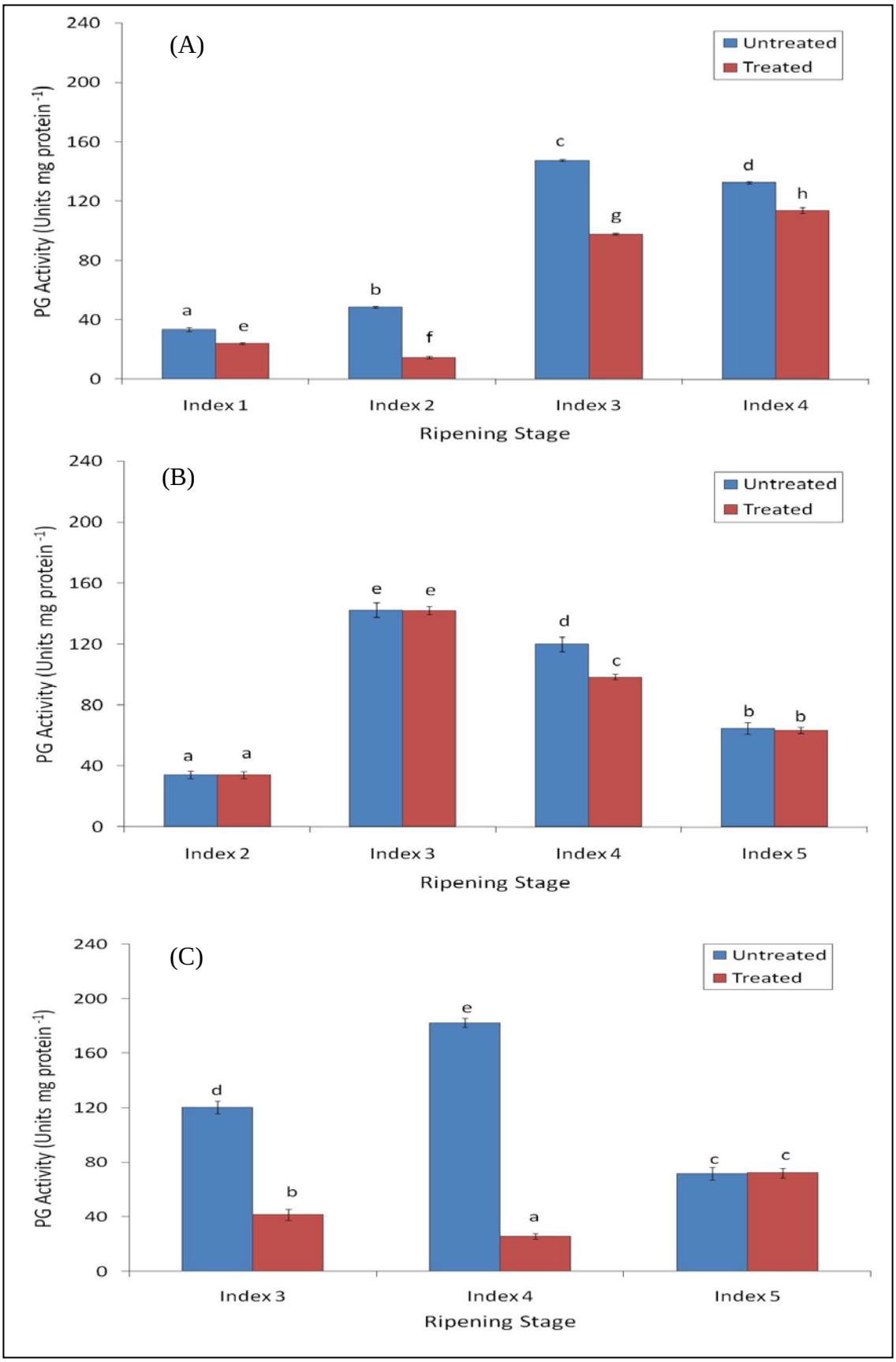


Figure 5.6: Polygalacturonase activity in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9).

5.3.4 Pectate Lyase (PL) Activity

The PL activity in Eksotika II papaya was greatly affected by the harvest maturity. H3 fruit at harvest had the highest PL activity, followed by H2 and H1 fruit (Figure 5.7). However, within one hour after treatment, all the treated H1, H2 and H3 fruit showed significantly lower level of PL activity than the untreated fruit with H2 and H3 fruit more severely affected than the H1 fruit. As shown in Figure 5.8, the activity of PL in treated and untreated Eksotika II papaya was low, within a small range of 0.002 U/mg to 0.306 U/mg throughout ripening. As for untreated H1 fruit, PL activity increased from 0.11 U/mg at Index 1 to 0.31 at Index 3 and declined to 0.29 U/mg at Index 4. Untreated H2 fruit showed an increase in PL activity at the early stages of ripening, but then decreased significantly at the later stages of ripening. PL activity in the untreated H3 fruit remained constant until Index 4 but then decreased slightly when it reached Index 5. Treated H1, H2 and H3 fruit showed significantly lower levels of PL activity compared to the untreated fruit at all ripening stages.

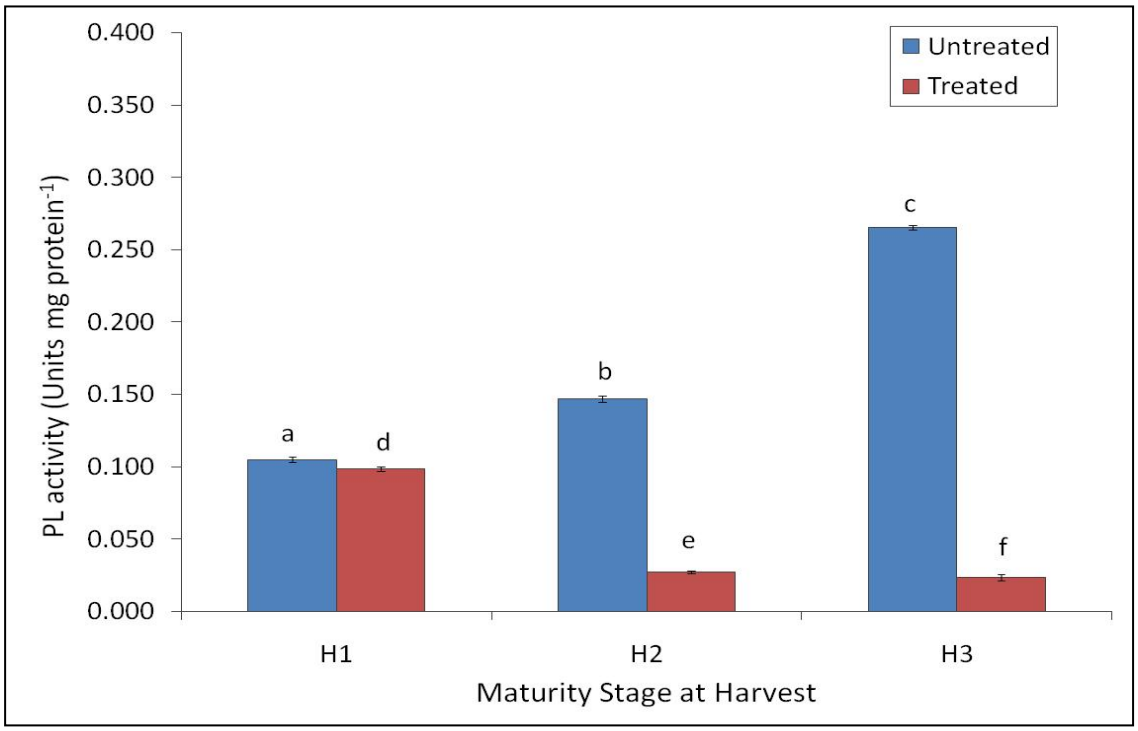


Figure 5.7: Initial pectate lyase activity of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9)

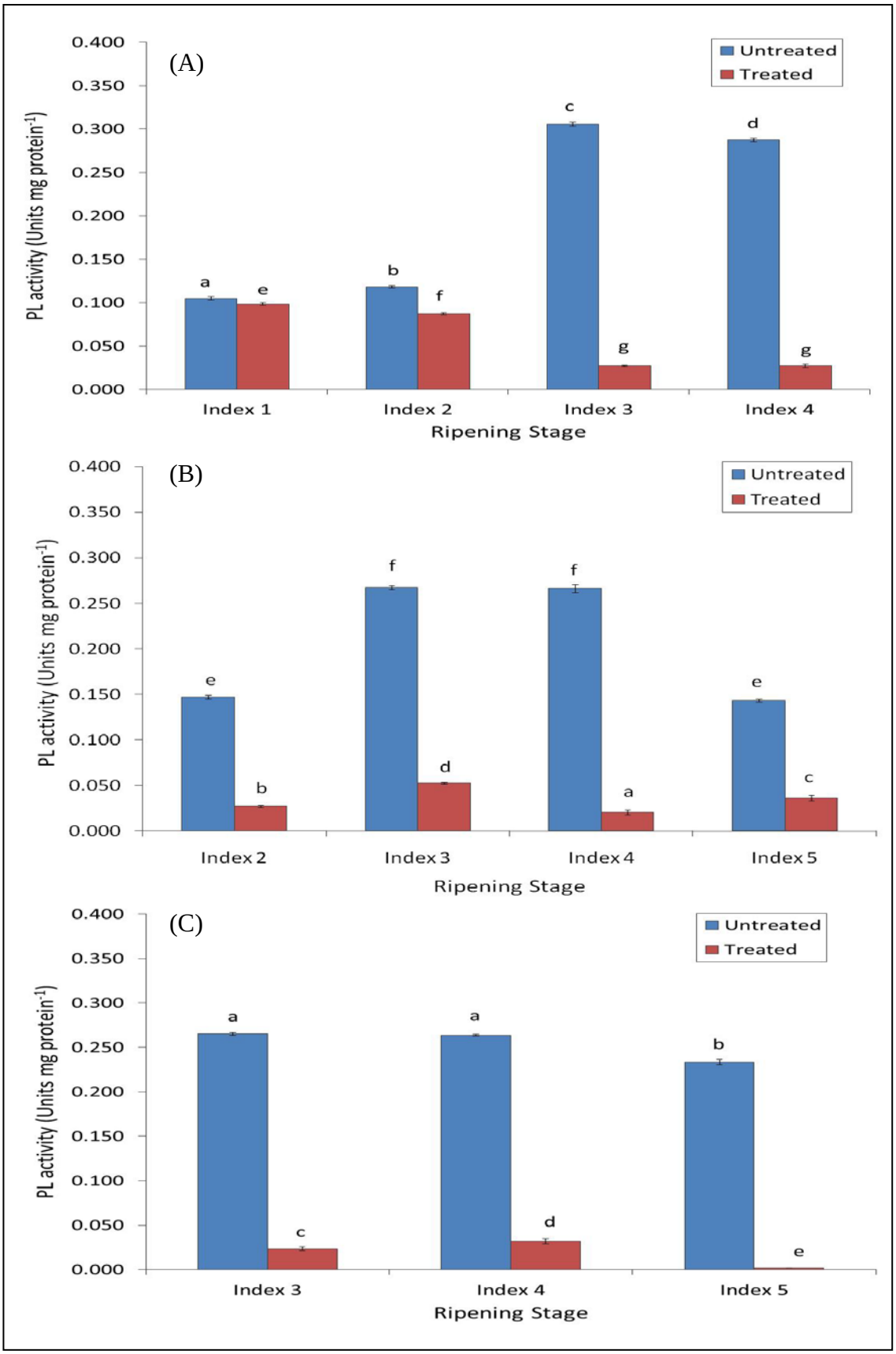


Figure 5.8: Pectate lyase activity in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9).

5.3.5 Cellulase Activity

As shown in Figure 5.9, at harvest, cellulase activity was highest in H3 fruit, followed by H2 and H1 fruit. Furthermore, within one hour after treatment, treated H1, H2 and H3 fruit showed lower level of cellulase activity than that in the untreated fruit. Cellulase activity was found to increase in untreated H1, H2 and H3 fruit throughout ripening (Figure 5.9). H3 fruit had the highest cellulase activity during ripening, followed by H2 and H1 fruit. As for treated H1 fruit, cellulase activity was slightly but significantly lower compared to the untreated fruit at Index 1 and Index 3. Subsequently, the cellulase activity recovered and reached almost the same level as in untreated fruit at Index 4. Heated H2 and H3 fruit exhibited significantly lower cellulase activity than untreated fruit at all ripening stages. In the heated fruit, cellulase activity was also found to increase during ripening, except in heated H3 fruit where at the later stage of ripening, cellulase activity decreased significantly.

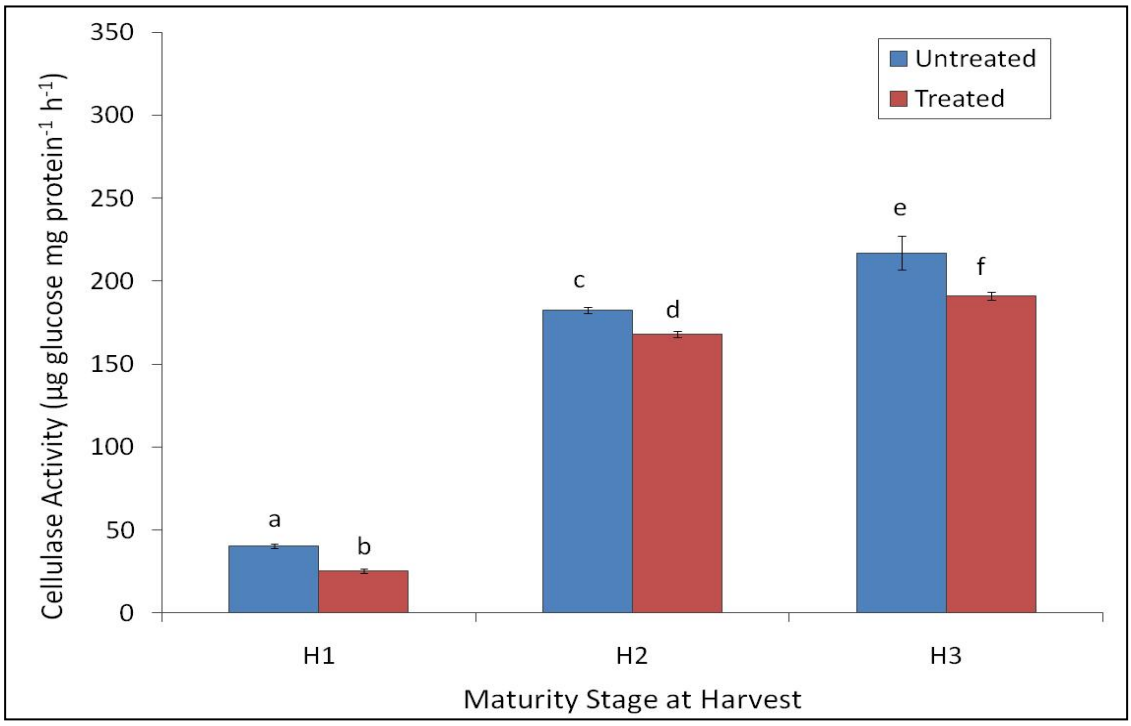


Figure 5.9: Initial cellulase activity of Eksotika II papaya fruit harvested at three different maturity stages H1, H2 and H3. Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9)

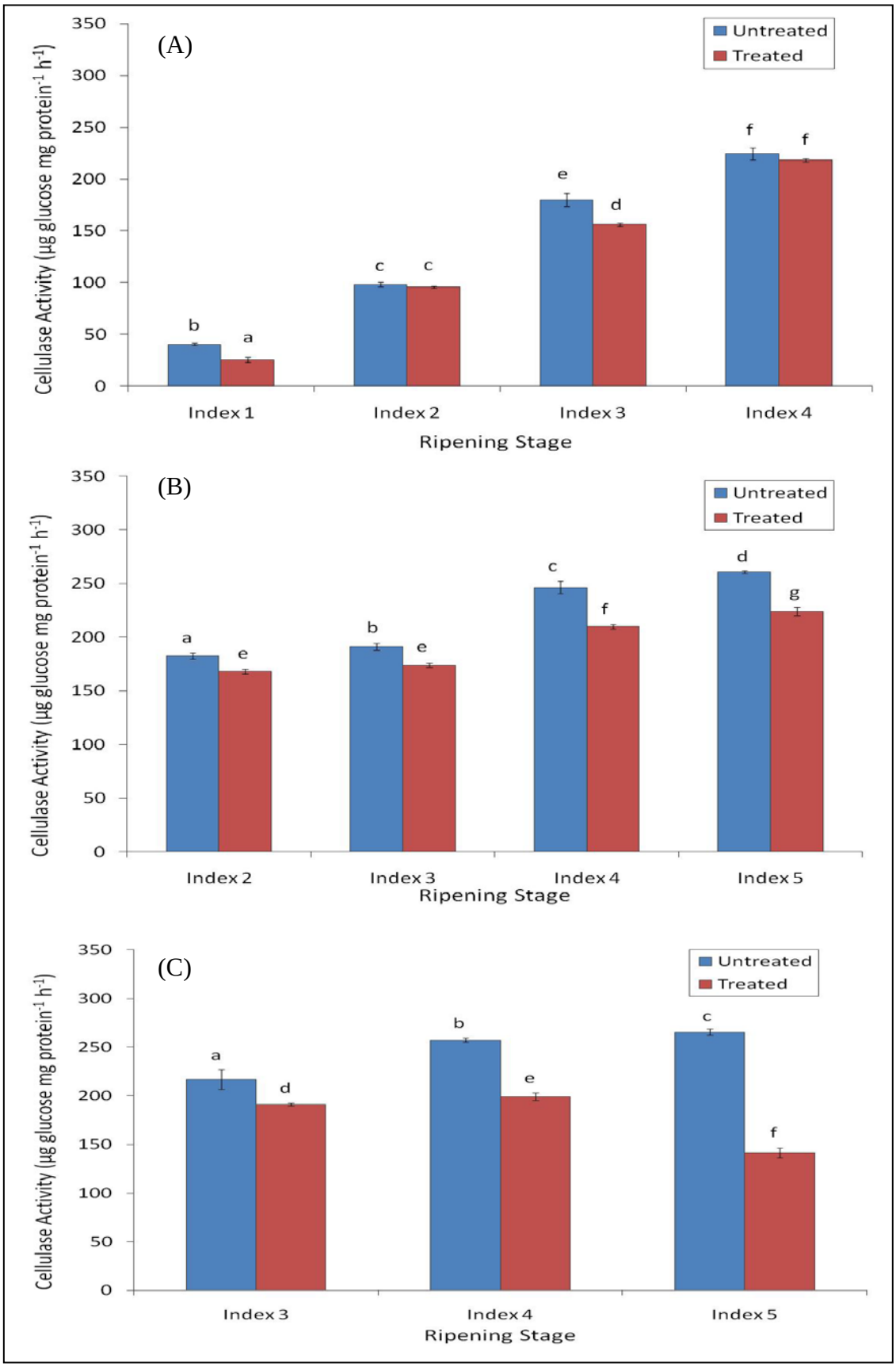


Figure 5.10: Cellulase activity in hot water treated and untreated Eksotika II papaya fruit harvested at different maturity stages during ripening. Fruit harvested at Index 1 (A) Fruit harvested at Index 2 (B) Fruit harvested at Index 3 (C). Different letters represent significant statistical differences by DMRT ($P < 0.05$). Vertical bars represent the standard error of the mean (n=9).

5.4 Discussion

The level of total extractable protein of untreated H1, H2 and H3 papaya fruit increased throughout ripening, probably indicating that particular proteins were being synthesized during ripening. It has been reported that the level of total protein in carambola fruit (Chin *et al.*, 1999) and guava (Abu-Goukh and Bashir, 2003) also increased during ripening. In addition to that, Paull and Chen (1983) had reported earlier that the level of proteinase activity decreased as the mature green 'Sunsrise' papaya ripened. Therefore, the increase in total extractable protein in the present study could be a consequence of a decrease in proteinase activity as reported in 'Sunrise' papaya by Paull and Chen (1983). In addition, the increase in the total protein content during ripening in the present study could also be attributed to the textural changes in the fruit during ripening. The increase in water content and the decrease in the cell wall material as the fruit ripened could results in more efficient solubilisation and extraction of the proteins such as reported in apples (Goulao *et al.*, 2007).

Most of the concepts and experimental evidence that explain heat injury in fresh produce involve protein denaturation, disruption of protein synthesis and loss of membrane integrity (Paull and Chen, 2000). Heat treated H3 fruit in the present study showed that probably the protein metabolism in the fruit has been disrupted following hot water treatment and the protein content was lower than in the untreated fruit throughout ripening. Nevertheless, higher protein content in heat treated H2 fruit within one hour following hot water treatment might be due to the synthesis of 'heat shock protein' (HSP) which resulted in tolerance to heat stress. It has been reported that the presence of translated polysomal RNA confirmed that new polypeptides are synthesized following heat shock induction in papaya (Paull and Chen, 1990). It has been suggested that an immediate response of high temperature, generally temperature above 35°C, is

disassociation of polyribosomes and then a reassocciation of some ribosomes into polyribosomes which preferentially translate the mRNA of HSP (Ferguson *et al.*, 1994). This response both down-regulates normal protein synthesis, even without degradation of the mRNAs and upregulate HSP synthesis (Lurie, 1998). In addition, in the present study, the temperature was raised slowly to 47°C which allows adaptation to heat shock and induction of the synthesis of HSPs.

It has been suggested that diverse structural changes in pectins, hemicelluloses and cellulose together may be responsible for the alteration of cell wall structure that occurs during fruit ripening (Huber, 1983a; Seymour *et al.*, 1990). Depending on the fruit species, different modifications may occur and to different extents. The pH in the fruit apoplast is known to decrease during ripening (Almeida and Huber, 1999). Therefore, enzymes with an alkaline pH optimum, such as PME, are expected to be involved in the early stages of softening. This coincides with the results of the present study where PME activity was found to be the highest in mature green papaya and also in papaya at Index 2 which is at the early stage of ripening. Subsequently, PME activity decreased as the fruit ripened. A similar trend was also observed in avocado, mango and pawpaw (*Asimina triloba*) where PME activity was found to decrease during ripening (Awad and Young, 1979; Ahmed *et al*., 2010; Abu-Sarra and Abu-Goukh, 1992; Ali *et al*., 2004; Koslanund *et al*, 2005). However, in contrast, there are several studies that have shown that PME activity in papaya increased throughout ripening (Paull and Chen, 1983; Ali *et al.*, 2004). PME is believed to have little effect on wall softening, but substantially affects tissue integrity during senescence (Brady, 1976; Brummel and Harpster, 2001). Demethylation of pectin by PME is necessary before PG can bring about any significant hydrolysis. Thus, PME may function to prepare the substrate for hydrolysis by PG. However, in the present study, PME is probably required not only for subsequent PG

activity, which was relatively low when the PME activity was high, but also to modify pH and cation exchange properties of the cell wall, which might impact on other cell wall enzymes activity for fruit softening (Micheli, 2001).

Heat treatment has been reported to increase the activity of PME in strawberry fruit as reported by Vicente *et al.* (2005). Similar results were observed in the present study, where PME activity of hot water treated H1 and H3 fruit were found to be higher than that in the untreated fruit during ripening. As for heat treated H2 fruit, PME activity was higher than that in the untreated fruit at the later stage of ripening. These results differ from data obtained with study on apples, where no difference in PME activity between control and heat treated fruit was found (Klein *et al.*, 1995). It has been suggested that heat allows demethylation of pectin by PME, resulting in the availability of anionic COO^- groups with which calcium can form salt bridge cross-links (Lara *et al.*, 2006). However, the response to heat treatment depends on the product and even on the cultivar used (Lurie, 1998).

In contrast to PME, PG activity in untreated H1, H2 and H3 papaya fruit in the present study tended to increase during ripening. Low PG activity was detected during the early stages of ripening and then it increased dramatically in the middle of ripening process. A similar trend was also observed in 'Sunrise Solo' papaya where PG activity was low in the early stages of ripening and then increased dramatically during ripening with peak activity when the fruit is 40% to 60% yellow (Paull and Chen, 1983). Ali *et al.* (2004) also reported that PG activity in 'Eksotika' papaya increased during ripening. During papaya ripening, there is an increase in soluble pectin (Paull *et al.*, 1999) which is the result of an increased activity of PG during ripening which has been implicated in pectin solubilization. This suggests that fruit softening is regulated by the accumulation of PG

and the rate of pectin breakdown (Prasanna *et al.*, 2007). In addition, an increase in PG activity is correlated with the production of the substrate by PME that facilitates PG activity on pectin (Lazan *et al*, 2004).

Heat treatment has been reported to interfere with PG accumulation in papaya. For example, thermal injury (46°C for 65 – 90 min) to papaya fruit during ripening is correlated with a 90% decrease in normal PG levels (Chan *et al.*, 1981). However, partial recovery to 25% of normal PG levels occurred after 6 days at 24°C (Chan *et al.*, 1981). Lazan *et al.* (1989) also reported that heat treatment at 48°C for 20 minutes reduced PG activity of papaya during ripening. In the present study, hot water treated H1 fruit showed lower PG activity compared to the untreated fruit. However, when heat treated H1 fruit reached Index 3 and Index 4, the PG activity increased, though in lower proportion than in the untreated fruit. This result correlates well with fruit softening in H1 fruit, where at Index 3 and 4, heat treated H1 fruit were found to be firmer than untreated fruit. On the other hand, PG activity of treated H2 and H3 papaya recovered from a decline in PG activity at Index 4 where it reach the same level as untreated fruit at Index 5. Similarly, heat treated tomato PG activity returned after a 6-day lag when returned to 25°C (Yoshida *et al.*, 1984).

PL probably plays a minor role in Eksotika II papaya fruit softening since only little activity of PL was detected in the present study. PL activity in untreated H1, H2 and H3 Eksotika II papaya increased during ripening and decreased slightly at the later stage of ripening. This result is in agreement with those obtained by Payasi and Sanwal (2003) where PL activity of banana increased steadily during ripening with a peak which coincides with the respiration climacteric peak of banana. Subsequently, the PL activity decreased throughout the postclimacteric stage. PL activity was also reported to increase

in banana during ripening by Lohani *et al.* (2004). Besides, PL activity has also been detected in ripe, soft apple fruit (Goulao *et al.*, 2007). The role of PL in ripening fruit has been addressed mainly based on the observation that there is mRNA accumulation of putative PL transcript in ripening strawberries (Medina-Escobar *et al.*, 1997) and bananas (Marín-Rodriguez *et. al*, 2003).

Hot water treatment applied in the present study clearly affected PL activity at all ripening stages, independent of the maturity stages at which papayas were harvested. The PL activity in H3 fruit was most severely affected by the hot water treatment. The disruption of PL activity in heat treated Eksotika II papaya might be due to the inhibition of PL enzymes, or its synthesis by heat stress. However, since PL was detected in only low amounts, it is possible to speculate that the rate of softening in Eksotika II papaya fruit is not dependent on PL activity.

Cellulase activity is known to increase with ripening in several fruits, such as avocado (Awad and Young, 1979), tomato (Hobson, 1968), papaya (Paull and Chen, 1983) and guava (Abu-Goukh and Bashir, 2003) which suggest a role in fruit softening. Similarly, in this study, cellulase activity increased during ripening with the highest activity observed in H3 fruit. This suggests that cellulase levels in the unripe fruit are generally low and it increases progressively during ripening. A similar trend was observed in 'Sunrise' papaya, where cellulase activity was low at early stage of ripening and then increased progressively during ripening (Paull and Chen, 1983).

Heat treatment has been shown to reduce cellulase activity in this study. However, cellulase activity in heat treated H1 fruit recovered when it reached Index 2 and then increased to the same level as that in untreated fruit until the end of the ripening period.

Heat treated H2 fruit showed a pattern of increasing cellulase activity although in significantly lower proportion than in the untreated fruit. Cellulase activity was more severely reduced in the more ripened fruit as can be seen in the treated H3 fruit. It has been reported in strawberry, that cellulase activity was reduced in the heat treated fruit (Vicente *et al.*, 2005; Martínez and Civello, 2008). The lower level of cellulase activity found in the heat treated fruit in this study, could be related to a less extensive hydrolysis of the xyloglucan structure in the cell wall since it has been reported that *in vitro*, cellulase are active against xyloglucans and non-crystalline cellulose (Brummell and Harpster, 2001).

In summary, hot water treatment markedly reduced the activities of the cell wall enzymes investigated except PME. However, the observed reduction did not elicit significant changes in tissue firmness because most of the enzymes recovered their activities as ripening progressed. However, the activity of PG correlated well with pulp firmness of H1 fruit where firmer fruit was obtained when the PG activity was reduced by the heat treatment. Thus, it is possible to suggest that PG might play a significant role in fruit softening in Eksotika II papaya. Nevertheless, it has been reported that other cell wall hydrolases which have not been included in this study, could also play a role in papaya fruit softening. A recent study by Thumdee *et al*. (2010) reported that the key hydrolase associated with papaya softening appears to be an endoxylanase. On the other hand, Lazan *et al*. (1995) and Lazan *et al*. (2004) suggested that in general, irrespective of enzyme distribution, tissue softening during papaya ripening was more closely related to changes in β-galactosidase activity than to PG or PME activity. Papaya β-galactosidase appears to be an important wall degrading enzyme and may contribute significantly to differential softening, perhaps by complementing the action of polygalacturonase (Lazan *et al*., 1995; Lazan *et al*., 2004).

However it has been shown that down-regulation of PG (Smith *et al*., 1988) and PME (Tieman and Handa, 1994) resulted in tomato fruit with no significant differences in pulp firmness, despite some characteristics of the cell wall having been modified. These results illustrate that individual enzymes are not sufficient to produce an effect on fruit softening, thus the action of all the enzymes should be investigated collectively so that we can obtain a more conclusive picture of the whole process.

Chapter 6: Conclusion

The ripening process of hot water treated and untreated Eksotika II papaya, harvested at three different harvesting maturities was investigated mainly in two different areas; physiology and biochemistry. Hot water treatment has been used widely as an insect disinfestation procedure in order to meet quarantine requirements by some countries such as Japan and China. Therefore it is important to understand whether the heat disinfestation treatment applied have any effect on the fruit quality.

This study has demonstrated that hot water treatment did not give any significantly adverse effect to the physiology parameters evaluated, such as L*a*/b* value, weight loss, TSS and pH. However, the pulp firmness of the treated Index 3 and Index 4 H1 fruit was slightly higher than untreated fruit suggesting that heat treatment applied might have affected the softening process. The chlorophyll fluorescence parameters investigated have shown that within one hour after treatment, the heat treated fruit experienced heat stress which was reflected in the lower chlorophyll fluorescence parameters values, Fv/Fm. The heat stress was found to be more severe in the riper fruit rather than in the mature green fruit. However, the Fv/Fm value in the affected fruit recovered at the subsequent stages of ripening following storage at ambient temperature. Furthermore, the total sugar and total reducing sugar content was not adversely affected by the hot water treatment. These observations indicated that the overall fruit quality is still maintained when the fruit is allowed to ripen at ambient temperature.

Moreover, the determination of skin colour using the L*a*/b* values provide an objective, non-destructive method of assessing papaya fruit maturity as there is an excellent correlation (R^2 = 0.997) between the L*a*/b* values and the conventional visually-determined maturity indices (Figure 3.5). Besides, the L*a*/b* values also can distinguish between the mature green and the similarly sized immature green papaya fruit.

This study has also shown that harvesting the fruit at Index 1 (H1) should be avoided since the fruit will ripen poorly. The TSS value of H1 fruit was significantly lower than those of the H2 and H3 fruit and the pulp firmness of the H1 fruit was lower than the H2 and H3 fruit when it ripened. The H1 fruit also failed to develop full colour and the pulp colour was often pale orange although the skin colour was not affected. The total sugar content of H1 fruit was also significantly lower than the H2 and H3 fruit during ripening. It is recommended that the Eksotika II papaya should be harvested at Index 2 for long distance market because of its longer shelf life. It can be stored for 10 days and maintain its quality when it ripens. For the local market, the Eksotika II papaya should be harvested at Index 3 which will ripen to an edible stage within 5 days.

The pattern of cell wall degrading enzymes activity showed that PME activity decreased during ripening, whilst PG, PL and cellulase activity increased progressively during ripening. It was also found that the application of the hot water treatment affected the cell wall degrading enzymes activity whereby it was found to be lower in the heat treated fruit. Nevertheless, pulp firmness was not severely affected since some of the enzyme activities managed to recover during the ripening period. It is also possible that, tissue softening during ripening is not dependent entirely on the activities of the cell wall degrading enzymes investigated in this study. Other enzymes such as β-

galactosidase and endoxylanase have also reported to be involved in the softening process of the papaya fruit (Lazan *et al*., 2004; Thumdee *et al*., 2010).

Overall, this study indicated that the hot water treatment at 47±1˚C fruit core temperature held for 10 minutes is able to maintain the Eksotika II papaya fruit quality when the fruit is allowed to ripen at ambient temperature and at the same time act as a good disinfestation treatment. However, further studies need to be carried out in order to fully understand the effect of the postharvest hot water treatment on the quality of Eksotika II papaya. Studies on the expression of the cell wall degrading enzymes related genes following hot water treatment would be useful in order to compare the expression level of the related genes between the hot water treated and untreated Eksotika II papaya. In addition, the possible effects of hot water treatment on nutritional quality of the fruit also should be investigated as the nutritional compounds will affect the quality of the fruit.