Flavor and Its Related Quality in Longkong (*Aglaia dookkoo* Griff.) during On-Tree Maturation and Storage

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy in Food Technology

Prince of Songkla University

2014

Thesis Title   Flavor and Its Related Quality in Longkong (*Aglaia dookkoo*
           Griff.) during On-Tree Maturation and Storage

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Academic Year   2013

ABSTRACT

Longkong is an important economic fruit that is gaining popularity in Thailand. The demand for this fruit is increasing tremendously compared to other Meliaceae family because longkong is juicy and has a pleasant taste. Longkong is a non-climacteric fruit which needs to develop its flavor character during on-tree maturation. In addition, longkong is a highly perishable fruit which has short shelf-life around 4 to 7 days under ambient condition. To extend the fruit shelf-life, an alternative tool such as modified atmosphere (both of passive and active MAPs) combined with optimal temperature was introduced. However, in long-term storage, the off-flavor such as ethanolic-flavor is formed. To discard unattractiveness character, intermittent warming (IW) treatment was also introduced in this research work.

The volatile flavor compounds in longkong were isolated by direct solvent extraction (DSE) and solid phase microextraction (SPME). The profiles were identified by chromatography mass spectrometry (GC-MS). Volatile flavor compounds in longkong were successfully isolated by DSE as using dichloromethane and SPME as using CAR/DVB/PDMS. The three abundant volatile flavor compounds isolated by dichloromethane were cis-linalool oxide, delta-germacrene and ethyl-3-hydroxy butyrate. They were representing for sweet-floral, herbaceous and fruity attributes, respectively. Green alcohols and aldehydes were identified in the profile isolated by CAR/DVB/PDMS. The green alcohols and aldehydes were cis-3-hexen-1-ol, cis-4-hexen-1-ol, n-hexanol, trans-2-hexenal and benzaldehyde. In addition, flavor compounds in longkong were occurred in a form of glycosidically bound flavor. The glycosidically bound flavors were hydrolyzed to release free volatile. The enzymatic hydrolysis was performed at 40ºC using β-glucosidase under different pHs (pH 3, 4, 5

and 3.8 (equal to pH of longkong)) and incubation times (4, 8, 12, 16 and 24 hours) conditions. In addition, acid hydrolysis under different pHs (pH 1, 2, 3, 4 and 5) and incubation temperatures (30, 50, 70, 90 and 100°C) for 60 min also performed. The suitable conditions were enzymatic hydrolysis under a condition of pH 5.0 for 24 hours or acid hydrolysis under pH 1 at 100°C. Both enzyme and acid hydrolysis exhibited similar types of free aglycones. The liberated aglycones were composed of butyl butanoate, 2-ethyl-1-hexanol, trans-2-hexanal and phenol, with the attributes of fruity, citrus, green and phenol characteristic, respectively.

Physical and chemical qualities of longkong at different stages of on-tree maturation (ripe (13 weeks after anthesis), medium ripe (14 weeks after anthesis) and full ripe (15 weeks after anthesis) stages) were evaluated. Longkong became light to bright yellow with stages of maturation (p<0.05). It can be indicated by the highest lightness (L*=64.21) and yellowness (b*=34.37) values presented in longkong at full ripe stage. Moreover, texture changed to soften with maturation (p<0.05). Fruit size and weight increased with stage of maturity but no significantly difference (p≥0.05). There were in a range of 3.17-3.30 cm in diameter and 19.94-21.36 g for each fruit weight. Moisture content was slightly increased with maturity stages (p<0.05). During on-tree maturation, titratable acidity and organic acids (citric, maleic and malic acids) decreased while pH, TSS and total sugar content increased. The main sugars found in longkong were sucrose, glucose and fructose, respectively. Sucrose content slowly increased from 9.88 to 11.44%. However, during the full ripe sucrose content was 7.03% (p<0.05). Glucose content significantly decreased from 1.98 to 1.46% during ripe to medium ripe stages of longkong then it increased again to 2.92% in full ripe stage (p<0.05). Similar to fructose content, it also decreased from 2.76 to 2.20% during ripe to medium ripe stages of longkong. After that it increased again to 3.39% in full ripe stage (p<0.05). The green aroma of 1-hexanol was only the volatile compound which found in ripe longkong. From ripe to full ripe stages, longkong presented more fruity and sweet characteristic. There were a lot of terpenes and their derivatives which presented fruity, floral, flower, herbaceous and sweet attributes.

Modified atmosphere packaging (MAP) extended the fruit shelf-life. The effective of passive MAP was related with fruit maturity stage (p<0.05). In this study, passive MAP at 18ºC was applied to store longkong at three different stages of

on-tree maturation (ripe, medium ripe and full ripe). Changes in longkong qualities were monitored at 6 day intervals. The extremely changes in fruit qualities was due to the stage of on-tree maturation (p<0.05). Longkong at full ripe stage presented the most sensitive on passive MAP condition. Fruit skin became darken rapidly. It was indicated by the lowest of L* (L*=31.81) and the highest of a* (a*=19.19) values. At the end of storage, longkong at full ripe stage showed the lowest fruit firmness (1,277.98 g) and the highest weight loss (1.98%). The highest concentration of CO_2 was 80.55% inside a full ripe longkong package. High CO_2 concentration enhanced the specific activity of ADH enzyme to 12.00 U/mg protein. The content of ethanol increased to 0.20 g/g FW with high specific activity of ADH enzyme throughout storage time (p<0.05). The total viable counts were 6.68×10^5 CFU/g at the end of storage. To enhance the effectiveness of passive MAP, the optimal temperature is required. Longkong was stored under passive MAP at three different storage temperatures at 4°C, 18°C and room temperature (~30°C). The quality of the longkong during storage was monitored at 3 day intervals. Longkong stored under passive MAP at 4°C for 36 days became darker and softened (p<0.05). Fruit skin color is represented by L*, a* and b* values. They were 25.57, 23.21 and 11.77, respectively. Fruit firmness decreased from 1849.30 to 1335.53 g. A slight increase in titratable acidity was found. It increased from 0.59 to 0.73%. High CO_2 concentration inside package enhanced the activity of ADH enzymes to 11.34 U/mg protein. The ethanol content increased during storage to 0.16 g/g FW. The microbial population increased with storage time to 4.31×10^5 CFU/g.

Active modified atmosphere packaging (Active MAP) is an alternative tool for prolonging the fruit shelf-life. To enhance the effectiveness of active MAP, an appropriate ratio of gases and an optimal temperature is required. Changes in longkong quality during storage under active MAP (5%CO_2:5%O_2, 5%CO_2:10%O_2 and 10%CO_2:5%O_2) at 18°C and room temperature (~30°C) were monitored. Throughout the storage time, the longkong lost its bright yellowness, fruit firmness of the fruit and fruit flavor. It also increased in acidity and perceived ethanolic-flavor. Longkong stored under active MAP (5%CO_2:5%O_2) at 18°C maintained fruit quality over the 24 days. At the end of storage, the longkong had lost its bright yellowness which was indicated by decreases in the L* and b* values. The titratable acidity

increased from 0.59 to 0.72%. The increase in perceived ethanolic-flavor and a decrease in flavor were also in agreement with the high levels of ethanol content at 0.21 g/g FW at the end of storage. The activity of the ADH enzyme increased from 0.54 to 13.52 U/mg protein. The microbial population was 7.53×10^5 CFU/g. However, longkong shows undesirable attributes such as brownish color, loss of fruit firmness and high ethanol accumulation during storage under active MAP condition. To reduce those attributes, the intermittent warming (IW) treatment has been introduced. The IW treatment was subjected to longkong in a different time during storage. Interestingly, longkong treated with a cycle of IW treatment to ambient condition in every 2 days showed a good quality with 0.73% weight loss. Under this condition, longkong had the lowest rate of fruit skin color changing. The L* and b* values on the 12th day were 40.14 and 27.10, respectively. They were correlated with high h° angle and C* values. The amount of 3-hydroxy-2-butanone, laevo-linalool and delta-germacrene were maintained by IW treatment. In addition, low concentration of ethanol at 0.21 g/g FW with the highest longkong-like flavor score was noticed.

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CHAPTER 1

INTRODUCTION AND REVEIW OF LITERATURE

1.1 Introduction

One of attractive and desirable attributes that create demand for fresh fruit is flavor characteristic which is the most noticeable to consumer (Knee, 2002). Consumer preference of fruit is strongly influenced by sweetness, acidity and characteristic flavors. Longkong is an important economic fruit that is gaining popularity in Thailand. The demand for this fruit is increasing tremendously compared to other Meliaceae family because the fruit is juicy and has a pleasant taste (Sapii *et al*., 2000). Native to Southeast Asia, longkong (*Aglaia dookkoo* Griff.), a non-climacteric tropical fruit belongs in the same family (Meliaceae) as the tropical fruits langsat, (or lanzones of the Philippines), duku-langsat and duku and is often described as being more aromatic and better sweet taste than langsat. The origin of this fruit is the South of Thailand, Indonesia, Philippines and the Malau Islands. The longkong pulp is juicy with a typically aromatic smell and a sweet but slightly sour taste (Sapii *et al*., 2000; Sabah, 2004; Paull, 2004). However, reports on flavor characteristics of longkong are very limited.

Recently, logkong becomes the one of popular fruit to be exported. It is generally sold as a raceme. However, insect infection inside a raceme is always found and delicate to sort it out. Moreover, some symtomps such as black rot and microorganism infection such as *Phomopsis sp*. and *Lasiodiplodia theobromae* is also found (Sangchote *et al*., 2010). They are the major problems encontured the demand of this fruit. Therefore, the individual longkong by cutting off the fruit from the raceme is created. Individual form is quite easy for cleaning, screening and sortting out some defects off. All experiments in this research will focus on an individual fruit.

Volatile flavor compound is an important factor determining final sensory quality of fruit. Volatile compounds formation is a dynamic process, because volatile substances are continuously synthesized and developed during fruit maturation. Generally, flavor quality of fresh fruits is maximal at harvest especially for non-climacteric fruits such as longkong. They produce volatile compounds as they

reach optimum eating quality. These fruits do not synthesize volatile compounds that are as aromatic as those in climacteric fruits due to their respiration pattern after harvested (Will *et al*., 1998). Non-climacteric fruits should be picked when fully ripe to ensure good flavor quality (Kader, 1999). Fruit flavor development during its on-tree maturation is different from its formation after harvested. In addition, the investigation of flavor characteristic in longkong has not been reported yet. Moreover, flavor changes during on tree maturation are also very limited. Therefore, all information regarding to longkong behaviors need to be explored. In general, fruit flavors are present either in free form or bound to sugars in the form of glycosides. The free form of flavor can release immediately, giving fruit aroma, but bound form is flavorless. Recently, flavor precursors and intermediates, especially glycosides in fruit have received increasing interest and attention. Since glycosides can enhance the aromatic profile by release free volatile from the glycoside. It was found that β-glucosidase enzyme and acid can hydrolyze fruit glycosides, resulting in enriched aroma characteristic.

Fruit volatile flavor or aroma compounds could be isolated by various methods such as direct solvent extraction (DSE) and headspace solid phase microextraction (HS-SPME). Each method has different advantages. One of the simplest and most efficient approaches for flavor or aroma isolation is DSE. It is widely applied due to its efficiency in isolating a broad range of volatiles. But, many reports have also noted problems with DSE of low recovery of compounds that having high volatility. Therefore, analytes loss with artifact is formed. The HS-SPME is introduced as a fast, simple, convenient sample preparation and solvent-free method, which has attracted widespread for many fruits analysis. HS-SPME can be used for the simultaneous isolation concentration of volatile compounds present in the headspace without artifact formation due to temperature or solvent effect. Headspace technique provided a relationship between sensorial and volatile contents (Riu-Aumatell *et al*., 2004), caused of the compounds at headspace only are analyzed, related to human perception.

Fruit is a perishable produce. Since they are living biological systems after harvested, they will deteriorate. The rate of deterioration varies greatly between individual produce depending on their overall rate of metabolism, but for many fruits

can be rapid. Therefore, the need for postharvest techniques that allow quality to be retained during storage is important. The combination of modified atmosphere storage and low temperature storage is the most effective tool for extending the storage life of fresh fruit. As decrease O_2 and/or elevate CO_2 levels, changes in the concentrations of the respiratory gas may extend storage life. In addition, the major reason that postharvest life is extended by cooling is that metabolism is slowed under low temperature during storage. Therefore, an important economic fruit such as longkong, which high rate of deterioration need for those postharvest techniques. However, under atmosphere levels different from normal air, showing insufficient O_2 and chilling temperatures, fruits are response by failure incomplete combustion and ripen abnormally. Resulting in, chemical compositions change especially the composition which affect on its organoleptic, such as off-flavors (aldehydes and alcohols) are formed, thus, poor quality, resulting in unacceptable of consumers. Aside from avoiding off-flavor formation and temperature stress or any undesirable qualities, intermittent warming (IW) technique has been introduced, especially in non-climacteric fruits. This technique acts as an effective tool on many commodities, for example, peach (Fernández-Trujillo and Artés, 1998), orange (Schirra and Cohen, 1999). The IW treatment involves placing the fruit immediately in low temperature storage condition after that removing them to warming (~15-20°C) or ambient condition interval during storage. The potential for reversal varies with species, fruit maturity, length of time under stresses conditions and temperature to which fruits exposed.

In addition, fruit flavor is one of the most important factors to determine consumer acceptance. Flavor compounds either aromas or off-flavors could then be used as an indicator for quality. Nevertheless, flavors of fresh longkong and its flavor quality affected by postharvest technology have not yet been investigated. Therefore, the objectives of this study are to identify flavors in longkong. Flavor development in longkong during on tree maturation is monitored. Moreover, their flavor profiles changing and possible off-flavor development storage under modified atmosphere conditions are also explored. The results from this work will benefit for all farmers, exporters and of course to consumers.

1.2 Review of Literature

    1.2.1 Longkong

        Longkong (*Aglaia dookkoo* Griff.) is a non-climacteric tropical fruit. It belongs to the Meliaceae family. Its common names are lanson, lanzone, duku, dukong and ayer-ayer (Paull *et al*., 1987). Longkong has its origin in the South of Thailand, Indonesia, Philippines and the Malau Islands (Paull, 2004). In Thailand, longkong is originally grown in Narathiwat province, located in the southern of Thailand which gave the best quality since the pulp has mild plesant aroma and almost seedless. So far, it has been introduced to eastern and northern Thailand. Longkong is roughly 3-4 cm globular shape and soft, comes in racemes which are 15-20 fruits. Fruit is almost seedless, free of latex, smooth with thin bright yellow skin. Skin of young fruit is green and turns to yellowness when it matures. Pulp is containing with five segments of hard white translucent and covered on1 to 5 green seeds (Paull *et al*., 1987; Paull, 2004) Fruit is juicy with a typically aromatic smell. Taste is sweet but slightly sour (Sabah, 2004; Paull, 2004).

    1.2.2 Grade classification of longkong

        Generally, longkong was traded in local maket by fruit size and weight. However, standard of longkong is provided for export market, for example, standard of Tanyongmat market in Narathiwat province. Longkong is classified into 3 grades (A, B and C) according to fruit size and its appearance. Longkong grade A must be in a raceme, has its diameter at least 3 cm or above. Fruit skin is bright yellow without any defects and damages. The length of raceme is approimately 25 cm or above and no fruit drop. Fruit must have a typical aroma and good sweet taste. Longkong grade B must have its diameter varied between 2-3 cm. Fruit skin is bright yellow with slight defects and damages. The length of raceme is varied between 20-25 cm. Fruit taste is sweet with a slight sour. Longkong grade C can be found as a fruit drop. Fruit skin can has some defects and symptoms. Fruit taste is sweet as well as has its slightly sour. In addition, longkong can be classified according to National Bureau of Agricultural Commodity and Food Standards (2006) as follows. Longkong is classified into 3 classes (Extra class, Class I and Class II) according to fruit size and its appearance. Longkong in extra class must be superior quality. It must be free of

defects with the exception of very slight superficial defects. However, these slight superficial defects need to be not affecting to the general appearance of this class. Meanwhile, longkong in class I must have good quality, but, slight defects may be allowed such as bruising, scratches or other mechanical damage. However, the defects can not exceed to a total area of 0.5 cm^2 of each longkong fruit. However, these defects need to be not affecting to the appearance of this class. Lower quality than longkong in class I can be found in longkong class II. Defects such as bruising, scratches or other mechanical damage can not exceeding to a total area of 0.5 cm^2 of each longkong fruit. However, these defects need to be not affect to the general appearance of this class.

1.2.3 Fruit respiration

A major metabolic process taking place in any living organisms is respiration. Fruit respiration is still there even after postharvest. It is initiated in the cytoplasm and completed in the mitochondria. It can take place in both of aerobe and anaerobe conditions. Generally, term of respiration can be described as the oxidative breakdown of sugars. Moreover, organic acids and fatty acids may be also used as a respiration substarte (Kays, 1991).

1.2.3.1 Aerobic respiration

Aerobic respiration is the most common type of respiration. It is started in the process of glycolysis and followed by two major stages of tricarboxylic acid (TCA) or Krebs cycle and the electron transport system. Aerobic respiration includes stepwise of energy-releasing by oxidation of organic compounds as indicated in Equation 1, it cannot be completed without oxygen.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + energy \text{ ------ Equation 1}$$
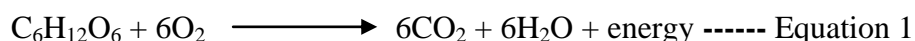
Aerobic respiration involves 3 major interacting pathways. They are consisted of glycolysis, tricarboxylic acid and electron transport system. Initial pathway of respiratory oxidation is glycolysis. In this pathway, starch or sucrose is broken down to glucose. Sequentialy break down of glucose to pyruvic acid. The pyruvic acid produced by the glycolysis pathway is further breaken down in TCA cycle. The TCA cycle is occasionally known as the Krebs cycle or citric acid cycle. It takes place in the matrix located between the cristae membranes of mitochondria. In

the initial step, pyruvic acid from glycolysis loses CO_2 to form 2-carbon acetyl group. Acetyl group combines with Coenzyme A (CoA) to form 2-carbon compounds of acetyl CoA. The acetyl CoA enters to TCA cycle by combines with oxaloacetic acid to form citric acid. Citric acid moves through a series of reaction ending with the formation of oxaloacetic acid. In this cycle, small amounts of energy and hydrogen are successively removed from a series of organic acids as well as energy-storing compounds are formed. The energy-storing compounds are $NADH_2$ and $FADH_2$. They will be further oxidized through electron transport system. The $NADH_2$ and $FADH_2$ from TCA cycle are oxidized through electron transport system. The $NADH_2$ and $FADH_2$ are passed along an electron transport to release some energy and ATP is formed. Finally, water is formed by the combination of hydrogen ions ($2H^+$) and oxygen (O_2) (Salisbury and Ross, 1985; Kays, 1991). A summary of aerobic respiration process in fruit is showed in Figure 1.

1.2.3.2 Anaerobic respiration

The respiration is also taken even limited of O_2 support or high CO_2 accumulation. It called anaerobic respiration. The equation of anaerobic respiration is indicated in Equation 2.

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + energy \text{ ------ Equation 2}$$
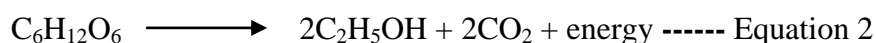
Insufficient of O_2 conditions such as fruit at late maturation or postharvest conditions of modified atmosphere storage, glucose is converted to pyruvic acid by glycolysis pathway, but, pyruvic acid can not passes through TCA cycle to produce energy-storing compounds like an aerobic condition due to the absent of oxidative enzyme in TCA cycle. Therefore, pyruvic acid is metabolized into ethanol and energy releasing by anaerobic respiration or fermentation. Under anaerobic respiration, ethanol is formed and leads to off-flavors in fruits (Baile and Young, 1981).

Glycolysis

Glucose

P   Fructose   P

P   P

PGAL   PGAL

Pyruvic acid   Pyruvic acid

To Krebs cycle (aerobic respiration) or fermentation

Fermentation

Pyruvic acid   Pyruvic acid

CoenzymeA   NAD $NADH_2$ $NADH_2$
CO_2 $NADH_2$ NAD   NAD

Acetal CoA   Ethanol

CoA CO_2 CO_2

Citric acid CO_2

4-carbon acid   NAD
Oxaloacetic acid

NAD   **Krebs cycle** $NADH_2$

$NADH_2$ **5-carbon acid**

CO_2

4-carbon acid   **4-carbon acid**

FAD $FADH_2$

Electrontransport chain

$NADH_2$ 3ADP+P

NAD   3ATP

Cytochrome and other accceptors

$FADH_2$ 2ADP+P

FAD   2ATP

H_2O ½ O+2H

Final oxidation

Figure 1 A summary of aerobic respiration process

Source: Knee (2002)

1.2.4 Maturity of longkong and its quality changes

Longkong is a non-climateric fruit, which should be picked when reaches full ripe stage to ensure good quality (Paull, 2004). The season of longkong

production, mainly is between August and September (Pantuvanid, 1985). Nevertheless, the harvesting time of longkong is vary with the areas, For example, the east of Thailand, longkong is harvested from middle of November to middle of February, and then, followed by the beginning of July to the middle July. On the other hand, in the southern of Thailand, harvested longkong is started from Apirl to middle of June, and then, followed by August to the end of October (Pantuvanid (1985). During longkong development, fruit physical and chemical qualities such as fruit weight, fruit size, fruit skin color, total soluble solid, total acidity and sugar content are changed (Sapii *et a*l., 2000). Pantuvanid (1985) suggested that the development stage of longkong can be devided into three stages based on fruit color and weight. The first stage is between the 1st and 7th week after anthesis. During the 1st to 4th week after anthesis, longkong development is slow. However, in the 5th week after anthesis, fruit weight is changed rapidly. After that, cell division and cell enlargement are increased. Fruit skin develops to yield bright yellow skin, but some fruit are still green. Longkong flesh at this stage is white and opaque and it has sour taste. Second stage is between the 7th and 13th week after anthesis. At this stage, the flesh and skin weights are approximately 74% and 24% of the total fruit weight, respectively. Third stage is started between the 13th and 16th week after anthesis. At this stage, fruit is full mature with its stable size and weight. The flesh is become transparent, soften and sweet. Third stage is the suitable time for optimal harvesting.

Both of physical and chemical qualities of longkong are changes with maturation. Changes in fruit skin color are the vital quality that can be used as an indicator for determining the harvesting time. A non-climacteric fruit such as longkong, also exhibit a marked loss of green color when it reaches the optimum quality. Skin of young longkong is pale green and turns to be yellow when full mature. At full maturation, longkong flesh turns to white translucent and astringency decreased. Moreover, loss of fruit firmness with increasing in soluble structure components of cell wall is found in full ripe longkong (Paull *et al*., 1987; Sapii *et al*., 2000; Paull, 2004). Much quantitative change associated with maturation is the breakdown or synthesis of chemical quality. The breakdown of carbohydrate polymers, especially starch converts to sugars and the reduction of organic acids are alters on fruit taste. Paull *et al* (1987) reported that the astringency of longkong at full

mature decreased with increasing in sugar at six folds. The percentage of total soluble solid (TSS) which represents for sweet taste in longkong increased with maturation. The TSS increased from 5% in longkong at 6th week to 17% at 15th week after anthesis, due to starch can be converted to sugars during maturation. At full ripe stage, longkong should have total soluble solid at least approximately 15°Brix according to National Bureau of Agricultural Commodity and Food Standards (2006).

1.2.5 Postharvest technology and sustaining fruit quality

Fruit is highly perisable produce. It has high moisture content, ranging from 70% to 95%. The fruit shelf-life under ambient conditions is very limited. The shelf-file of longkong is limited to about 4-7 days under room temperature with changes in fruit skin browning, soften texture and off-flavor formation (Paull, 2004). Fruit respiration plays a major role in the postharvest life of fresh fruit. To prolong fruit shelf life, several postharvest techniques such as modified atmosphere packaging, low temperature during storage is introduced. These techniques are really useful for fruit and widely used in worldwide.

1.2.5.1 Modified atmosphere packaging

Modified atmosphere packaging (MAP) is a technique used to prolong the shelf life of fresh fruit. MAP is created when fruit sealed in plastic film with a relatively low permeablility to gases. Consequently, as the fruit respire, the O_2 level decreases and the CO_2 level increases inside the bags (Kader, 2002). Under these atmospheric conditions, the respiration rate of fruit is decreased. The consumption of respiration substrates such as sugars or organic acids are retarded (Tano *et al*., 2007). The MAP can be devided into two types, namely, passive and active MAP. Modified atmosphere can passively evolve within a hermetically sealed package. In case of a respiration characteristic are properly matched to film permeability, then a beneficial modified atmosphere can be passively created within a package (Castro e*t al*., 2008).The active MAP consists essentially of gas flushing to quickly establish equilibrium condition within the package (Kader, 2002). Meenune and Janthachum (2004) reported that the longkong raceme which was stored under MAP has longer shelf life than that at atmospheric condition. In addition, under MAP condition

(5%CO_2 and 5%O_2), longkong raceme that treated with 1.5% citric acid for 5 min prior to storage under MAP at 18ºC had its shelf life extension for 30 days.

1.2.5.2 Low temperature

Temperature is an important factor governing the maintenance of postharvest quality in fruits. Poor temperature control can lead to the deterioration of a packaged produce due to an increase in metabolism and growth of spoilage organisms. Low temperature has been widely used to extend shelf-life of fruits. It can minimize fruit weight loss, fruit biological reaction, and microbial load. Low temperature is beneficial because respiration rates and metabolism reactions are reduced. Fruit deterioration such as skin desiccation, color changes and/or losses, firmness and disease development can be delay by low temperature during storage (Kays, 1991). Low temperature at 20°C can prolong longkong shelf-life for 8 days with only 1% weight loss, meanwhile, longkong has its shelf life for 4 days at 28.5°C (Pantuvanid, 1985). In addition, Lichanporn *et al* (2008) reported that longkong stored at low temperature at 13°C could maintain a better quality.

1.2.6 Fruit flavor and its biogenesis

Fruits produce a vast array of volatile flavors that are detected by oral and nasal senses. Volatile flavor compounds give each fruit a sensory fingerprint that is unique (Kays and Wang, 2000; Baldwin *et al*., 2000). Fruit flavors involve both volatile and non-volatile compounds.

Non-volatile flavor compounds in fruit can be detected by taste, namely, sugars, organic acids, terpenoid lactone and tannin. They provide sweetness, sourness, bitterness and astringency, respectively. Sweetness is related mainly sucrose, glucose and fructose and even sorbitol, which persists in ripe fruits (Knee, 2002). Sourness is mainly contributed to organic acids such as citric, tartaric and malic acids (Baldwin, 1993). Bitterness depends upon terpenoid lactone such as limonin, naringin (Baldwin, 1993; Maier, 1969). Astringency derives from flavonoids, alkaloids and tannin (Taylor, 1993).

Volatile flavor compounds are presented in concentrations that can be perceived by the human nose. They contribute to fruit aromas. Fruit aromas or volatile

flavor compounds are formed by a complex group of chemicals, namely, aldehydes, alcohols, esters, acids, lactones, terpenes and ketones. The typical fruit volatile compound is not present during early stage fruit maturation. But it develops entirely during maturation (Knee, 2002). During maturation, metabolism of fruit changes to catabolism. Mainly, lipids, carbohydrates and proteins, are enzymatically converted to simple fatty acids, sugars and amino acids which are metabolized and converted to volatile compounds. Details of volatile compounds form fatty acid, amino acid and carbohydrate metabolisms will be discussed as follows.

1.2.6.1 Fatty acid metabolism

Fatty acids are major precursors of aroma volatiles in most fruits. The most prevalent pathways for metabolites of fatty acid are β-oxidation and oxidation via lipoxygenase enzyme. The β-oxidation of fatty acids (linoleic and linolenic acids) is the primary pathway providing alcohols and acetyl CoA for ester formation. The esters are formed via the action of alcohol acyltransferase (AAT) enzyme (Shalit *et al*., 2000) (Figure 2)**.**

Figure 2 Formation of volatile esters by acylation of alcohols and acetyl CoA by AAT enzyme

Source: Shalit *et al* (2001)

In addition, an oxidation of fatty acids via lipoxygenase enzyme is a secondary pathway to form green aldehyde. Lipoxygenase enzyme catalyzes hydroperoxidation of polyunsaturated fatty acids (linoleic and linolenic acids) to give the green aroma in fruit. Reineccius (2006) suggested that hexanal and (*E*)-2-hexenal from oxidation of linoleic acid and linolenic acids which called a green aldehyde. The

green aldehyde such as hexanal and (*Z*)-3-hexenal) can be converted to green alcohol by the action of alcohol dehydrogenase (ADH) enzyme.

1.2.6.2 Amino acid metabolism

Amino acid metabolism generates several aroma compounds such as aromatic, aliphatic and branched chain of alcohols, acids, carbonyls and esters. Valine, leucine and iso-leucine are the main amino acid can be converted to short chain alcohols, carbonyls, and esters (Reineccius, 2006). Tressl and Drawert (1973) reported that 3-methyl butylrate was the impact compound in banana. It was formed from a deamination of leucine followed by decarboxylation. Rowen *et al.* (1996) reported that iso-leucine is a precursor of 2- methyl butanoate which was an ester in 'Granny Smith' apples. In addition, aromatic amino acids can be converted to the odor characterized as phenolic via oxidation and reduction reactions. The aromatic amino acids, tyrosine and phenylalanine are formed by the shikimic acid pathway. Many aromatic flavor compounds from spices such as eugenol (cloves), cinnamaldehyde and coumarin (cinamon) are generated from these metabolisms via deamination, oxidation and reduction reactions (Tressl and Drawert, 1973; Kays, 1991).

1.2.6.3 Carbohydrate metabolism

A large variety of volatile flavors compounds can be traced to carbohydrate metabolism. Fruit flavors come indirectly from carbohydrate metabolism since the other entire flavor precursors come from carbohydrate metabolism. Most terpenes are derived from carbohydrate metabolism, expecially for mevalonate pathway (Mahmoud and Croteau, 2002; Rodriguez-Concepcion and Boronat, 2002). The biosynthesis pathway proposed for the synthesis of isopentenyl diphosphate (IPP, isoprene building block) (Figure 3a). This pathway leads to the formation of the C_5-isopentenyl diphosphate (IDP) units, allylic isomer dimethylallyl diphosphate (DMADP) (Figure 5a) and called isoprene building block (IPP). In both of IPP and DMAPP are used by prenyl transferases in condensation reactions to produce larger prenyl diphosphates, such as the monoterpene precursor (geranyl diphosphate (GPP)), the sesquiterpene precursor (farnesyl diphosphate (FPP)) and the

diterpene and C_{40} carotenoid precursor (geranylgeranyl diphosphate (GGPP)) (Figure 3b).
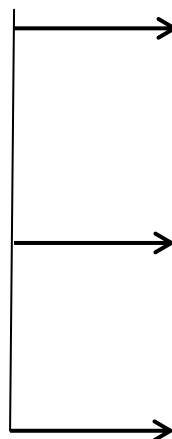
Figure 3 Biosynthesis pathways for the formation of isopentenyl diphosphate (IPP)

(a) and formation of different families of terpenes from IPP (b)

Note: x = isoprene unit

Source: Adapted from Little and Croteau (1999)

1.2.7 Glycosidically bound volatile compounds (glycoside)

Volatile flavor compounds in fruit are not only in a form of free volatile but also including in a form of bound compounds. The bound compounds are called glycosides. Volatile flavor compounds from glycosides can be released to enhance fruit flavor profile by maturation, storage, processing or aging by enzymes, acids or heat. Glycosides are carbohydrate acetals in which the hemiacetal or the reducing group of a sugar (the glycone) links through a hydroxyl group of a non-sugar component (the aglycone) either the α- and β-glycoside configuration (Karlson, 1968). Naturally, glycosides are colorless, crystalline, natural compounds, usually soluble in water alcohol or acetone (McIlroy, 1951).

Glycones may consist of monosaccharides, disaccharides or polysaccharides linking with an aglycone. Monosaccharides include both pentoses

and hexoses. The pentose glycosides are D- and L-arabinose, D-xylose and D-ribose. The hexose glycosides are D-glucose, D- and L-galactose, D-mannose, D-fructose and L-rhamnose. D-glucose is the most widely distributed sugar in plant glycosides. The majority of natural plant glycosides are β-glycoside (Emmanuel-Sarry and Günata, 2004). The common structure of glycoside is glycopyranosyl unit attached through a β-glycosidic linkage to an aglycone. It called β-D-glucosides (Winterhalter and Skouroumounis, 1997). The formation of glycoside is illustrated in Figure 4.
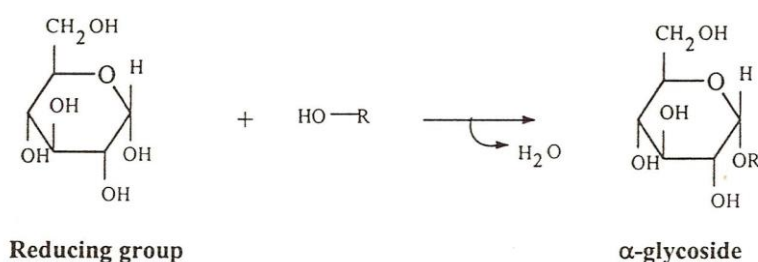
Figure 4 Formation of a glycoside linkage

Source: Karlson (1968)

1.2.7.1 Hydrolysis of glycoside

The glycosidically bound volatile compounds or glycosides can be liberated by enzymatic hydrolysis, especially by β-glucosidase, and acid hydrolysis (McIlroy, 1951; Williams *et al*., 1989). The volatile flavor compounds in fruits usually present at extremely low concentrations. But, free volatile flavor compounds from the extracted glycosides can be liberated by β-glucosidase hydrolysis.

The β-glucosidase is an effective enzyme for liberate free volatile flavor compounds. Generally, optimum pH activities are 4-6 and the optimum temperature activities are 40-50°C (Günata *et al*., 1992). Step of enzymatic hydrolysis is a sequential mode. Firstly, one of the following exoglycosidase makes the cleavage of the inter-sugar linkage liberating corresponding sugars (glycones) and β-D-glucosides. Secondly, beta-glucosidase catalyzes the hydrolysis of β-D-glucosides and liberates the corresponding aglycone and glucose (Emmanuel-Sarry and Günata, 2004). Mainly commercial glucosidases are from almond beta-glucosidase. The beta-glucosidase activity as it is a key enzyme in flavor release from glycosidic flavor precursors. Thermal denaturation is accelerated at temperature above 50°C

(Emmanuel-Sarry and Günata, 2004). The aglycones structure of beta-D-glucosides has a great effect on the activity of beta-glucosidase. The beta-D-glucosides of primary and secondary alcohols are good substrates for plan beta-glucosidase. All plant enzymes show an ability to transfer glucose to primary alcohols. Almond enzyme shows low transglucosylation activity for secondary alcohols (Günata *et al*., 1992). Enhancing of free volatile in lychee (*Litchi chinensis* Sonn.) was done by hydrolysis its bound fraction by almond β-glucosidase. The free volatile flavor compounds were enriched up to 50%. The profile of volatile flavor compounds in lychee would be changed to more floral and lemon-like (Chyau *et al*., 2003). In acerola fruit (*Malphigia glabra* L.), only free form of 17 volatile flavor compounds were identifiled. However, 42 volatile flavor compounds after β-glucosidase hydrolysis can be identified. The 3-methyl-but-3-en-1-ol was the most abundant (Boulanger and Crouzet, 2001).

In addition, acid hydrolysis is an alternative way one of an efficient technique for releasing free volatile flavor compounds from fruit glycosides (Williams *et al*., 1989). Acid hydrolysis reacts by splitting the bond between glycosyl group (glycone) and oxygen atom of the bridge (Figure 5).

Methyl beta-D-glucopyranoside   Beta-D [1-^{18}O] glucopyranose
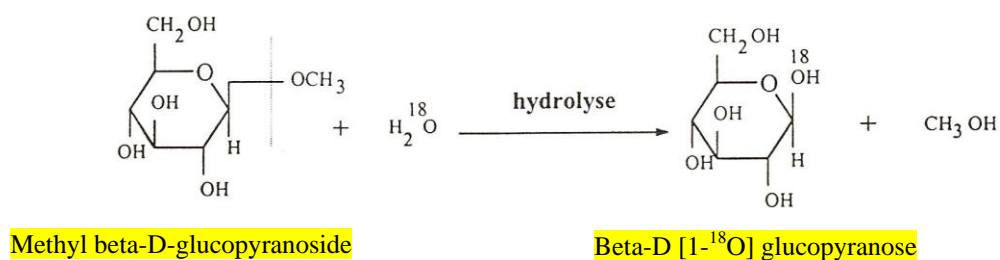
Figure 5 Hydrolysis of Methyl-D glucopyranoside to -D glucose and methanol
Source: Barnett (1981)

The hydrolysis of glycosides by acid hydrolysis increases with decreasing pH (Buttery *et al*., 1990). Different patterns of volatile flavor compounds are produced when hydrolyzed at different pH values. A Hydrolysis of the glycoside fraction was carried out in fresh tomato at pH 2.5, 3.0, 4.1 and 5.0. It was found that major volatile flavor compounds, 4-(2,3,6-trimethylphenyl)-3-buten-2-one was

identified under the hydrolysis condition at pH 2.5 and 3. However, it was not found under in the products from hydrolysis condition at pH 4.1 and 5 (Buttery *et al*., 1990). Another study was done by investigation free volatile flavor compouns released from glycoside fraction of "Tempranillo" grapes using mild acid hydrolysis of tartaric acid at pH 2.5 under 50°C for 4 weeks. The result showed that, most important volatile flavor compounds released were hexanal, octanol, 1-octen-3-one, *cis*-2-heptenal, *trans,trans*-2,4-Decadien-1-al, gamma-nona, -deca- and undecalactones, delta-decalactone and delta–dodecalactone, guaiacol, 2-phenylethanol, ethyldihydrocinnamate, ethylcinnamate, 2,6-dimethoxyphenol, 4-vinylphenol, isoeugenol, phenyl acetic acid, and vanillin (López *et al*., 2004).

1.2.8 Ethanolic-flavor

The profiles of fruit volatile flavor can be changed during storage. Mainly off-flavor likes an ethanolic flavor can be found in fruit during storage under postharvest condition such as modified atmosphere storage (Porat *et al*., 2005). In the condition of oxygen is low or not available, the build up of an anaerobic condition in fruit occours. It enhances anaerobic metabolism with increase in production of off-flavor, mainly, ethanolic flavor. The intensity of ethanolic flavor is depending upon the content of ethanol in fruit (Shi, 2005). The acetaldehyde (AA) presents in fruit acting as a natural flavor or precursor of flavor compound. It accumulates during ripening but to a much greater extent under partially or totally anaerobic conditions (Fidler, 1968). The formation of AA in fruits is from carbohydrate metabolism by using pyruvate as a substrate which was oxidized by pyruvate decarboxylase enzyme. Then, AA is converted to form ethanol. Ethanol is the most off-flavor characteristic in fruit. It obtained from fermentation of carbohydrates. Ethanol fermentation is a two-step process in which pyruvate is first decarboxylated by pyruvate decarboxylase (PDC) enzyme and gets AA. Then AA is subsequently converted to ethanol by alcohol dehydrogenase (ADH) enzyme (Cossins, 1978; Ke *et al*., 1994) (Figure 6). The activity of ADH and PDC enzymes is accelerated by high CO_2 or low O_2 of MA conditions. Under this condition, pyruavate (substrate) increases. The content of pyruvate in mandarin orange increased 1.4 folds with enhanced the accumulation of

AA and ethanol during exposed to anaerobic conditions for 24 hours (Shi *et al.*, 2007).
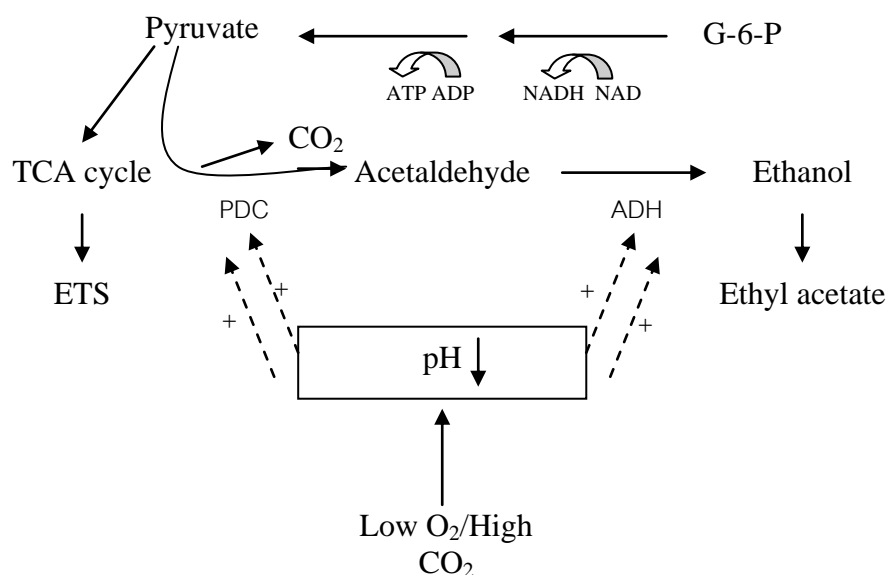
Figure 6 Ethanol fermentation pathways

Note: G-6-P=glucose-6-phosphate; PDC=pyruvate decarboxylase; ADH=alcohol dehydrogenase; TCA cycle=tricarboxylic acid cycle; ETS electron transport system; ATP=adenosine triphosphate; ADP=adenosine diphosphate; NAD=nicotinamide adenine dinucleotide; NADH= reduced nicotinamide adenine dinucleotide; $-\overset{+}{-}-\blacktriangleright$ = induction and/or activation

Source: Ke *et al*. (1994)

1.2.9 Flavor analysis

An appropriate way to determine flavor profile in food is using the combination of chemical analysis and sensory evaluation. The objective of chemical analysis is dependent upon sampling techniques and sample handing techniques and/or separation methods before measurement. Meanwhile, sensory evaluation looks at the whole sample, is very reproducible and the analysis is usually done by averaging individual responses of trained judges (Fisher and Scott, 1997).

1.2.9.1 Isolation method

Most of the techniques used in volatile flavor isolation take advantage of either solubility or volatility of flavor compounds. Inherently, flavor compounds

must be volatile to be sensed. Thus, it is logical that volatility is a common basis for separation from food matrix. Likewise, volatile flavor compounds tent to be more soluble in an organic solvent than an aqueous solution (food system). Therefore, the isolation of volatile flavor compound may be prepared by solvent extraction. The most common procedures for the isolation of the volatile flavor compounds are headspace solid phase microextraction and solvent extraction (Chin *et al*., 2007).

1.2.9.1.1 Headspace

For recovery of flavor compounds, headspace (HS) technique is a technique that has been recognized. HS techniques are frequently divided in terms of static headspace (SHS), dynamic headspace (DHS) or purge and trap. The fundamental principle in HS techniques in each case is the same, which is volatile analytes from a solid or liquid material are sampled by investigation of the atmosphere adjacent to the sample, leaving the actual sample material behind (Wampler, 2002). Different approaches have been employed, either using direct HS analysis or by collection of volatile compounds in the headspace using sorbent devices or cold traps (Augusto, 2003).

Static headspace (SHS) is a direct analysis of the equilibrium headspace above sample. This equilibrium is affected by environmental temperature, sampling size and equilibrium time. The method analyses exactly what the nose receives. This method is very simple and gentle. Sample is placed in a closed vessel and then volatile components are allowed to undergo equilibrium distributing between the sample matrix and the headspace in a sealed container under a controlled temperature. After that, headspace sample was withdrawn via a septum. In SHS techniques, a small amount of sample usually 1 ml of atmosphere around the sample (Wample, 2002) was used. Advantages of SHS include simple preparation, elimination of organic solvents and low risk of artifact formations. However, Reineccius (2006) has illustrated the primary limitation of SHS which give inadequate sensitivity. The concentration of volatile above sample generally ranges from about 10^{-4} to 10^{-10} g/l. Only the most abundant volatile flavor compound will be detected by direct headspace technique. The second disadvantage is that is not efficient towards the components with low volatility (Wample, 2002). The efficiency of headspace technique can be greatly improved by application of a trapping step to enhance the

sensitivity. This technique is generally referred to dynamic headspace (DHS) or purge and trap.

DHS involves moving the analytes away from the sample matrix in the headspace phase. Instead of allowing the sample volatile come to equilibrium between the sample matrix and the surrounding headspace. The atmosphere around the sample is constantly swept away by flowing with carrier gas (Wampler, 2002). Moreover, since the sample is being purged with a flow of carrier gas and the analytes were trapped via cryogenic, Tenax, charcoal or other trapping system for analysis, this technique is also frequently called purge and trap. In general, the terms purge and trap is used to refer to liquid samples analyzed by bubbling the carrier through the liquid while DHS is used when the sample is a solid (Wampler, 2002). The advantage of DHS or purge and trap is the increase the amount of volatile analytes and sample should be transferred to the GC for a single analysis. DHS technique offers many of the same advantages as SHS technique, including simple preparation, elimination of organic solvents and low risk of artifact formations. In addition, trapping stage of this analysis technique offers an increased sensitivity by increasing of the amount of analytes. This is accomplished by venting the carrier gas of the dynamic headspace through a collection trap, which retains the analytes while letting the carrier pass through. In this way, the analytes from a large headspace volume are concentrated in trap which called sample concentrator. Volatile present at parts per billion (ppb) levels can be detected. Furthermore, sorbents offer some selectivity of specific analytes while venting others, thus simplifying the analysis (Wampler, 2002). A major disadvantage is not efficient towards the components with low volatility. DHS is difficult to do quantitative analysis, since there is no equilibrium (Wampler, 2002). Enrichment of the HS techniques may also be accomplished through the addition of soluble salts such as NaCl (Chin *et al*., 2007).

1.2.9.1.2 Direct solvent extraction

One of the simplest and most efficient approaches for flavor isolation is direct solvent extraction caused of most compounds tend to be lipophilic. Majority of these compounds will partition into the oil phase (the organic solvent in an extraction). Flavor extraction with organic solvents has been used as a standard laboratory method in flavor research (Sugisawa, 1981). Direct solvent extraction

(DSE) is widely applied due to its efficiency at isolating a broad range of volatiles. DSE can be used as its simply as putting sample and adding solvent, finally extraction with solvent. However, various studies have noted the problems with DSE of low recovery of compounds with having high volatility. Analyte which has small molecule size losses during concentration and artifact formation can be occurred. In addition, DSE is most useful on foods that do not contain any lipids (Sugisawa, 1981; Sides, 2000). Solvent selection is an important factor to be considered. Solvents are usually selected on the basis of selectivity and boiling point. The solvent most commonly used in fruit flavor extraction is dichloromethane (Zabetakis *et al*., 2000; Vendramini and Turgo, 2000; Tokitomo *et al*., 2005; Ong *et al*., 2006). In addition, other solvents used are pentane, the mixture of pentane and dichloromethane, diethyl ether, the mixture of diethyl ether and pentane or hydrocarbon (Flores *et al*., 2002; Chassagne *et al*., 1995; Mookdasanit *et al*., 2002; Elss *et al*., 2005). To aid in extraction, NaCl may be added to the aqueous phase to salt out the organics when low density solvents are employed (Parliment, 2002).

1.2.9.1.3 Solid phase microextraction

Solid phase microextraction (SPME) is a fast, simple, convenient sample preparation and solvent-free method. SPME is an equilibrium method, similar to solvent extraction. SPME is dependent upon the extraction phase. The recovery analytes from an aqueous solution are selected by a stationary phase which coated on a fiber. The low volatility or partition coefficient analytes are problematic for this extraction. This method has attracted widespread popularity for the analysis volatile flavor compounds in of aroma various foods. Rapid analysis constituents may improve the standardization of quality and provide a relationship between sensorial and volatile contents (Riu-Aumatell *et al*., 2004). SPME is especially suitable for qualitative and quantitative analysis with a calibration or optimization of SPME conditions. It always required for increasing the sensitivity and accuracy of the analysis (Penton, 1999). The SPME techniques requires no solvent and can be performed without heating the sample, the formation of chemical artifacts is greatly reduced. Therefore, it is suitable for fruit flavor analysis. SPME involves the adsorption of analytes onto a fused silica fiber coated with suitable stationary phases and their subsequent desorption immediately before chromatographic analysis. The

target analytes can be adsorbed on the fiber by immersing it in the sample or by exposing it to the sample headspace (HS-SPME) (Harmon, 1997; Pawliszyn, 2001). SPME fibers are commercially available in several thicknesses and are coated with polymer ranging from the polydimethylsiloxane (PDMS) to the more polar Carbowax (CW). Combination of CW, PDMS, Carboxen and divinylbenzene (CAR/DVB) copolymer are also available, which provide benefit for the extraction of specific compound types (Camara *et al*., 2007). Types of coatings available can be classified as non-polar, polar and semi-polar fiber. PDMS (100 µm) is a non-polar fiber, CAR/PDMS (75 µm) is a bi-polar fiber and CW/DVB (65 µm), DVB/CAR/PDMS (50/30 µm) and PA (85 µm) are a polar fiber. Fibers with different polarity, provides high extraction selectivity and reduce the possibility of extracting interferences. For a specific application, the coating is chosen based on the polarity of the target analytes. There is no single fiber coating that will extract the analytes to the same extent. Polar fibers are effective for extracting polar analytes and non-polar fibers are effective for extracting the non-polar ones, from different matrices.

1.2.9.2 Identification of volatile flavor profile

Flavors are the combination of volatile and non-volatile compounds. Flavors are a complex of chemical substances. Flavors are usually analyzed by chromatograph techniques. Chromatograph is a general term applied to a wide variety of separation techniques based on the partitioning of distribution of a sample (analyte) between a mobile phase and a stationary phase (Fisher and Scott, 1997). Gas chromatograph (GC) or High performance liquid chromatography (HPLC) are the common instrument used for flavor analysis. Nowadays, GC is the single most widely used technique in flavor studies, especially volatile flavor componds. GC has tremendous separating power, advance in column and detector, have extremely high resolution and sensitivity possible. These attributes are essential for the separation of complex flavor (Reineccius, 2002). In GC, the mobile phase is a gas that flow through the stationary phase, which could be a solid or a liquid coated onto a solid matrix. The separation of compounds is determined by the relative rate of the reversible absorbtion or volatilization of the analyte into and out of the stationary phase (Fisher and Scott, 1997). Analysis of non-volatile derivatives of flavor compounds (e.g.,

sugar, organic acids) has been achived via HPLC approach. Mobile phase in HPLC is a liquid that flows through the stationary phase and the separation of compounds were determined by the relative solubility of the analytes into two phases (Fisher and Scott, 1997).

1.2.9.2.1 Gas chromatograph-mass spectrometry

Gas chromatograph (GC) is ideally suited to aroma studies since it has excellent separate powers and extremes sensitivity. GC is separation technique that has more efficiency than other chromatographic techniques due to its greatest number of theoretical plate when capillary column is used as stationary phase (Grant, 1996). A limitation of this technique, however, is that the sample has to be volatile and thermally stable under the analytical conditions. GC compositions are composed of injector port, column and detector. The injection port is maintained at a higher temperature than the boiling point of the least volatile component in the sample matrix. Separating components with a wide range of boiling point is accomplished by starting at low oven temperature and increasing the temperature over time to elute the high boiling point components. Column in GC is mainly divided into two types, namely, capillary column and packed column. Stationary phase in packed column is coated on the wall of column. Whereas, stationary phase in packed column are coated on inert support solid that packed in the column. After the analytes are separated using GC column, the effluent from the GC is subsequently identified by the detector. Mainly, mass detector (MS) is useful to identify unknown volatile flavor compounds. Other detectors can be used such as flame ionization detector (FID). However, they need the standard compounds to confirm. Mass spectrometer (MS) is an instrument that measures the mass-to-charge ratio (m/z) of unknown in gas phase ions and provides a typical fragmentation pattern which may use as a "fingerprint" for comparison with reference files of known spectra. The combination of GC and MS provide structural information and selective detection. Comprehensive MS libraries and efficient searching algorithms make identification simple. The MS will provide a best match for any unknown irrespective of the validity of the match. The MS is operated in either the selected ion mode (SIM) or full scan mode, depending on the objective (Fisher and Scott, 1997).

1.3 Objectives

1. To isolate and identify volatile flavor compounds in longkong using different isolation methods and GC analysis.
2. To identify free volatle flavor compounds in longkong by means of enzymatic and acid hydrolysis of glycosidically bound flavors.
3. To study the changes of longkong quality and its volatile flavor compounds during on-tree maturation.
4. To study the effect of postharvest technology (passive and active modified atmosphere conditions) on the quality changes and possibly off-flavor accumulation in longkong during storage.
6. To study the effect of intermittent warming on the quality the quality changes and possibly off-flavor accumulation in longkong during storage.
5. To investigate the correlation between instrumental analysis and sensory evaluation of aroma and off-flavor in longkong during storage.

CHAPTER 2

ISOLATION AND IDENTIFICATION OF VOLATILE FLAVOR COMPOUNDS IN LONGKONG

2.1 Abstract

Profiles of volatile flavor compounds in longkong were isolated by direct solvent extraction (DSE) and solid phase microextraction (SPME). The analytes were identified by chromatography mass spectrometry (GC-MS). Three types of solvent, dichloromethane, pentane and a mixed solution of dichloromethane and pentane (1:2 v/v) were used. Two types of 50/30 µm CAR/DVB/PDMS and 100 µm PDMS were used for SPME isolation. A different method of isolation gives different profiles of volatile flavor compounds. Volatile flavor compounds in longkong were successfully isolated by DSE as using dichloromethane and SPME as using CAR/DVB/PDMS. The three abundant volatile flavor compounds isolated by dichloromethane were cis-linalool oxide, delta-germacrene and ethyl-3-hydroxy butyrate. They were representing for sweet-floral, herbaceous and fruity attributes, respectively. Green alcohols and aldehydes were identified in the profile isolated by CAR/DVB/PDMS. The green alcohols were cis-3-hexen-1-ol, cis-4-hexen-1-ol and n-hexanol. The green aldehydes were trans-2-hexenal and benzaldehyde. Similar to the profile of DSE, fruity and sweet-floral attributes were identified in the profile of SPME as using CAR/DVB/PDMS. Main volatile flavor compounds were benzaldehyde, ethyl butyrate, cis-linalool oxide and laevo-linalool.

2.2 Introduction

Volatile flavor compounds in fruit can be isolated by various methods. The methods used in isolation of volatile flavor compounds take the advantage of either solubility or volatility. One of the simplest methods for volatile flavor compound isolation is direct solvent extraction (DSE). It is a broad range of isolation which based on solubility. Because of volatile flavor compounds tend to dissolve in the organic solvent phases, therefore, organic solvents such as dichloromethane were

commonly used in fruit volatile flavor compounds isolation (Zabetakis *et al.*, 2000; Vendramini and Turgo, 2000; Ong *et al.*, 2006).

The problems of DSE, such as low recovery of high volatility compounds, loss of a small size compounds during concentration, and artifact formation, have been reported. To overcome these problems, solvent-free isolation method called solid phase microextraction (SPME) is developed (Sugisawa, 1981; Sides, 2000). It is a method of simultaneous isolation of volatile compounds in headspace without artifact formation. The SPME method involves the adsorption of analytes and subsequent desorption immediately prior to chromatographic analysis. The SPME fibers coated are commercially available in various thicknesses and polarity, such as non-polar of PDMS (100 µm), bi-polar fiber of CAR/PDMS (75 µm) and polar of DVB/CAR/PDMS (50/30 µm) fibers (Harmon, 1997; Pawliszyn, 2001). Longkong has its unique aromatic smell (Sapii *et al.*, 2000; Sabah, 2004; Paull, 2004). However, reports on its volatile flavor compounds are limited. Most of studies are mainly concentrated on its physical attributes and the non-volatile fractions (Narkviroj and Srivatanavorachai, 1981; Sapii *et al.*, 2000). Only the study undertaken by Chairgulprasert *et al.* (2006), who identified the chemical constituents of essential oils in longkong, was found. This result reported that oleic acid (14.80%), alpha-copane (11.15%), delta-germacrene (9.16%), delta-cadinene (6.74%), (+) spathulenol (5.72%) and palmitic acid (5.49%) were the major constituents. However, the overall volatile flavor profile of longkong fruit has not yet been reported. Therefore, the aim of this study was to isolate and identify volatile flavor compounds in longkong by using two different methods, either DSE or SPME.

2.3 Material and methods
Chemicals

Dichloromethane and sodium chloride was obtained from LabScan Asia Co., Ltd (Bangkong, Thailand). Pentane was purchased from BDH Laboratory Supplies (Poole, England). Sodium sulfate anhydrous was purchased from Fisher Scientific (Leicestershire, England).

Plant material

Lonkong at 15 weeks after anthesis (full ripe stage) was obtained from a contact garden in Natawee district (Songkhla province, Thailand). Fruit was harvested in the morning and immediately transported on the same day to the Food Analysis Laboratory, Faculty of Agro-Industry (approximately 2 hours). A fruit free from any apparent skin damage and of a uniform size (grade A, approximately 3-3.5 cm in diameter, in according to Tanyongmut market) was selected for analysis.

Sample preparation

Longkong flesh (de-seeded) and 100 g in weight was mixed with NaCl (30% w/w), and blended at constant speed, using a blender for 3 min at 4°C. After that it was filtered through the stainless steel sieve, yielding the homogenate. The homogenate was clarified by using refrigerated centrifugation (4ºC) at 10,000×g for 30 min (Adapted from Chyau *et al*., 2003; Soares *et al*., 2007). Clear solution of longkong juice was obtained for isolation of the volatile flavor compounds.

Isolation of volatile flavor compounds

Volatile flavor compounds were isolated by using direct solvent extraction (DSE) and solid phase microextraction (SPME) as follows.

Direct solvent extraction (DSE)

Three different solvents, dichloromethane, pentane and mixed solution of dichloromethane and pentane (1:2 v/v), were alternatively in this work. The 100 ml clear solution of longkong juice was mixed with 100 ml of each solvent. After that, the mixture was gently shaken for 90 min and left equilibrates for 30 min at room temperature (~30°C). Solvent phase was collected and re-extracted twice. The solvent phase extracts was dried on anhydrous Na_2SO_4, and was kept overnight at -20°C, cold- filtered and concentrated by purging of nitrogen gas to produce the concentrate. The concentrate was kept at -20°C prior to analysis. The 1 μl was directly injected into GC-MS (Adapted from Flores *et al*., 2002; Elss *et al*., 2005; Ong *et al*., 2006).

Headspace solid phase microextraction (HS-SPME)

The methods of HS-SPME, two types of fiber; 100 μm PDMS (poly (dimethylsiloxane)), and 50/30 μm DVB/CAR/PDMS (divinylbenzene/Carboxen on poly (dimethylsiloxane)) (Supelco, Bellafonte, PA, USA) were applied. The 20 ml clear solution of longkong juice was placed into a 125 ml vial fitted with a rubber septum. The vial was kept at 30°C and left as equilibrates for 60 min. After equilibration, the fiber was inserted and exposed into the headspace for 15 min while maintaining the sample at 30°C. Finally, the fiber was introduced into a GC injection port, at 240°C, and left for 5 min to allow the thermal desorption of the analytes (Adapted from Lalel *et al.*, 2003; Wanakhachornkrai and Lertsiri, 2003; Camara *et al.*, 2007).

Identification of volatile flavor compounds

The volatile flavor compounds were identified by GC-MS. A chromatograph Hewlet-Packard 6890 (Palo Alto, CA, USA) was used with HP-FFAP column (25 m×0.20 mm; film thickness 0.20 mm). The inlet temperature was set at 240°C and the column temperature was maintained at 40°C for 2 min, followed by an increase to 240°C at the rate of 5°C/min and held for 13 min. The carrier gas was applied by ultra high purity helium at a constant flow of 1.5 ml/min. A Hewlet-Packard 5973 A mass spectrometer with a quadrupole mass filter was coupled to the GC. A mass spectrum (MS) was obtained at 70 eV in the electronic impact (EI) and positive modes. MS was scanned in range *m/z* 40-350 at 1 s intervals. The integration of peaks was done on HP chemstation software (Hewlett-Packard). The minimum peak area for detection level was 100,000 counts (Adapted from Wanakhachornkrai and Lertsiri, 2003).

2.4 Results and discussion

2.4.1 Profiles of volatile flavor compounds in longkong isolated by direct solvent extraction (DSE)

The DSE isolation showed 16 volatile flavor compounds. Their chromatograms are presented in Figure 7. The volatile flavor compounds were classified into groups of ester, alcohol, terpene and its derivative, ketone and phenol

(Table 1). The results show that a group of terpenes and their derivatives was identified as abundances. Among the 3 types of solvents, dichloromethane shows as the most suitable for DSE. It was probably due to the various fruity-volatile flavor compounds were identified in the profile. The cis-linalool oxide, delta-germacrene and ethyl-3-hydroxy butyrate were identified as abundances. The profile of volatile flavor compounds using pentane as a solvent extraction is shown in Table 1 and Figure 7B. The results showed that only a group of terpenes and their derivatives was identified in the pentane profile. It might be due to a non-polarity system of pentane unavailable for polar volatile flavor compounds. The delta-germacrene and laevo-linalool represent herbaceous and fruity flavors were identified as abundances. The profile of volatile flavor compounds of using mixed solution of dichloromethane and pentane (1:2 v/v) as a solvent extraction is shown in Table 1 and Figure 7C. A few different between a profile of pentane and mixed solution of dichloromethane and pentane (1:2 v/v) was observed. A higher polarity of this mixed solution than pentane, therefore, a group of esters such as ethyl-3-hydroxy butyrate and phenol was identified. The abundance volatile flavor compounds of delta-germacrene and para-cymene were found in a mixed solution extraction. They represent herbaceous and citrus, and flavors, respectively.

2.4.2 Profiles of volatile flavor compounds in longkong isolated by solid phase microextraction (SPME)

The SPME showed 19 volatile flavor compounds. Their chromatograms are shown in Figure 8. The volatile flavor compounds were classified into a group of ester, alcohol, aldehyde, terpene and its derivative (Table 2). A group of terpenes and their derivatives was identified as abundances. Two types of SPME fiber, namely, PDFS and CAR/DVB/PDMS, were used in this work. It was found that CAR/DVB/PDMS was a suitable fiber for the extraction of volatile flavor compounds in longkong. Because of the various types of volatile flavor compounds represent for the fruity characteristic were also identified in its profile. The green aldehydes and alcohols were identified by CAR/DVB/PDMS, namely trans-2-hexenal and cis-3-hexen-1-ol. In addition, volatile flavor compounds of fruity ester, such as ethyl butyrate and sweet aldehyde such as benzaldehyde, were identified in the profile of

CAR/DVB/PDMS. The beta-caryophyllene and beta-carene were identified as abundances in the PDMS profile. Their attributes are fruity and orange peel, respectively. However, esters and aldehydes were not identified by PDMS. It might be due to non-polar of PDMS coating.

2.4.3 Comparing between volatile flavor compounds in longkong isolated by direct solvent extraction (DSE) and solid phase microextraction (SPME)

The different types of volatile flavor compounds and their amounts in both DSE and SPME isolation methods were monitored. The method of DSE was chosen due to the advantage in terms of the solubility of the compounds. Many groups of volatile flavor compounds were identified in the DSE profile as can be seen in Tables 1. Groups of ketone and phenol were presented only in the DSE profiles. The 3-hydroxy-2-butanone was identified in the dichloromethane profile which gives the sweet characteristic (Table 1). The higher amount of many terpenes and their derivatives were identified in SPME profiles (Table 2). The trans-ocimene, beta-carene, alpha-gurjunene, cis-linalool oxide, alpha-copene, beta-caryophyllene, alpha-humulene, alpha-muurolene, delta-germacrene and delta-cadinene were present only in the SPME profiles. According to the free solvent SPME and performed by without the concentration step, high-volatility compounds were still identified in the SPME profile. In addition, the formation of chemical artifacts is greatly reduced by using the SPME method as suggested by Harmon, 1997 and Pawliszyn, 2001. It was aligned with the smooth and clear chromatogram obtained by SPME isolation as can be seen in Figure 2.

Table 1 Volatile flavor compounds and their attributes identified in longkong extracted by direct solvent extraction (DSE) method with GC-MS

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]		
				Types of solvent		
				Dichloromethane	Pentane	Dichloromethane:Pentane (1:2)
Esters						
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	15.21	nd	2.01
Alcohols						
n-butanol	6.19	1157	fruity	5.33	nd	nd
n-hexanol	11.05	1347	green	2.97	nd	nd
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	3.83	nd	nd
benzyl alcohol	23.77	1895	floral, sweet	11.24	nd	nd
phenylethyl alcohol	24.45	2134	floral	13.20	nd	nd
Terpenes and their derivatives						
cis-linalool oxide	14.39	1479	floral, sweet	28.04	16.11	20.54
laevo -linalool	16.35	1559	fruity, sweet	14.41	48.11	30.00
beta-caryophyllene	17.12	1591	fruity	-	15.81	29.21

Table 1 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]		
				Types of solvent		
				Dichloromethane	Pentane	Dichloromethane:Pentane (1:2)
Terpenes and their derivatives						
alpha-humulene	18.30	1640	woody	nd	7.26	3.31
alpha-muurolene	19.63	1701	woody	nd	17.05	8.92
delta-germacrene	19.75	1706	herbaceous	15.78	52.42	31.79
delta-cadinene	20.84	1756	herbaceous	2.60	18.05	19.75
para-cymene	32.56	2370	citrus	nd	nd	42.01
Ketones						
3-hydroxy-2-butanone	9.78	1298	sweet	4.98	nd	nd
Phenols						
phenol	26.38	2027	phenol	13.09	nd	4.71

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = total ion current; nd = not detected

Abundance (A) Dichloromethane

delta-germacrene

ethyl-3-hydroxy butyrate

cis-linalool oxide

450000
400000
350000
300000
250000
200000
150000
100000
50000

5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00

Time-->

Abundance (B) Pentane

delta-germacrene

laevo-linalool

1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000

5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00
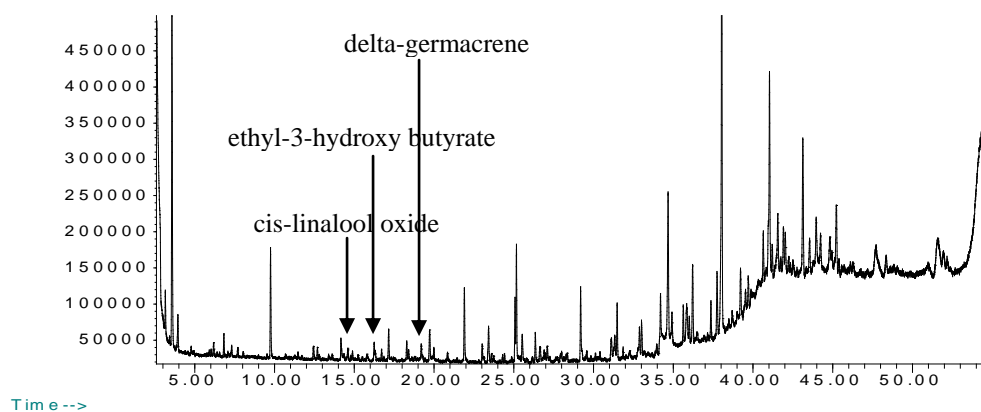
Time-->
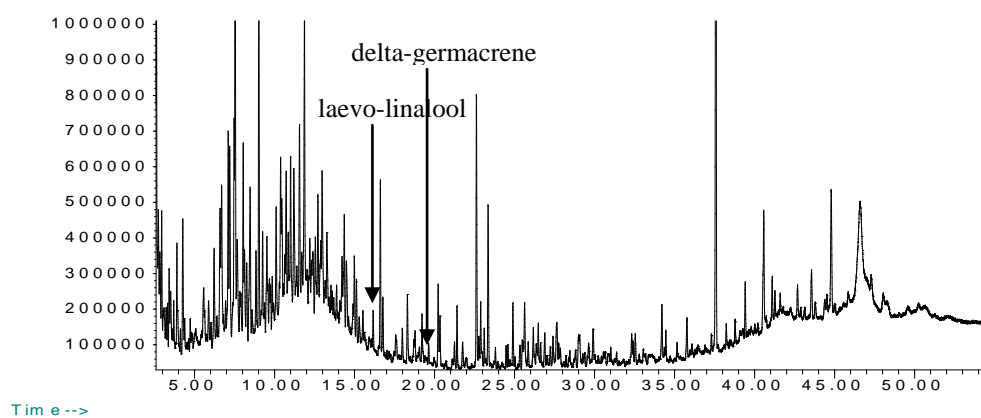
Abundance (C) Dichloromethane: Pentane (1:2 v/v)

phenol

ethyl-3-hydroxy butyrate

1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000

5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00

Time-->
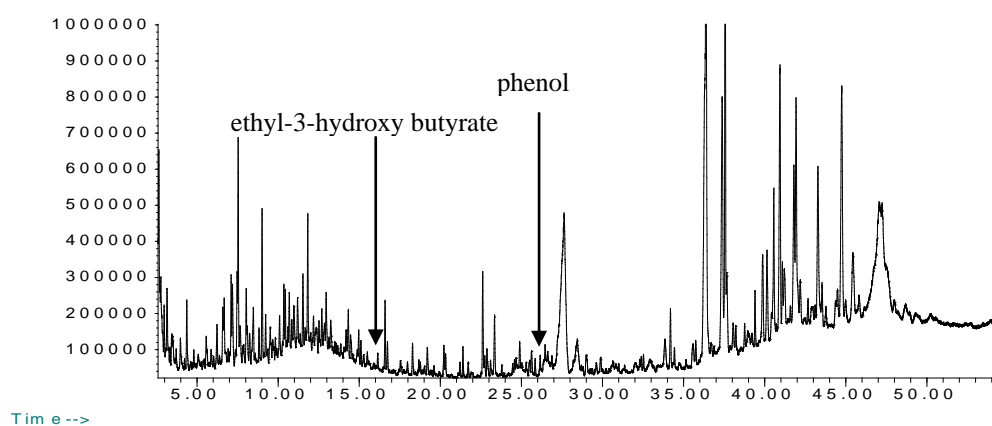
Figure 7 Chromatogram of volatile flavor compounds of longkong extracted by using

different types of solvents

Table 2 Volatile flavor compounds and their attributes identified in longkong extracted by solid phase microextraction (SPME) with GC-MS

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]	
				Types of SPME	
				CAR/DVB/PDMS	PDMS
Esters					
ethyl butyrate	3.36	1049	fruity	11.42	nd
Alcohols					
ethanol	3.00	1002	alcohol	14.91	21.59
cis-3-hexen-1-ol	8.76	1258	fresh, green	39.99	nd
cis-4-hexen-1-ol	9.48	1300	fresh,green,purgentgreen	9.49	nd
n-hexanol	11.05	1347	green	11.95	1.14
Aldehydes					
trans-2-hexenal	6.39	1165	green, leaf	38.23	nd
n-hexanal	9.48	1286	grass	32.53	nd
benzaldehyde	11.53	1482	fruity	19.14	nd
Terpenes and their derivatives					
trans-ocimene	6.81	1183	floral, sweet, herbaceous	3.41	nd
beta-carene	10.37	1421	orange peel	nd	37.16

Table 2 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]	
				Types of solvent	
				CAR/DVB/PDMS	PDMS
Terpenes and their derivatives					
alpha-gurjunene	11.30	1457	woody, balsamic	nd	31.17
cis-linalool oxide	14.39	1479	floral, sweet	25.12	6.77
alpha-copene	14.58	1486	woody	5.03	3.21
laevo -linalool	16.35	1559	fruity, sweet	23.39	17.41
beta-caryophyllene	17.12	1591	fruity	nd	36.06
alpha-humulene	18.30	1640	woody	nd	10.85
alpha-muurolene	19.63	1701	woody	nd	3.10
delta-germacrene	19.75	1706	herbaceous	7.09	15.22
delta-cadinene	20.84	1756	herbaceous	7.23	4.80

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = total ion current; - = not detected

CAR/DVB/PDMS = 50/30 µm divinylbenzene/Carboxen on poly (dimethylsiloxane)

PDMS = 100 µm poly (dimethylsiloxane)

(A) CAR/DVB/PDMS

Abundance

(B) PDMS

Abundance
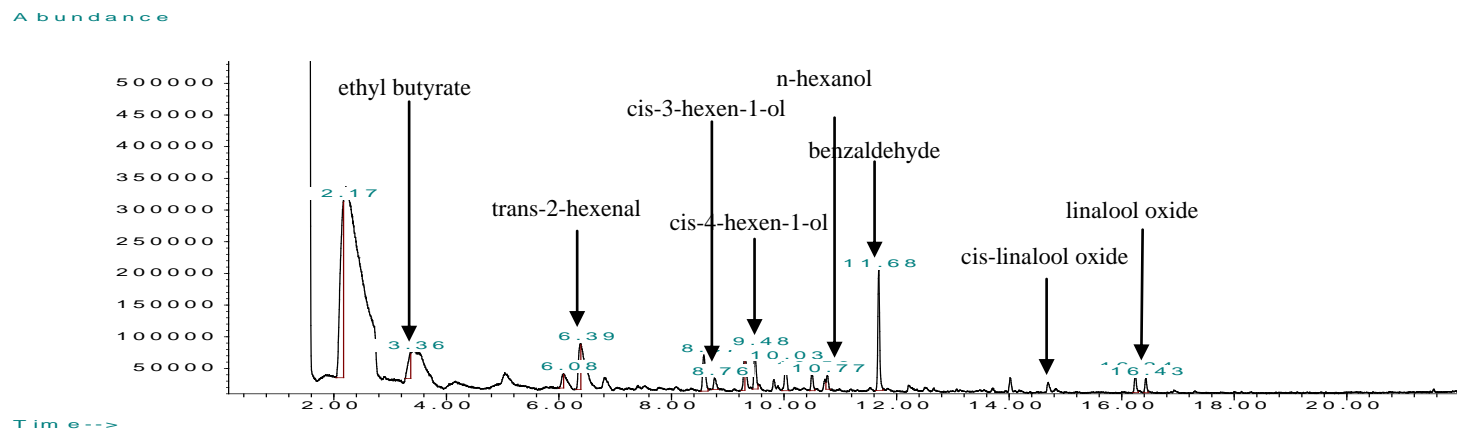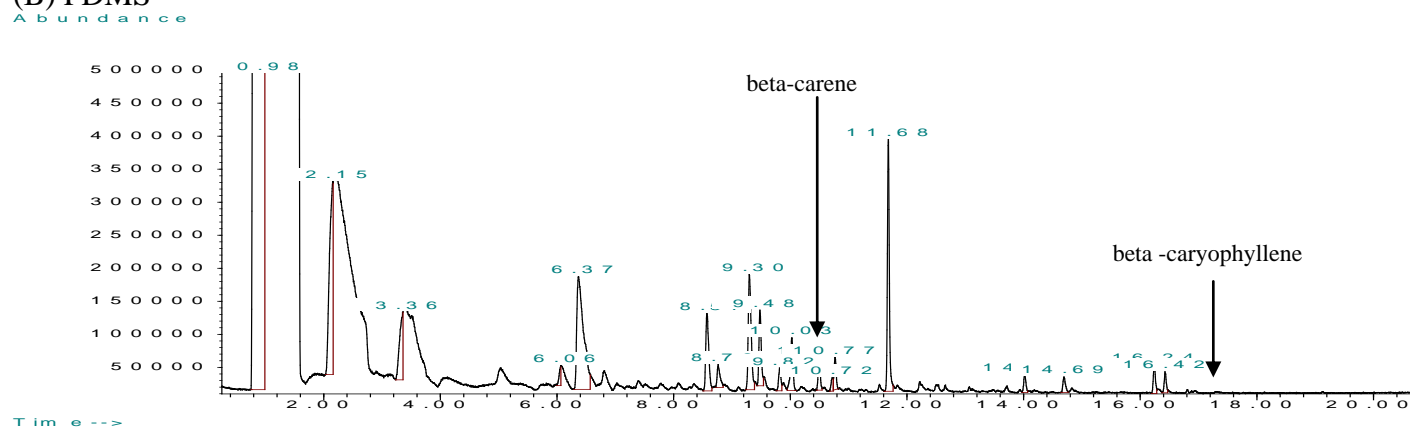
Figure 8 Chromatogram of volatile flavor compounds of longkong extracted by using different types of SPME

Note: CAR/DVB/PDMS = 50/30 μm divinylbenzene/Carboxen on poly (dimethylsiloxane)

PDMS=100μm poly (dimethylsiloxane)

2.5 Conclusion

Volatile flavor compounds in longkong can be isolated by various methods, either by DSE or solvent free of SPME coupling with GC-MS. A different method of isolation gives different profiles of volatile flavor compounds. The high polarity method of DSE by using dichloromethane and SPME by using CAR/DVB/PDMS presented the suitable conditions for the isolation of volatile flavor compounds in longkong. A group of terpenes and their derivatives was identified as a major compound. The most abundance of cis-linalool oxide, delta-germacrene and ethyl-3-hydroxy butyrate were identified in the profile of DSE by using dichloromethane as a solvent for extraction. In addition, green aldehydes and alcohols were identified by CAR/DVB/PDMS namely trans-2-hexenal and cis-3-hexen-1-ol. Main volatile flavor compounds in longkong, such as ethyl butyrate as a fruity ester and benzaldehyde as a sweet aldehyde, were also identified in the profile of CAR/DVB/PDMS.

CHAPTER 3

IDENTIFICATION OF FREE VOLATILE FLAVOR COMPOUNDS IN LONGKONG AFTER HYDROLYSIS OF GLYCOSIDICALLY BOUND FLAVORS BY ENZYMATIC AND ACID

3.1 Abstract

Glycosidically bound flavors fraction of longkong was isolated by Amberlite XAD-2 adsorption followed by methanolic extraction. After hydrolysis aglycones (free volatile flavor compounds) and glycone (glucose residues) were determined by GC-MS and HPLC, respectively. The enzymatic hydrolysis was performed at 40°C using β-glucosidase enzyme under different pHs (pH 3, 4, 5 and 3.8 (equal to pH of longkong)) and incubation times (4, 8, 12, 16 and 24 hours). The acid hydrolysis under different pHs (pH 1, 2, 3, 4 and 5) and incubation temperatures (30, 50, 70, 90 and 100°C) for 60 min also performed. Enzymatic hydrolysis under a condition of pH 5 for 24 hours was an optimal condition for the releasing of the highest amount of aglycones. In addition, acid hydrolysis under a condition of pH 1 at 100°C gives the highest amount of aglycones. Both of enzyme and acid hydrolysis exhibited similar types of aglycones. A higher amount of aglycones was found in the system of acid hydrolysis. The liberated aglycones were composed of butyl butanoate, 2-ethyl-1-hexanol, trans-2-hexanal and phenol, with the attributes of fruity, citrus, green and phenol characteristic, respectively.

3.2 Introduction

Longkong is a well known and important commercial fruit in the southern of Thailand. It has a unique aromatic smell (Sapii *et al*., 2000; Sabah, 2004; Paull, 2004), giving a pleasant taste. Volatile flavor compounds in fruit are normally presented in both of free and bound form with sugar in the form of glycosidically bound flavors or glycosides. Analytical researches on the volatile flavor compounds in free form of longkong were carried out by Meenune *et al*. (2009) and Chairgulprasert *et al*. (2006). The characteristic of longkong aroma arises from a complex mixture of compounds, terpenes, their derivatives and esters. In the previous (chapter 2), free

volatitle flavor compounds in longkong were identified. They were cis-linalool oxide, delta-germacrene and ethyl-3-hydroxy butyrate which were representing for sweet-floral, herbaceous and fruity attributes, respectively.

The presence of glycosidically bound flavors or glycosides which was shown a potential enhanced of fruit sensory profile has been reported (Pabst *et al*., 1991; Wu *et al*., 1991; Groyne *et al*., 1999; Boulanger and Crouzet, 2001). Glycosidically bound flavor can be released free forms either acid or enzymatic hydrolysis (Williams, 1993; Chassagne *et al*., 1999）. The release of aromatic aglycones from the extracted glycosides has been affected by beta-glucosidase. The plant β-glucosidases are generally specific to natural glycosides and almond is a common source for extraction commercial beta-glucosidase. Infusion of fruit tissue with β-glucosidase, glycoside flavor precursors could be conceivably converted into free aroma volatiles (Jerković and Mastelić, 2001). Chyau *et al*. (2003) investigated on the effect of almond β-glucosidase on recovery of free and glycosidicall bound volatile compounds in lychee (*Litchi chinensis* Sonn.) The aroma would be enriched by 50% as the volatile compounds in the bound fraction were released by enzymatic hydrolysis. The aroma characteristics would be changed to more floral and lemon-like due to high amount of geraniol and geranial in bound fraction. Acid hydrolysis is an alternative way to release free form of volatile flavor compounds in fruit. It was be the most efficient technique for sugar release from fruit glycoside (Williams *et al*., 1995). López *et al*. (2004) investigated in odorants released from odorless fraction from "Tempranillo" grapes by mild acid hydrolysis. The odorless fraction was hydrolyzed by tartaric acid in a model wine (13% v/v ethanol, 6 g/l tartaric acid, pH 3.2) maintained for 4 weeks at 50°C. The result showed that 98 odor-active regions were detected. The most important odorants released were unsaturated fatty acid derivatives, such as hexanal, octanol, laevo-octen-3-one, cis-2-heptenal, trans-2-trans-4-decadienal. In the system of acid hydrolysis, different patterns of volatile compounds are produced when they are hydrolyzed at different pH values (Buttery *et al*., 1990; Nolen and Friend (1994). Until now, the information about glycosidically bound flavors or glycosides in longkong is not available. Therefore, this work is initiated gaining a better understanding of some liberated aglycones (free volatile

flavor compounds) from glycosidically bound flavor through the beta-glucosidase enzymatic and acid hydrolysis.

3.3 Material and methods

Chemicals

Dichloromethane and sodium chloride was obtained from LabScan Asia Co., Ltd (Bangkok, Thailand). Anhydrous sodium sulfate was purchased from Fisher Scientific (Leicestershire, England). Almond beta-glucosidase enzyme was obtained from Sigma Chemical Co. (St. Louis, MO). Dibasic sodium phosphate and citric acid were purchased from Fisher Scientific (Leicestershire, England). Acetonitrile and water (HPLC grade) were obtained from Prolabo (Paris, France). The D-glucose was purchased from Fluka (Messerchmittstr, Switzerland).

Amberlite XAD-2 resin

Amberlite XAD-2 resin adsorbent was purchased from Sigma Chemical Co. (St. Louis, MO). The XAD-2 resin was washed successively with methanol and dichloromethane (each solvent for 8 hours). After that, it was stored in methanol according to Amberlite XAD-2 polymeric adsorbent product specification.

Plant material

Longkong was obtained from a contact garden in Natawee district (Songkhla province, Thailand). The maturity stage of 13 weeks after anthesis was chosen. Fruit was harvested in the morning and immediately transported on the same day to the Food Analysis laboratory, Faculty of Agro-Industry (approximately 2 hours). Fruits free from any apparent skin damage and uniform size (grade A about 3-3.5 cm in diameter, according to Tanyongmut market) were selected for analysis.

Sample preparation

Longkong flesh (de-seeded) 100 g was mixed with NaCl (30% w/w), and blended at constant speed, using a blender for 3 min at 4°C. After that it was filtered through the stainless steel sieve, yielding the homogenate. The homogenate was clarified by using refrigerated centrifugation (4°C) at 10,000×g for 30 min. Clear

solution of longkong juice was obtained for free and bound fractionation (Adapted from Chyau *et al*., 2003; Soares *et al*., 2007).

Free and bound fractionation

The 200 ml of clear solution was subjected onto a 25×1 cm, i.d. glass column filled with Amberlite XAD-2 at 1.5 ml/min. In this step, the column was rinsed with 300 ml of distilled water to remove free sugars and other polar contents. Subsequently, the column was rinsed with 300 ml of dichloromethane to elute free fraction with adsorbed on the column. The bound fraction has finally been collected by eluting with 300 ml of methanol (Adapted from Groyne *et al*., 1999).

Hydrolysis of bound fractionation

Enzymatic hydrolysis (Beta-glucosidase enzyme)

The 10 ml of bound fraction was concentrated to final volume of 1 ml by gentle purging with nitrogen gas. The concentrate (1 ml) was re-dissolved in 4 ml of 0.1 M phosphate-citrate buffer in different pH ranges (pH 3, 4, 5 and 3.8 (equal to natural pH of longkong)). After that, 1 ml of almond beta-glucosidase solution (5 unit/ml in 0.1 M phosphate-citrate buffer, pH 5) was added. The mixture was incubated at 40°C for 4, 8, 12, 16 and 24 hours.

Acid hydrolysis

The 10 ml of bound fraction was concentrated to final volume of 1 ml by gentle purging with nitrogen gas. The concentrate (1 ml) was re-dissolved in 4 ml of 0.1 M phosphate-citrate buffer in different pH ranges (pH 1, 2, 3, 4 and 5). The mixture was incubated at 30, 50, 70, 90 and 100°C for 60 min.

Isolation and identification of aglycone

Aglycones obtained from bound fraction hydrolysis were isolated by direct solvent extraction (DSE). They were isolated by 5 ml of dichloromethane. The mixture was gentle shaken for 90 min and left equilibrates for 30 min at room temperature (~30°C). Solvent phase was collected and re-extracted twice. The solvent phase was dried on anhydrous Na_2SO_4, kept overnight at -20°C, cold-filtered and

concentrated by purging of nitrogen gas to produce the concentrate. The concentrate was kept at -20°C prior to analysis. The 1 µl was directly injected into GC-MS (Adapted from Flores *et al*., 2002; Elss *et al*., 2005; Ong *et al*., 2006).

The analytes were identified by GC-MS. A chromatograph Hewlet-Packard 6890 (Palo Alto, CA, USA) was used with HP-FFAP column (25 m×0.20 mm; film thickness 0.20 mm). The inlet temperature was set at 240°C and the column temperature was maintained at 40°C for 2 min, followed by an increase to 240°C at the rate of 5°C/min and held for 13 min. The carrier gas was applied by ultra high purity helium at a constant flow of 1.5 ml/min. A Hewlet-Packard 5973 A mass spectrometer with a quadrupole mass filter was coupled to the GC. A mass spectrum (MS) was obtained at 70 eV in the electronic impact (EI) and positive modes. MS was scanned in range *m/z* 40-350 at 1 s intervals. The integration of peaks was done on HP chemstation software (Hewlett-Packard). The minimum peak area for detection level was 100,000 counts (Adapted from Wanakhachornkrai and Lertsiri, 2003).

Glycones (glucose residues) determination

After aglycone isolation, the fraction of glycone or glucose residues was obtained. The concentration of glucose residues was determined by HPLC (Shimadzu, CR 6A Chromatopac) with Hypersil APS-2 column. Injection volume was 10 µl with an isocratic flow rate 1 ml/min and refractive index detector. The 90% acetonitrile was used as a mobile phase. Their concentrations were quantified by comparing retention time and peak area of the samples with glucose standards (Adapted from Ong *et al*., 2006).

Statistical analysis

The experiment was performed by a completely randomized design (CRD) with factorial treatment structure. The enzymatic hydrolysis, a two-way factorial treatment structure (5 levels of pH × 5 levels of incubation times) was performed. The acid hydrolysis, a two-way factorial treatment structure (5 levels of pH × 5 levels of incubation temperature) was performed. Significant differences between means were estimated by Duncan's new multiple range test (DMRT), with a level of significance of 0.05. Statistical analyses were performed by using the

Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

3.4 Results and Discussion

Enzymatic and acid hydrolysis had affected on the liberation of glycone and aglycone fractions. The results were reported and discussed as follows.

3.4.1 Enzymatic hydrolysis

The beta-glucosidase enzymatic hydrolysis was significantly affected on glycone and aglycone liberate ($p < 0.05$). The different pH and incubation times had a significantly play role on aglycone released ($p < 0.05$). Large amounts of aglycones were identified after treated with enzymatic hydrolysis. The esters, alcohols, aldehydes, acids and phenol were identified by GC-MS. The 4 alycones of butyl butanoate, 2-ethyl-1-hexanol, trans-2-hexanal and phenol were identified. They give the attributes of fruity, citrus, green and phenol characteristic, respectively. The hydrolysis with beta-glucosidase under the condition of pH 5 for 24 hours was the best condition for releasing the aglycones in the highest amounts (Table 3). In addition, slightly different of the amounts of aglycones was found in the condition of pH 4 and pH 5. It probably due to the optimum pH which enhances the activities of plant beta-glucosidase is ranged from 4 to 6 (Gunata *et al*., 1992). However, The 4 main aglycones were released and increased in their amounts when giving a longer incubation time and using the pH near the optimum pH of beta-glucosidase enzyme. In addition, glycones (glucose residues) were determined after enzymatic hydrolysis by HPLC. It was found that, the combination of pH and incubation times had a significant effect on the amount of glucose residues ($p < 0.05$) (Table 4). The amount of glucose residues increased with incubation time ($p < 0.05$). Increasing in glucose residues was positive relation with the increasing in aglycones moiety as presented in Table 3.

Table 3 Volatile flavor compounds in bound fraction after a methanolic extract (Amberlite XAD-2) and enzymatic hydrolysis

	Peak areas (×10⁴) TIC[A]																								
pH	Control (no enzyme)					pH 3					pH 4					pH 5					pH 3.8 (equal to pH of longkong)				
Incubate time (hours)	4	8	12	16	24	4	8	12	16	24	4	8	12	16	24	4	8	12	16	24	4	8	12	16	24
Esters																									
butyl butanoate	nd	nd	nd	nd	nd	nd	nd	2.15	3.14	6.41	nd	5.01	7.88	10.00	12.50	4.21	8.48	9.09	14.05	15.61	nd	3.31	5.05	7.11	10.04
Alcohols																									
2-ethyl-1-hexanol	nd	nd	nd	nd	nd	nd	nd	nd	2.03	3.01	nd	nd	6.06	7.08	9.42	nd	5.01	7.07	9.00	10.76	nd	nd	nd	4.87	5.00
Aldehydes																									
trans-2-hexanal	nd	nd	nd	nd	nd	nd	nd	1.01	2.00	2.50	1.43	2.00	3.06	4.01	4.04	3.02	4.02	4.56	5.01	5.00	nd	nd	2.78	3.11	3.50
Phenol																									
phenol	nd	nd	nd	nd	nd	nd	nd	3.11	5.01	14.27	nd	5.05	8.41	14.02	17.01	8.99	13.05	21.01	28.44	29.00	nd	15.23	18.88	27.07	28.00

Note: [A] TIC = total ion current; nd = not detected

butyl butanoate (fruity, 1841[RI]); RI = retention index

2-ethyl hexanol (citrus, floral, sweet, 1499[RI])

trans-2-hexanal (green, leaf, 1165[RI])

phenol (phenol, 2027[RI])

Table 4 Glucose residues (glycones) in bound fraction after a methanolic extract
(Amberlite XAD-2) and enzymatic hydrolysis

pH	Incubation time (hours)	Glucose residues (%)
Control	4	2.03 ± 0.01^k
	8	2.07 ± 0.00^l
	12	2.10 ± 0.02^j
	16	2.19 ± 0.02^f
	24	2.21 ± 0.00^e
3	4	2.07 ± 0.01^k
	8	2.13 ± 0.00^i
	12	2.14 ± 0.02^i
	16	2.18 ± 0.02^{fg}
	24	2.18 ± 0.00^{fg}
4	4	2.13 ± 0.01^i
	8	2.17 ± 0.00^{hg}
	12	2.26 ± 0.00^d
	16	2.28 ± 0.02^c
	24	2.30 ± 0.00^c
5	4	2.15 ± 0.01^{hg}
	8	2.16 ± 0.02^{hg}
	12	2.22 ± 0.02^e
	16	2.26 ± 0.00^c
	24	2.47 ± 0.01^a
3.8 (equal to pH of longkong)	4	2.13 ± 0.01^i
	8	2.17 ± 0.01^{hg}
	12	2.18 ± 0.00^{fg}
	16	2.26 ± 0.00^d
	24	2.37 ± 0.01^b

Note: Data are mean $\pm$ standard deviation (in four replicates).

Mean values in the same column with different superscript letters indicate that
there are significant different among pH and incubation time ($p < 0.05$).

3.4.2 Acid hydrolysis

After XAD-2 fractionation, acid hydrolysis of bound fraction in longkong juice was performed. The effect of the combination of pH and incubation temperatures on the release of aglycones and glycones was determined. Types and amount of liberated aglycones after acid hydrolysis were closely dependent on the pH and incubation temperature ($p < 0.05$). The hydrolysis of glycosides by acid hydrolysis increased with decreasing in pH and increasing in incubation temperature. The mixtures of liberated aglycones were composed of esters, alcohols, aldehydes, acids and phenol. The 4 alycones of butyl butanoate, 2-ethyl-1-hexanol, trans-2-hexanal and phenol were identified (Table 5). They give the attributes of fruity, citrus, green and phenol characteristic, respectively. Slightly different of the amount of aglycones released at the condition of pH 1, incubated temperatures at 90 and 100°C was found. However, acid hydrolysis under the condition of pH 1 at 100°C showed the best condition for released aglycones with the highest amounts (Table 5). It could be explained by strong acid condition quite easy liberate free volatile bound from sugar molecules. In addition, glycones or glucose residues were determined after acid hydrolysis by HPLC. It was found that, the combination of pH and incubation temperature had a significant effect on the amount of glycone residues ($p < 0.05$). The increasing in glycone residues after hydrolysis was in agreement with the increasing in aglycones moiety releases. The contents of glucose were reported in Table 6.

Table 5 Volatile flavor compounds identified in longkong after a methanolic extract (Amberlite XAD-2) and acid hydrolysis

	Peak areas (×10⁴) TIC[A]																								
pH	pH 1					pH 2					pH 3					pH 4					pH 5				
Incubation temp. (°C)	30	50	70	90	100	30	50	70	90	100	30	50	70	90	100	30	50	70	90	100	30	50	70	90	100
Esters																									
butyl butyrate	2.00	3.00	5.88	9.00	10.50	2.56	3.31	8.11	12.34	12.57	0.48	1.51	3.11	4.04	5.33	nd	nd	2.05	3.11	3.44	nd	nd	1.01	2.42	2.81
Alcohols																									
2-ethyl-1-hexanol	3.12	4.21	8.00	9.38	9.42	3.33	5.61	8.92	14.00	14.21	1.88	2.56	4.50	5.03	5.41	nd	nd	1.74	2.87	3.08	nd	nd	0.84	1.67	2.00
Aldehydes																									
trans-2-hexanal	2.01	3.00	4.06	4.51	4.64	1.41	1.88	2.09	3.57	4.00	0.63	1.00	1.53	1.80	3.30	nd	nd	0.78	1.51	2.50	nd	nd	nd	0.45	1.00
Phenol																									
phenol	10.02	11.23	14.08	33.07	38.00	6.39	10.15	18.01	22.14	23.00	4.14	5.05	10.41	14.02	16.51	4.00	4.82	5.31	5.34	6.84	2.03	2.34	4.11	5.41	6.27

Note: [A] TIC = total ion current; nd = not detected

butyl butanoate (fruity, 1841[RI]); RI = retention index

2-ethyl hexanol (citrus, floral, sweet, 1499[RI])

trans-2-hexenal (green, leaf, 1165[RI])

phenol (phenol, 2027[RI])

Table 6 Glucose residues (glycones) in bound fraction after a methanolic extract
(Amberlite XAD-2) and acid hydrolysis

pH	Incubation temp. (°C)	Glucose residues (%)
1	30	3.16 ± 0.00^e
	50	3.25 ± 0.04^d
	70	4.04 ± 0.10^b
	90	4.10 ± 0.00^a
	100	4.12 ± 0.01^a
2	30	2.99 ± 0.00^f
	50	3.00 ± 0.04^f
	70	3.14 ± 0.11^e
	90	3.31 ± 0.02^c
	100	3.31 ± 0.02^c
3	30	2.20 ± 0.01^j
	50	2.28 ± 0.04^i
	70	2.39 ± 0.11^h
	90	2.58 ± 0.02^g
	100	2.59 ± 0.02^g
4	30	2.28 ± 0.01^k
	50	2.29 ± 0.04^{jk}
	70	2.32 ± 0.11^i
	90	2.40 ± 0.02^i
	100	2.41 ± 0.02^h
5	30	2.13 ± 0.01^k
	50	2.17 ± 0.01^{jk}
	70	2.20 ± 0.10^i
	90	2.28 ± 0.00^i
	100	2.30 ± 0.02^i

Note: Data are mean $\pm$ standard deviation (in four replicates).

Mean values in the same column with different superscript letters indicate that
there are significant different among pH and incubation time ($p < 0.05$).

3.4.3 Comparing between enzymatic hydrolysis and acid hydrolysis

The 4 alycones of butyl butanoate, 2-ethyl-1-hexanol, trans-2-hexanal and phenol were released and identified by GC-MS after enzymatic and acid hydrolysis (Tables 3 and 5). Both of enzymatic and acid hydrolysis showed the similarly pattern of aglycones but significant different in term of their amount. The system of acid hydrolysis presented a higher amount of aglycones comparing with enzymatic hydrolysis. It probably due to strong acid quite easy liberates the glycidic linkage than the system of enzymatic. Since the system of enzymatic is more specific and required the optimal condition (optimal pH and temperature) to accelerate the hydrolysis reaction (Williams *et al*., 1995; Buttery *et al*., 1990).

3.5 Conclusion

Non-volatile or odorless precursors such as glycosidically bound flavors are the potential source of free volatile flavor compounds. Glycosidically bound flavors are rapidly hydrolyzed in acid condition or in the presence of an appropriate hydrolytic enzyme. The bound fraction of longkong juice was hydrolyzed by beta-glucosidase and acid hydrolysis. The potential volatile flavor compound of butyl butanoate, 2-ethyl-1-hexanol, trans-2-hexanal and phenol were identified. The released of some potential volatiles can enhance the flavor characteristic of longkong. This information is useful as a basic knowledge. It provides the way to enhance the aromatic of the products that produced by longkong based products such as wine, juice.

CHAPTER 4

CHANGES IN FRUIT QUALITY AND VOLATILE FLAVOR COMPOUNDS DURING ON-TREE MATURATION OF LONGKONG

4.1 Abstract

Longkong (*Aglaia dookkoo* Griff.) is a non-climacteric fruit, juicy with typically aromatic smell and sweet but slightly sour taste. The optimal harvest period was started from 13 to 15 weeks after anthesis. The physical and chemical qualities of longkong at different stages of on-tree maturation were evaluated as follows: ripe (13 weeks after anthesis); medium ripe (14 weeks after anthesis); and full ripe (15 weeks after anthesis). It was found that longkong became light to bright yellow with the stages of maturation ($p < 0.05$). This can be indicated by the highest lightness (L*=64.21) and yellowness (b*=34.37) values shown in longkong at the full ripe stage. Moreover, the texture changed and softened with maturation ($p < 0.05$). The fruit size and weight increased with the stage of maturity but there was no significant difference ($p < 0.05$). The fruit were in the range of 3.17-3.30 cm in diameter and 19.94-21.36 g in weight. The moisture content was slightly increased with the stage of maturity ($p < 0.05$). During on-tree maturation, from the ripe to full ripe stages, acidity and organic acids (citric, maleic and malic acids) decreased while pH, TSS and total sugar content increased. The main sugars found in longkong were sucrose, glucose and fructose, respectively. From the ripe to medium ripe stages of longkong maturation, the sucrose content slowly increased from 9.88 to 11.44%. However, during the full ripe stage the sucrose content was 7.03% ($p < 0.05$). The glucose content significantly decreased from 1.98% to 1.46% during the ripe to medium ripe stages of the maturing longkong and then it increased again to 2.92% at the full ripe stage ($p < 0.05$). Similarly to fructose content, it also decreased from 2.76 to 2.20% during the ripe to medium ripe stages of the maturing longkong. After that it increased again to 3.39% at the full ripe stage ($p < 0.05$). The green aroma of 1-hexanol was the only volatile compound which was found in ripe longkong. From the ripe to full ripe stages, the longkong had more fruity and sweet characteristics. There were many

terpenes and their derivatives. The key volatile flavor compounds were delta-germacrene and 3-hydroxy-2-butanone. They had herbaceous and sweet attributes.

4.2 Introduction

Longkong (*Aglaia dookkoo* Griff.), is a well known and important commercial fruit grown in southern Thailand. It belongs to the Meliaceae family and has its origin in the South of Thailand, Indonesia, the Philippines and the Malau Islands (Paull, 2004). The demand for this fruit has increased tremendously. Because this fruit is juicy, has a pleasant taste and contains a variety of nutrients. The Office of Agricultural Economics (2009) reported that farm values of longkong increased from 4,521 in the year 2000 to 5,092 million baht in the years 2007. However, the farm values of longkong have decreased to 3,000 million baht in the year 2013. It was probably due to the insurgency in the south of Thailand. Longkong is a non-climacteric tropical fruit, which comes in racemes. The fruit is round; roughly 3-4 cm across and soft. It has a smooth, thin and bright yellow skin. There are 15-20 fruits per raceme, and are almost seedless and free of latex. Longkong pulp is white, juicy with a typically aromatic smell and a sweet but slightly sour taste (Sabah, 2004; Paull, 2004). Normally, physical or chemical qualities such as color changes, acidity changes and total soluble solid (TSS) changes can be used as a harvesting index for longkong (Pantuvanid, 1985; Bumrugrak, 1992). The ripe stage (13-15 weeks after anthesis) is the optimal stage for harvesting. The fruit skin becomes a full bright yellow and its flesh becomes transparent and it has typical aromas and a sweet taste (Bumrugrak, 1992).

In addition, the National Bureau of Agricultural Commodity and Food Standards (2006) reported that the characteristics of longkong at the full ripe stage should be a full bright yellow skin color, soft flesh and 15ºBrix of total soluble solid. The chemical constituents that affect the organoleptic profiles and characteristic of fruit flavors are the important quality standards for fresh fruit. Sweetness is related mainly to types and concentrations of sugars. Organic acids mainly contribute to the sourness. During maturation, sugars increase and organic acids decline. This makes the fruit much sweeter (Wills *et al*., 1998; Soares *et al*., 2007; Vendramini and Trugo, 2000; Sapii *et al*., 2000). Volatile flavor compounds mainly contribute to the fruity

aroma and are synthesized during the maturation of the fruit. The unique flavor characteristic of longkong seems to directly influence the demand for this fruit. However, the formation of the flavor profile in longkong during on-tree maturation has not yet been investigated. Therefore, this study was undertaken to establish the changes in longkong quality, in physical or chemical qualities and volatile flavor compounds, during on-tree maturation.

4.3 Material and Methods

Chemicals

The D-glucose, D-fructose, sucrose were purchased from Fluka (Messerchmittstr, Switzerland). Acetonitrile and water (HPLC grade) were obtained from Prolabo (Paris, France). Sodium hydroxide, copper sulfate pentahydrate, trichloroacetic acid and sodium sulfite were obtained from Merck (Darmstadt, Germany). Potassium sodium tartrate, lead acetate and potassium oxalate were obtained from Riedel-deHaen (Seelze, Germany). Dichloromethane was obtained from LabScan Asia Co., Ltd (Bangkok, Thailand).

Plant material

Longkong was obtained from a contact garden in Natawee district (Songkhla province, Thailand). It was harvested at different stages of on-tree maturation; at 13 (ripe), 14 (medium ripe) and 15 (full ripe) weeks after anthesis. Fruit was harvested in the morning and immediately transported on the same day to the Food Analysis laboratory, Faculty of Agro-Industry (approximately 2 hours). Fruits free from any apparent skin damage and uniform size (grade A about 3-3.5 cm in diameter, according to Tanyongmut market) were selected for analysis.

Fruit quality evaluation

Both physical (fruit color, fruit firmness, fruit size and fruit weight) and chemical (total soluble solid; TSS, sugars (reducing sugars, total sugars and type and concentration of sugars, titratable acidity; TA, pH, moisture content, type and concentration of organic acids and volatile flavor compounds) qualities were evaluated. For chemical quality analysis, the homogenate was prepared for analysis. It

was prepared by flesh (de-seeded) blending. For physical quality analysis, the fruit was cut of raceme to form an individual fruit. The 10 individual fruits were used to measure in each quality.

Fruit color

Two opposite sides of longkong fruit skin were quantified in terms of CIE lightness (L*), redness (a*) and yellowness (b*) values using a Color Flex, Hunter Lab colorimeter. CIE values were calculated in terms of Hue (h°) angle = artangent b*/a* and Chroma (C* value) = $(a*^2+b*^2)^{1/2}$ (Apai, 2010).

Fruit firmness

Two opposite sides of the fruit were measured by using a TA-XT2i Texture Analyzer (Stable Micro System, UK) equipped with a 2 mm diameter cylinder probe (P/2). The penetrometric method was applied. The results were expressed as gram force (Adapted from Sapii *et al*., 2000).

Fruit size

The fruit diameter was measured by using a SV-02 Stainless Steel Dial Vernier Calipers.

Fruit weight

The fruit weight was evaluated by weighing with a Sartorius BP310S analytical balance.

Moisture content

The moisture content was determined gravimetrically by drying at 105°C (A.O.A.C., 2000).

TSS

The TSS was determined using an Atago 1E (Japan) hand refractometer at 25ºC and expressed as a percentage (Ong *et al*., 2006).

Total and reducing sugars

    The 25 g of the longkong homogenate and 100 ml of distillated water were mixed and clarified by 45% neutral lead acetate (2 ml) and 22% potassium oxalate (2 ml). The sample volume was adjusted to 250 ml with distillated water and filtered through filter paper No 1. The total sugar and reducing sugar content was quantified by titration with Fehling's reagents according to Lane and Eynon (A.O.A.C., 2000).

Titratable acidity

    The titratable acidity (TA) was quantified by titrating 10 ml of the homogenate to an end point of pH 8.2 with 0.1 N NaOH with 1% (v/v) phenolphthalein as an indicator. The result was calculated as a percentage of citric acid (Ong *et al*., 2006).

pH

    The pH was measured using a Sartorius PB-20 (Germany) digital pH meter (Ong *et al*., 2006).

Types and concentrations of sugars

    Types and concentrations of sugars (sucrose, fructose and glucose) were determined by HPLC (Shimadzu, CR 6A Chromatopac) with a Hypersil APS-2 column. For sample preparation, the homogenate was clarified by using refrigerated centrifugation (4ºC) at 10,000×g for 30 min. Clear solution of longkong juice was obtained. The clear solution was filtered through 0.45 μm pore size membrane filters and was kept at -20°C until analysis. The injection volume was 10 μl with an isocratic flow rate of 1 ml/min and a refractive index detector. The 90% acetonitrile was used as a mobile phase. Their concentrations were quantified by comparing the retention times and peak areas of the samples with sucrose, fructose and glucose standards (Adapted from Chyau *et al*., 2003; Ong *et al*., 2006; Soares *et al*., 2007).

Types and concentrations of organic acids

Organic acids (citric, maleic and malic acids) were determined by HPLC (Agilent 1100 series HPLC) with Hypersil ODS 4.0×250 mm, 5 μm reverse phase column. For sample preparation, the homogenate was clarified by using refrigerated centrifugation (4°C) at 10,000×g for 30 min. Clear solution of longkong juice was obtained. The clear solution was filtered through 0.45 μm pore size membrane filters and was kept at -20°C until analysis. Injection volume was 10 μl with an isocratic flow rate 0.5 ml/min and diode array detector was set at 210 nm. Mobile phase was 0.05 disodium hydrogen phosphate, pH 2.5. Their concentrations were quantified by comparing retention time and peak area of the samples with known standards (Adapted from Chairgulprasert *et al*., 2006).

Volatile flavor compounds

Volatile flavor compounds were extracted by direct solvent extraction and identified by GC-MS. The 100 ml clear solution of longkong juice was mixed with 100 ml of dichloromethane. Next, the mixture was gentle shaken for 90 min and left equilibrium for 30 min at room temperature (~30°C). The solvent phase was collected and re-extracted twice. The solvent phase was dried on anhydrous Na_2SO_4, kept overnight at -20°C, cold- filtered and concentrated using purging nitrogen gas. The concentrate was kept at -20°C prior to analysis. The 1 μl was directly injected into GC-MS (Adapted from Flores *et al*., 2002; Elss *et al*., 2005; Ong *et al*., 2006).

The volatile flavor compounds were identified by GC-MS. A chromatograph, Hewlet-Packard 6890 (Palo Alto, CA, USA), was used with an HP-FFAP column (25 m×0.20 mm; film thickness 0.20 mm). The inlet temperature was set at 240°C and the column temperature was maintained at 40°C for 2 min, followed by an increase to 240°C at the rate of 5°C/min and held for 13 min. The carrier gas was applied by ultra high purity helium at a constant flow of 1.5 ml/min. A Hewlet-Packard 5973 A mass spectrometer with a quadrupole mass filter was coupled to the GC. A mass spectrum (MS) was obtained at 70 eV in the electronic impact (EI) and positive modes. The MS was scanned in the range m/z 40-350 at 1 s intervals (Adapted from Wanakhachornkrai and Lertsiri, 2003).

Statistical analysis

The experiment was performed by completely randomized design (CRD). Significant differences between means were estimated by Duncan's new multiple range test (DMRT), with a level of significance of 0.05. Statistical analyses were performed by using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

4.4 Results and Discussion

The physical qualities (fruit color, firmness, size and weight) of longkong at three different stages of on-tree maturation (13 weeks (ripe), 14 weeks (medium ripe) and 15 weeks (full ripe)) were evaluated. The results were reported and discussed as follows.

4.4.1 Physical quality

Fruit Color

The data on fruit skin color were recorded and quantified in terms of CIE (lightness (L*), redness (a*) and yellowness (b*)) values. A high L*, a* and b* values refer to lighter, more reddish and more yellowish surface color of longkong. Fruit skin color was found to differ significantly with the different stages of on-tree maturation (p<0.05). It was found that the fruit skin at the ripe stage was pale yellow with a tinge of green, and the L*, a* and b* values were 43.16, 3.69 and 23.36 respectively (Figures 9A, 9B and 9C). The L* and b* values increased significantly with on-tree maturation (p<0.05). The significant increase in L* value at 58.43 and b* value at 23.95 (p<0.05) was found when the fruit matured to the medium ripe stage (Figures 9A and 9B). At the full ripe stage, the longkong was lighter and more yellow indicated with sharpen increased in L* and b* values. The L* and b* values were 64.21 and 34.37, respectively (Figure 9A and 9B). Changes in fruit skin color from pale yellow with tinge of green to bright yellowness could be explained by degrade of chlorophyll (Sapii *et al*., 2000). However, the significant highest a* value at 5.21 was observed in full ripe longkong (Figure 9C). An increase in browning was an evidenced by an increase in a∗ value (Sapii *et al*., 2000). The brownish color becomes due to enzymatic reaction. The browning enzyme interacted with substrate as phenolic compounds and promote fruit skin darken (Venkatachalam and Meenune, 2012). The

results were in agreement with the study undertaken by Venkatachalam and Meenune (2012) who reported that a significant increase in a∗ value was observed during maturation stages.
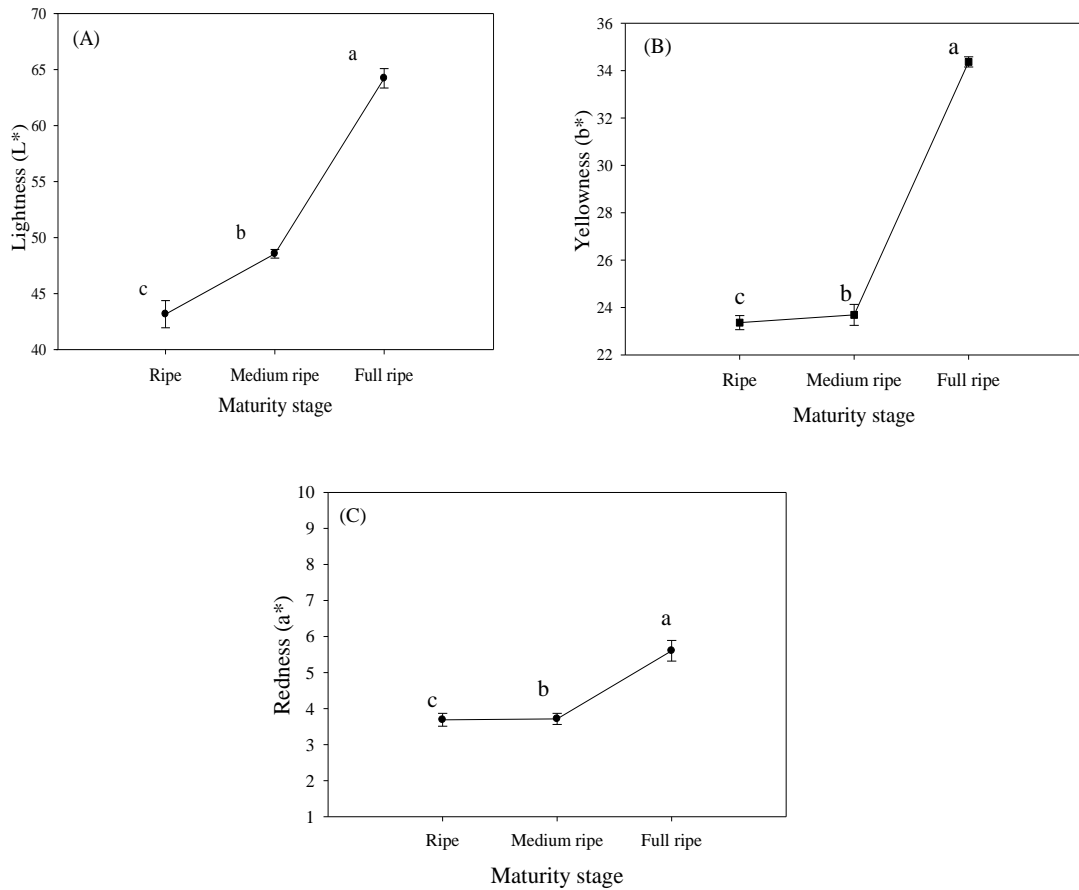
Figure 9 Lightness (A), yellowness (B) and redness (C) values in longkong skin at different stages of on-tree maturation

Note: Data are mean ± standard deviation (in ten replicates).

Means with different superscript letters indicate that there are significant differences among maturity stages (p<0.05).

More appropriate measures of fruit skin color can be obtained from the calculation of the hue (h°) angle and chroma (C*) value. The fruit skin color of longkong at different stages of on-tree maturation in terms of h° and C* value are shown in Figure 10. The increases in the angle of h° and C* value at the mature stages of longkong were observed (p<0.05). The angle of h° of the longkong at the ripe,

medium ripe and full ripe stages was 80.74, 81.02 and 81.09. The angle of h° shown at nearly 90° indicates that the longkong skin becomes nearly yellow (h°, 90° = yellow). The results show a positive correlation with changes in CIE yellowness (b*) which increased with the maturation of the fruit (Figure 9B). The chroma C* value increased with the maturity of the longkong (p<0.05). The C* values of ripe, medium ripe and full ripe longkong were 23.65, 23.98 and 34.82, respectively. The highest C* value was because of the high saturation from yellowness.
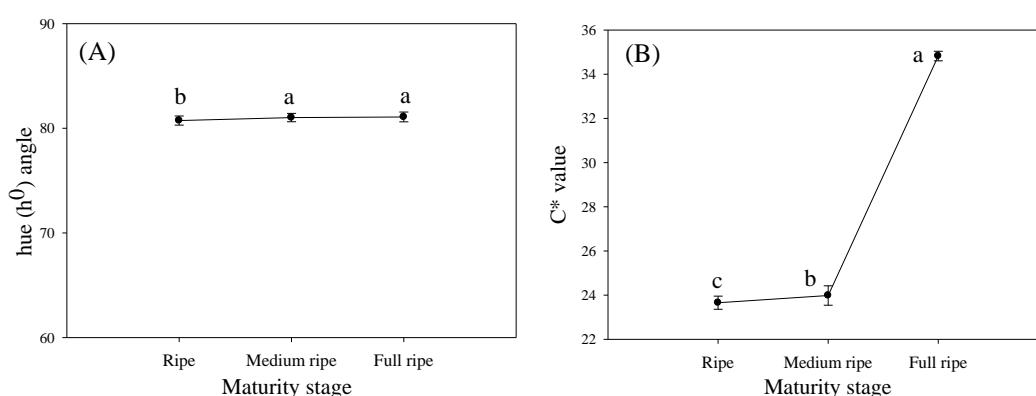
Figure 10 Hue (h°) angle (A) and Chroma (C* values) (B) of longkong at different
stages of on-tree maturation

Note: Data are mean ± standard deviation (in ten replicates).
Means with different superscript letters indicate that there are significant differences among maturity stages (p<0.05).

Fruit firmness

The loss of firmness in the fruit during fruit maturation is a major factor determining fruit quality. Changes in fruit firmness were associated with the stages of on-tree maturation. In ripe longkong, the fruit texture is firmer than that in the medium and full ripe stages. This can be indicated when using the highest force to penetrate into longkong at the ripe stage, which was 2,054.7 g. During the medium ripe to full ripe stages, fruit firmness decreased from 1,928.3 to 1,658.8 g (Figure 11). These phenomena could be explained by the degradation of the structural

carbohydrates, cell wall disassembly and modifications to the pectin fraction by the role of pectin enzymes (White, 2002; Seymour and Gross, 1996).
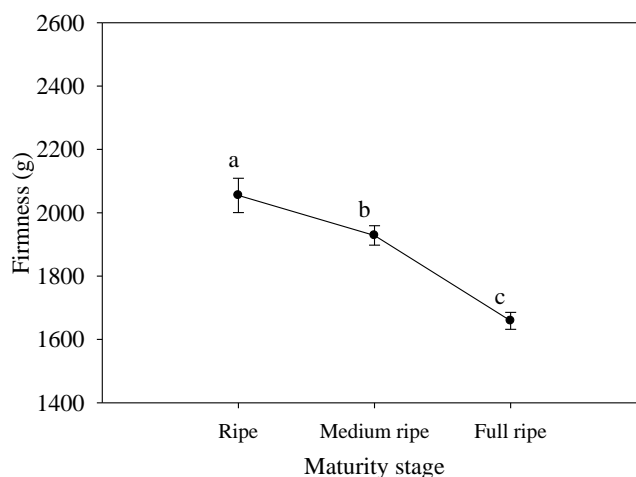
Figure 11 Firmness of longkong at different stages of on-tree maturation

Note: Data are mean ± standard deviation (in ten replicates).

        Means with different superscript letters indicate that there are significant
        differences among maturity stages (p<0.05).

Fruit size and weight

            The size and weight of the longkong slightly increased from the ripe to full ripe stages (p<0.05). The almost constant diameter of the longkong was observed in the ranges from 3.17 to 3.30 cm (ripe to full ripe stages) (Figure 12). This is in agreement with the standards of Tanyongmat the market in Narathiwat province. It prescribes that longkong grade A has a diameter of at least 3 cm or above. The fruit weight was found to be in the range from 19.94 g to 21.36 g (ripe to full ripe stages) (Figure 4). This is similar to the study of Bumrugrak (1992) which monitored the quality changes in longkong during its on-tree maturation (2 to 16 weeks after fruit set). It was found that at the ripe to full ripe stages (12 to 16 weeks after fruit set), the fruit weight and size remained almost constant. This is probably because a constant cell enlargement at these stages.
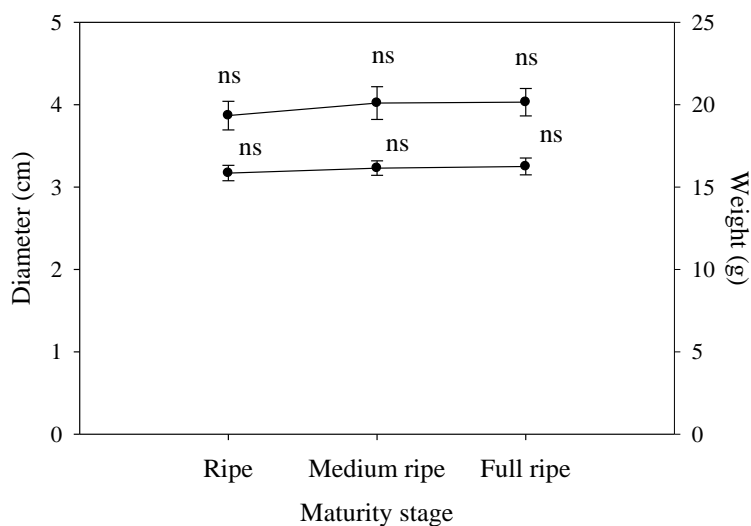
Figure 12 Diameter and weight of longkong at different stages of on-tree maturation

Note: Data are mean ± standard deviation (in ten replicates).

        ns = no significant differences among maturity stages (p<0.05).

4.4.2 Chemical quality

Changes in chemical quality are important factors in determining the stage of fruit maturation. These are useful to determine the optimal harvesting time, such as the harvesting index. In this study, the chemical qualities (moisture content, pH, titratable acidity, total soluble solid and sugars) of longkong were evaluated during the edible stages of on-tree maturation (ripe, medium ripe and full ripe). The results are presented in Table 7.

Moisture content

The moisture content in the longkong varied significantly from the ripe to full-ripe stages during on-tree maturation (p<0.05). The highest moisture content was observed at the full ripe stage. It was probably due to the movement of water from root throughout xylem into fruit cell during fruit maturation on-tree (Kays, 1991). In addition, an increase in the amount of the components such as sugars and acids in longkong during maturation might be affected on turgor pressure resulting in osmotic flow of water from area of low solute out into cell (Campbell and Reece, 2008).

Table 7 Moisture content, pH, titratable acidity (TA), total soluble solid (TSS), reducing and total sugars in longkong at different stages of on-tree maturation

Maturity stage	Moisture content (%)	pH	TA (% as citric acid)	TSS (%)	Reducing sugar (%)	Total Sugar (%)
Ripe	81.37 ± 0.02^c	3.85 ± 0.06^c	0.95 ± 0.00^a	14.08 ± 0.10^c	4.76 ± 0.02^c	12.78 ± 0.03^c
Medium ripe	81.64 ± 0.10^b	4.00 ± 0.01^b	0.77 ± 0.00^b	16.65 ± 0.06^b	5.05 ± 0.01^b	15.13 ± 0.01^b
Full ripe	82.03 ± 0.14^a	4.27 ± 0.01^a	0.61 ± 0.00^c	17.50 ± 0.08^a	5.38 ± 0.04^a	15.59 ± 0.01^a

Note: Data are mean $\pm$ standard deviation (in four replicates)

Mean values in the same column with different superscript letters indicate that there are significant differences between the variety ($p<0.05$).

pH and TA

The pH increased significantly with on-tree maturation ($p<0.05$). The pH values increased from 3.85 at the ripe stage to 4.27 at the full ripe stage (Table 7). The lowest TA was found in longkong at the full ripe stage, which decreased significantly ($p<0.05$) from 0.95 to 0.61%. This result was in agreement with the study undertaken by Sapii *et al*. (2000). They monitored the changes in the quality of longkong at different stages of ripeness (4, 7, 10 and 14 days after the fruit yellowed). It was found that the TA was significantly higher in the fruit harvested at the earlier stage. The decline of acid in fruit during maturation may impart a significant portion of the characteristic flavor. Loss of acids in most fruit during maturation is due largely to the utilization of these compounds as respiratory substrates and as carbon skeletons for the synthesis of new compounds during ripening (Kays, 1991).

TSS and sugars

There was a significant difference in the TSS, reducing and the total sugar contents in longkong at the different stages of on-tree maturation ($p<0.05$). Table 7 shows that the TSS gradually increased significantly at the more mature

stages of the ripening of the longkong (p<0.05). The TSS increased significantly from 14.08 to 17.50%. During maturation, reducing and the total sugar contents tended to increase (p<0.05). The reducing sugar content increased from 4.76 to 5.38% during the ripe to full ripe stages. This finding indicated an increase in the total sugar content. The total sugar content increased from 12.78 to 15.59% during the ripe to full ripe stages (Table 7). The increase in the TSS could be attributed to the decomposition of the cell walls which caused the release of water-soluble components. In addition, increases in the TSS were probably due to the solubilization of neutral sugars from carbohydrate polymer residues (Beirao-da-Costa et al., 2006). Increases in the TSS may be affected by the increase in the acids (Brady, 1987).

Types and concentrations of sugars

The main sugars in longkong were sucrose, fructose and glucose. The sucrose content increased slowly from 9.88 to 11.44%, during the ripe to medium ripe stages, and then gradually decreased to 7.03% at the full ripe stage (p<0.05). The glucose content decreased from 1.98 to 1.46% from the ripe to medium ripe stages and increased to 2.92% in the full ripe stage. This was similar to the fructose content which decreased in the ripe to medium ripe stages, from 2.76 to 2.20%, and then increased to 3.39 at the full ripe stage (Figure 13). The decrease and increase in the glucose and fructose contents at the different stages of on-tree maturation could be explained by: the decrease was due to the rapid consumption of sugars in the respiration process to produce the energy required for the synthesis of the components. However, an increase in the glucose and fructose content at the full ripe stage may be due to the higher inversion reaction of the sucrose to glucose and fructose molecules (Ong *et al*., 2006).
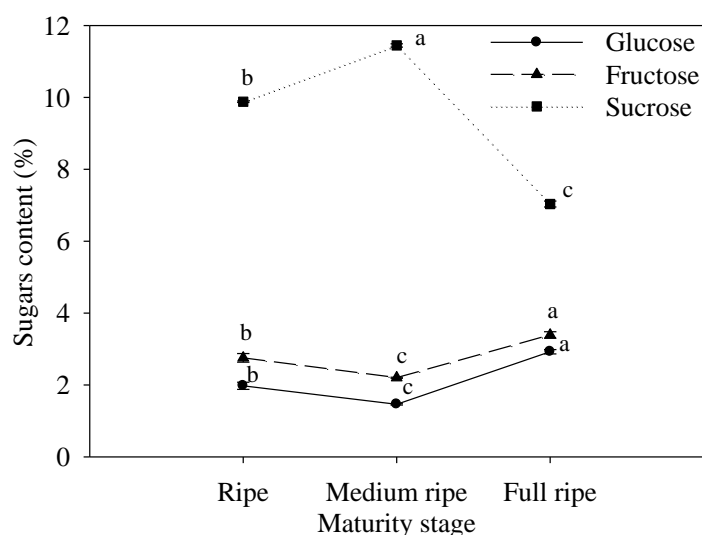
Figure 13 Types and concentrations of sugars content in longkong at different stages
of on-tree maturation

Note: Data are mean ± standard deviation (in four replicates).

Means with different superscript letters indicate that there are significant
differences among maturity stages (p<0.05).

Types and concentrations of organic acids

Citric, maleic and malic acids were monitored by HPLC in accord with
Chairgulprasert *et al.* (2006). A reduction of the concentration of organic acids was
observed during on-tree maturation (p<0.05) (Table 8). The concentration of citric
acid in the ripe stage was 1.22% and this was reduced from 1.19 to 0.79% in the
medium ripe to full ripe stages. The maleic acid also decreased with on-tree
maturation (p<0.05). It was 0.40% in the ripe stage and then decreased to 0.36 and
0.05% in the medium ripe and full ripe stages, respectively. This was similar to the
decrease of malic acid which decreased from 0.20 to 0.13% from the ripe to the
medium ripe stages. However, the malic acid was not detected at the full ripe stage.
This was probably due to the quick consumption of malic acid by it being conversed
to pyruvate and being used as a substrate for the respiration process (Knee, 2002).

Table 8 Some organic acids in longkong at different stages of on-tree maturation

Maturity stage	Organic acid (%)		
	Citric acid	Maleic acid	Malic acid
Ripe	1.22 ± 0.01^a	0.40 ± 0.01^a	0.20 ± 0.01^b
Medium ripe	1.19 ± 0.01^b	0.36 ± 0.01^b	0.13 ± 0.01^a
Full ripe	0.79 ± 0.01^c	0.05 ± 0.02^c	ND

Note: ND = not detected

Data are mean $\pm$ standard deviation (in four replicates).

Mean values in the same column with different superscript letters indicate that there are significant differences between the variety (p<0.05).

Volatiles flavor compounds

Fruit volatile flavor profile is directly influenced by fruit maturation. The formation of volatile flavor compounds in fruit is a dynamic process. Volatile flavor compounds are continuously synthesized and developed both qualitatively and quantitatively during fruit maturation. The volatile flavor compounds developed in longkong at the three different stages of on-tree maturation (ripe, medium ripe and full ripe) are shown in Table 9. Their chromatograms are shown in Figure 14. The volatile flavor compounds included alcohols, terpenes, ketones and phenol. The terpenes and their derivatives were the major constituents. They promote the fruity, floral, flowery, herbaceous characteristics in longkong, which are normally found in a fruit aroma. In the ripe stage, the volatile flavor compounds which have fruity, floral, flowery, and herbaceous characteristics were not detected. Green aroma C_6-alcohol (1-hexanol) was only the volatile flavor compound that was found in the ripe longkong. Thus it has a green aroma at the ripe stage. The longkong also showed some herbaceous and sweet characteristics by showing the lowest constituent levels of delta-germacrene and 3-hydroxy-2-butanone. When the fruit became more mature, more terpenes and their derivatives were detected. As a consequence, more fruity and sweet characteristics were found.

Table 9 Volatile flavor compounds and their attributes identified in longkong at different stages of on-tree maturation

Compounds	Rt[A]	RI[B]	Attributes[C]	Peak area ($\times 10^4$) TIC[D]		
				Maturity stage		
				Ripe	Medium ripe	Full ripe
Alcohols						
1-hexadecanol	5.08	1116	floral, flower	nd	nd	1.64
n-butanol	6.19	1157	fruity	nd	nd	3.70
n-hexanol	11.05	1347	green	0.83	nd	nd
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.89	5.61	nd
benzyl alcohol	23.77	1895	floral, sweet	nd	nd	1.53
Terpenes and their derivatives						
ortho-xylene	6.03	1150	geranium	nd	4.73	9.55
cis-linalool oxide	14.39	1479	floral, sweet	nd	nd	1.42
alpha-copaene	14.58	1486	woody	nd	nd	1.18
laevo-linalool	16.35	1559	fruity, sweet	nd	1.48	3.09
beta-caryophyllene	17.12	1591	fruity	nd	nd	4.08
delta-germacrene	19.75	1706	herbaceous	4.56	15.68	26.54
bicyclogermacrene	20.29	1731	woody	nd	nd	2.01
delta-cadinene	20.84	1756	herbaceous	nd	1.49	2.97
para-cymene	32.56	2370	citrus	nd	4.47	nd
Ketones						
3-hydroxy-2-butanone	9.78	1298	sweet	14.83	16.48	26.75
Phenols						
phenol	26.38	2027	phenol	18.30	76.85	74.40

Note: [A] Rt = Retention time (min);   [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html,

http://www.flavornet.org/flavornet.html

[D] TIC = total ion current;   nd = not detected
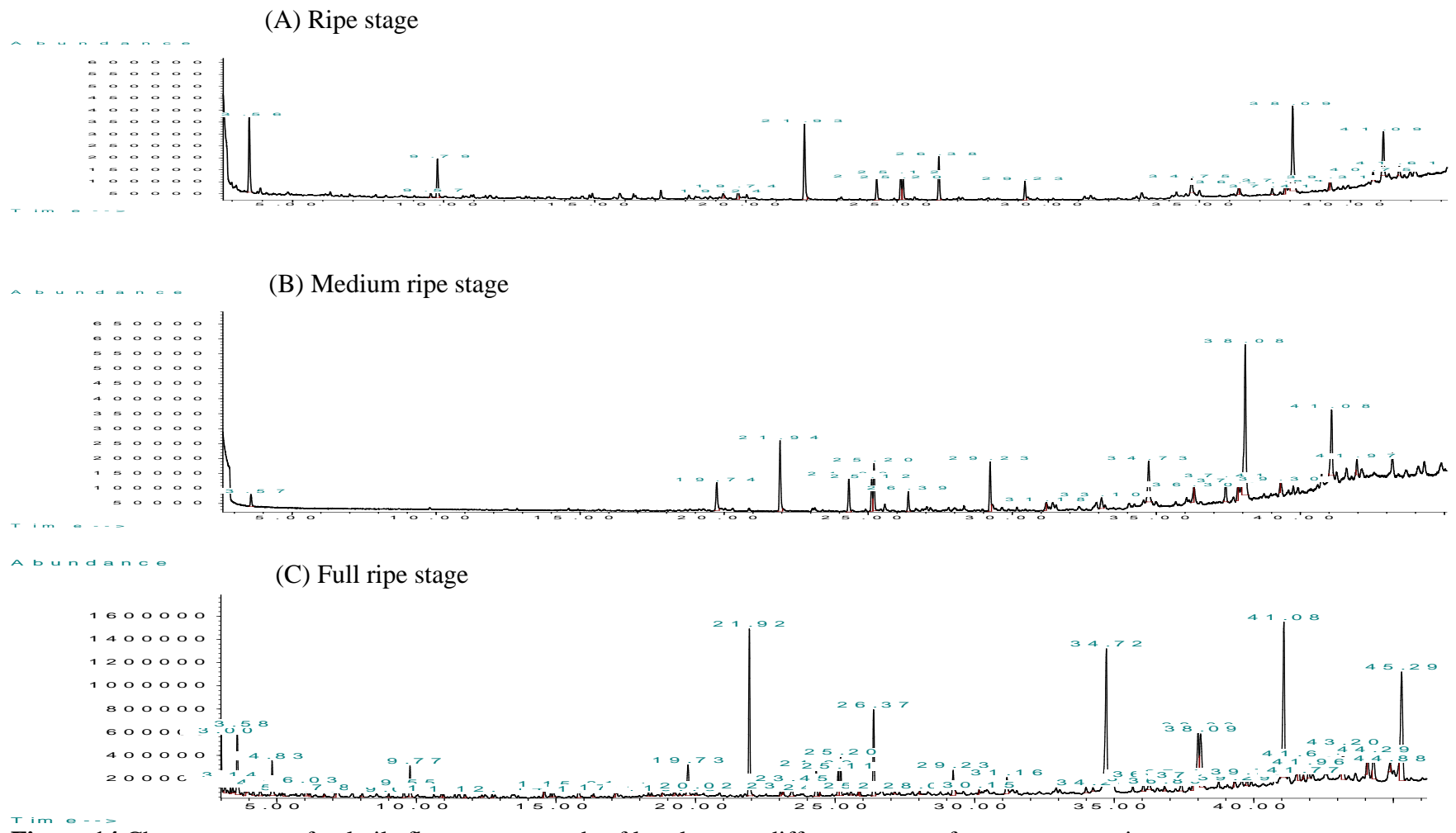
Figure 14 Chromatogram of volatile flavor compounds of longkong at different stages of on-tree maturation

4.5 Conclusions

During on-tree maturation, the physical, chemical and volatile flavor compounds in longkong are still changing. This information could indicate suitable harvesting times and the edibility of the fruit. The fruit skin became lighter and yellower with a softer flesh at the full ripe stage. Furthermore, the fruit size and weight was almost constant during the ripe to full ripe stages. The TSS and sugars increased while the acidity decreased with on-tree maturation, and this makes the fully ripe longkong more palatable. Organic acids such as citric, maleic and malic acids declined during on-tree maturation. The volatile flavor compounds were synthesized during on-tree maturation. The green aroma of 1-hexanol was the only volatile compound found in ripe longkong. The delta-germacrene and 3-hydroxy-2-butanone were the major compounds in the medium and fully ripe longkong. These compounds were the components that gave the longkong more fruity and sweet characteristics.

CHAPTER 5

CHANGES IN FRUIT QUALITY, VOLATILE FLAVOR COMPOUNDS AND POSSIBLY OFF-FLAVOR ACCUMALATION IN LONGKONG AT DIFFERENT STAGES OF ON-TREE MATURATION DURING STORAGE UNDER PASSIVE MODIFIED ATMOSPHERE PACKAGING

5.1 Abstract

Passive modified atmosphere packaging (passive MAP) is an alternative way to extend fruit shelf-life. However, the effective of passive MAP was related with fruit maturity stage ($p<0.05$). In this study, passive MAP under the optimum temperature at 18ºC was applied to store longkong at three different stages of on-tree maturation (ripe, medium ripe and full ripe). Changes in longkong qualities were monitored at 6-day intervals. The extremely changes in fruit qualities was due to the stage of on-tree maturation ($p<0.05$). Longkong at full ripe stage presented the most sensitive on modified atmosphere packaging condition. Fruit skin became darken rapidly. This result was indicated by the lowest of L* and the highest of a* values. They were 31.81 and 19.19, respectively. At the end of storage, longkong at full ripe stage showed the lowest fruit firmness at 1,277.98 g and the highest weight loss (1.98%). During 18 days under passive MAP, full ripe fruit showed the highest rate of fruit respiration as shown by the lowest of O_2 and the highest of CO_2 concentrations inside package. The highest concentration of CO_2 was 80.55% inside a full ripe longkong package. High CO_2 concentration enhanced the specific activity of ADH enzyme to 12.00 U/mg protein. The content of ethanol increased with high specific activity of ADH enzyme and storage time ($p<0.05$). Ethanol content in full ripe longkong was 0.20 g/g FW at the end of storage. The microbial population increased with storage time ($p<0.05$). At the end of storage, total viable counts were 6.68×10^5 CFU/g.

5.2 Introduction

Harvested fruit still needs energy for its metabolism. The energy is produced by fruit respiration process. The fruit respiration process is a major role in fruit shelf life and its quality. It rapidly deteriorates with high rate of fruit respiration. Therefore, alternative tool to diminish rate of fruit respiration needs to be applied. Passive modified atmosphere packaging (passive MAP) is one of the most effective tools used for shelf-life extension in fruit (Kader *et al*., 1989). It maintains its optimal adequate atmosphere inside package by fruit respiration and gas permeation of the package film. Fruit store in passive MAP has low rate of its respiration by low O_2 inside package. Even passive MAP is a direct effect on fruit quality, however, fruit maturation is an one factor which enhances the effective of this condition. It was suggested by Pesis *et al*. (2002), who reported that quality of full mature or over mature fruit rapidly dropped during passive MAP storage. The undesirable quality occurs such as darken flesh and peel, unpleasant flavor. In addition, high accumulation of ethanol content in fruit can be occurred.

Longkong is a highly perishable fruit which has its shelf life around 4 to 7 days under room temperature. An alternative tool as passive MAP combined with low temperature was introduced to prolong longkong shelf life by many researchers. The study on longkong quality during storage under modified atmosphere condition was undertaken by Bunsiri *et al*. (2006). Longkong was stored at 18°C in polystyrene tray cover by plastic bag with ethylene absorber. Under this condition, the satisfied quality and less fruit drop was presented within 4 weeks. Similarly to the study undertaken by Piyasaengthong *et al*. (1997), who reported that longkong stored at 18°C had its shelf life for 21 days with 60% fruit drop. Previous study, research work was focused on the changes in longkong quality during storage under passive MAP. The effect of maturity stages of longkong on its changes during storage under passive MAP has not yet been investigated. Therefore, the objective of this study was to focus on stages of maturity of longkong on changes in its quality, volatile flavor compounds and possibly off-flavor accumulation during storage under passive MAP.

5.3 Materials and Methods

Chemicals

The pyruvate decarboxylase and alcohol dehydrogenase enzymes were obtained from Fluka, Sigma Chemical Co. (St. Louis, MO), Aldrich Chemicals Co. (Milwaukee, WI) and Merck Co. (Darmstadt, Germany). D-glucose, D-fructose, sucrose were purchased from Fluka (Messerchmittstr, Switzerland). Acetonitrile and water (HPLC grade) were obtained from Prolabo (Paris, France). Sodium hydroxide, copper sulfate pentahydrate, trichloroacetic acid and sodium sulfite were obtained from Merck (Darmstadt, Germany). Potassium sodium tartrate, lead acetate and potassium oxalate were obtained from Riedel-deHaen (Seelze, Germany). Dichloromethane was obtained from LabScan Asia Co., Ltd (Bangkok, Thailand). Plate count agar (PCA) for total viable counts was obtained from Merck Co. (Darmstadt, Germany).

Plant material and fruit preparation

Longkong was obtained from a contact garden in Natawee district (Songkhla province, Thailand). The maturity stages of 13 (Ripe), 14 (Medium ripe) and 15 (Full ripe) weeks after anthesis were choosen. Fruit was harvested in the morning and immediately transported on the same day to the Food Analysis laboratory, Faculty of Agro-Industry (approximately 2 hours). Fruits free from any apparent skin damage and uniform size (grade A about 3-3.5 cm in diameter, according to Tanyongmut market) were selected for analysis. Fruit were prepared in an individual by cut of raceme, cleaned with brush and submerged into mixed solution of 500 ppm benomyl and 1.5% citric acid for 5 min to reduce microbial load and anti-browning, drying at room temperature (~30°C) for 15 min.

Passive modified atmosphere packaging and storage conditions

Each maturity stage, 12 individual fruits were placed into a plastic tray. A tray was covered with 0.08 mm thickness nylon laminated with linear Low Density Polyethylene (nylon/LLDPE) bag; size 7x11 inch. with permeability to CO_2 and O_2 of 4.7 and 2.6 cm^2/m^3/day at 25°C and 0% RH, respectively. It immediately sealed by

using heat sealer. Sample bags were stored at 18ºC. At 6-day intervals during storage, fruit quality was evaluated until the end of storage.

Fruit quality evaluation

The physical qualities as fruit color, fruit firmness, and weight loss were measured in 10 replicates (10 fruits) at 6-day intervals. The chemical qualities as total soluble solid (TSS), sugars (reducing sugars, total sugars and type and concentration of sugars), titratable acidity (TA), pH, alcohol dehydrogenase (ADH) activity and volatile flavor compounds were also evaluated at 6 day intervals. The homogenate flesh (de-seeded) was used for chemical quality analysis. The methods of physical and chemical analysis were explained below.

Fruit color

Two opposite sides of longkong fruit skin were quantified in terms of CIE lightness (L*), redness (a*) and yellowness (b*) values using a Color Flex, Hunter Lab colorimeter. CIE values were calculated in terms of Hue (h°) angle = artangent b*/a* and Chroma (C* value) = $(a*^2+b*^2)^{1/2}$ (Apai, 2010).

Fruit firmness

Two opposite sides of the fruit were measured by using a TA-XT2i Texture Analyzer (Stable Micro System, UK) equipped with a 2 mm diameter cylinder probe (P/2). The penetrometric method was applied. The results were expressed as gram force (Adapted from Sapii *et al*., 2000).

Fruit weight

The fruit weight was evaluated by weighing with a Sartorius BP310S analytical balance.

Total soluble solid (TSS)

The TSS was determined using an Atago 1E (Japan) hand refractometer at 25ºC and expressed as a percentage (Ong *et al*., 2006).

Total and reducing sugars

The 25 g of the longkong homogenate and 100 ml of distillated water were mixed and clarified by 45% neutral lead acetate (2 ml) and 22% potassium oxalate (2 ml). The sample volume was adjusted to 250 ml with distillated water and filtered through filter paper No 1. The total sugar and reducing sugar content was quantified by titration with Fehling's reagents according to Lane and Eynon (A.O.A.C., 2000).

Titratable acidity

The titratable acidity (TA) was quantified by titrating 10 ml of the homogenate to an end point of pH 8.2 with 0.1 N NaOH with 1% (v/v) phenolphthalein as an indicator. The result was calculated as a percentage of citric acid (Ong et al., 2006).

pH

pH was measured using a Sartorius PB-20 (Germany) digital pH meter (Adapted from Ong et al., 2006).

Types and concentrations of sugars

Types and concentrations of sugars (sucrose, fructose and glucose) were determined by HPLC (Shimadzu, CR 6A Chromatopac) with a Hypersil APS-2 column. For sample preparation, the homogenate was clarified by using refrigerated centrifugation (4ºC) at 10,000×g for 30 min. Clear solution of longkong juice was obtained. The clear solution was filtered through 0.45 μm pore size membrane filters and was kept at -20°C until analysis. The injection volume was 10 μl with an isocratic flow rate of 1 ml/min and a refractive index detector. The 90% acetonitrile was used as a mobile phase. Their concentrations were quantified by comparing the retention times and peak areas of the samples with sucrose, fructose and glucose standards (Adapted from Chyau et al., 2003; Ong et al., 2006; Soares et al., 2007).

Volatile flavor compound isolation by direct solvent extraction (DSE) and identification by GC-MS

Volatile flavor compounds were extracted by direct solvent extraction and identified by GC-MS. The 100 ml clear solution of longkong juice was mixed with 100 ml of dichloromethane. Next, the mixture was gentle shaken for 90 min and left equilibrium for 30 min at room temperature (~30°C). The solvent phase was collected and re-extracted twice. The solvent phase was dried on anhydrous Na_2SO_4, kept overnight at -20°C, cold- filtered and concentrated using purging nitrogen gas. The concentrate was kept at -20°C prior to analysis. The 1 µl was directly injected into GC-MS (Adapted from Flores *et al*., 2002; Elss *et al*., 2005; Ong *et al*., 2006).

The volatile flavor compounds were identified by GC-MS. A chromatograph, Hewlet-Packard 6890 (Palo Alto, CA, USA), was used with an HP-FFAP column (25 m×0.20 mm; film thickness 0.20 mm). The inlet temperature was set at 240°C and the column temperature was maintained at 40°C for 2 min, followed by an increase to 240°C at the rate of 5°C/min and held for 13 min. The carrier gas was applied by ultra high purity helium at a constant flow of 1.5 ml/min. A Hewlet-Packard 5973 A mass spectrometer with a quadrupole mass filter was coupled to the GC. A mass spectrum (MS) was obtained at 70 eV in the electronic impact (EI) and positive modes. The MS was scanned in the range m/z 40-350 at 1 s intervals (Adapted from Wanakhachornkrai and Lertsiri, 2003).

Headspace gas composition analysis

Changes in the headspace gas composition were monitored by gas chromatography (GC) coupling with a thermal conductivity detector (TCD-detector). The 1 ml of headspace gas was directly withdrawn from headspace inside the package and subsequently injected into Poropak N columns. Helium at flow rate of 50 ml/min was used as the carrier gas. The temperature oven and injection port was held at 60°C. The temperature TCD detector was held at 150°C. The internal package atmosphere was identified and quantified by comparison with external standard gas (Adapted from Tano *et al*., 2007).

Ethanol isolation by headspace-solid phase microextraction (HS-SPME) and identification by GC-FID

The ethanol content in longkong was extracted by headspace solid phase microextraction (HS-SPME). The extraction was done as follows; the 100 g of the flesh of longkong was mixed with 100 ml of 20% NaCl (cold solution). It was blended at a constant speed using a blender for 3 min. It was then filtered through a stainless steel sieve. This process produced the homogenate. The 20 ml homogenate was placed into a 125 ml vial followed by the addition of 3 g of NaCl and fitted with a rubber septum. The vial was kept at 30ºC and left equilibrates for 60 min.

Equilibrated ethanol in the headspace was isolated by using 50/30 µm DVB/CAR/PDMS (divinylbenzene/Carboxen on poly (dimethylsiloxane) (Supelco, Bellafonte, PA, USA). The equilibrated ethanol in the headspace was adsorbed for 15 min at room temperature (~ 30°C) and desorbed for 5 min in GC-FID (PerkinElmer, Autosystem XL, USA) (Adapted from Lara *et al.*, 2006).

The ethanol was analyzed by GC-FID. The apparatus was equipped with an Rtx-5 (Restek) column (30 m×0.25 mm; film thickness 0.25 µm). The carrier gas was helium of ultra high purity helium at a constant flow of 1.5 ml/min. The injector was kept at 240°C and set for the splitless mode. The column temperature was set at 35°C for 3 min and then programmed to 230°C at the rate of 8°C/min and held for 2 min. The ethanol concentration was calculated using standard aqueous solutions of ethanol under the same conditions as were used for the samples (Adapted from Lara *et al.*, 2006).

ADH enzyme extraction and ADH activity evaluation

The 100 mg of longkong flesh was homogenized in 1 ml of extracted solution. The extract solution contained 85 mM 2-(N-mor-pholino) ethane-sulfonic acid (MES) buffer, with a pH 6.0, 5 mM dithiothreitol (DTT) and 1% (w/v) polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged at 25000×g for 15 min at 4°C to recover the supernatant. The supernatant was kept in ice as crude enzyme extract (Adapted from Lara *et al.*, 2006).

The ADH activity was assayed by mixing 2.55 ml NADH solution (0.150 mM NADH in 85 mM MES, pH 6.0), 150 µl of acetaldehyde solution (80 mM

acetaldehyde in 85 mM MES, with a pH 6.0), and 300 µl enzyme extract. The ADH activity was measured every 1 min by the spectrophotometer technique at 340 nm. The results were expressed as specific activity (U/mg protein) (Lara *et al*., 2006). The total protein in the extract was determined according to the method described by Bradford (1976).

Microbiological determination

The microbial was determined at the beginning and the end of storage. Homogenized longkong flesh (de-seed) 25g was combined with 225 ml of sterile 0.1% peptone water in a sterile polyethylene bag. The sample was pummeled with a stomacher (Seward Stomacher 400, UK) for 2 min using a medium speed. The aliquot was used for the appropriate dilution.

The total viable counts were determined by pouring 1 ml of diluted sample onto plate count agar (PCA). The plates were incubated at 35°C for 48 hr, and the colonies were counted. The microbial counts were expressed in CFU/g of longkong (Koide and Shi, 2007).

Statistical analysis

The experiment was performed by a completely randomized design (CRD) with factorial treatment structure. A two-way factorial treatment structure (4 levels of storage times × 3 levels of maturity stages) was performed. Significant differences between means were estimated by Duncan's new multiple range test (DMRT), with a level of significance of 0.05. Statistical analyses were performed by using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

5.4 Results and Discussion

The visible mold growth on the longkong skin was used as a marker to reach the end of storage. Longkong stored under passive MAP for 18 days was noticed. The interaction between storage time and maturity stages had significant effect on longkong quality (p<0.05). The results are reported and discussed as follows.

5.4.1 Physical quality

Fruit color

The data on fruit skin color were recorded and quantified in terms of CIE (lightness (L*), redness (a*) and yellowness (b*)) values. Fruit skin color of longkong at different stages of on-tree maturation during storage under passive MAP is showed in Figure 15. Fruit skin color of longkong in all stages during storage under passive MAP became more darken throughout storage time ($p < 0.05$). The L* value of fresh longkong at ripe, medium ripe and full ripe stages were 50.34, 58.54 and 62.00. At the end of storage, the L* value decreased to 34.01 (ripe), 33.34 (medium ripe) and 31.81 (full ripe) (Figure 15A). A decrease in b* value also was observed. The b* value of fresh longkong at ripe, medium ripe and full ripe stages were 23.59, 28.42 and 30.75. At the end of storage, the yellowness (b* value) decreased to 19.21 (ripe), 18.21 (medium ripe) and 17.94 (full ripe) (Figure 15B). The increase in a* value was observed. The a* value of fresh longkong at ripe, medium ripe and full ripe stages were 3.07, 5.74 and 9.07. At the end of storage, the a* value increased to 14.19 (ripe), 16.89 (medium ripe) and 19.19 (full ripe) (Figure 15C). The lowest L* and b* value was noticed in full ripe longkong ($p < 0.05$). It was probably due to more sensitive on CO_2 stress of full ripe longkong, giving a result of rapidly loss on bright-yellowness skin. Fruit brownish color is associated with enzymatic browning reaction. The enzymatic browning can be accelerated by low O_2 or high CO_2. Under low O_2 or high CO_2, fruit becomes stress and loss its membrane integrity. Therefore enzymatic browning with phenol compound quite easy occurs (Sacher, 1962; Azevedo *et al*., 2008) and the darkening was found in full ripe longkong.

More appropriate measure of fruit skin color can be obtained from the calculation of hue (h°) angle and chroma (C* value). Changes in fuit skin color of longkong at different stages of on-tree maturation during storage under passive MAP in terms of h° angle and C* value are shown in Figure 16. The decrease in angle of h° and C* value with storage time in all treatments was observed ($p < 0.05$). The angle of h° in fresh longkong at ripe, medium ripe and full ripe stages was 82.59, 71.53 and 79.54. At the end of storage, the angle of h° decreased to 49.91 (ripe), 41.11 (medium ripe) and 50.66 (full ripe) (Figure 16A).
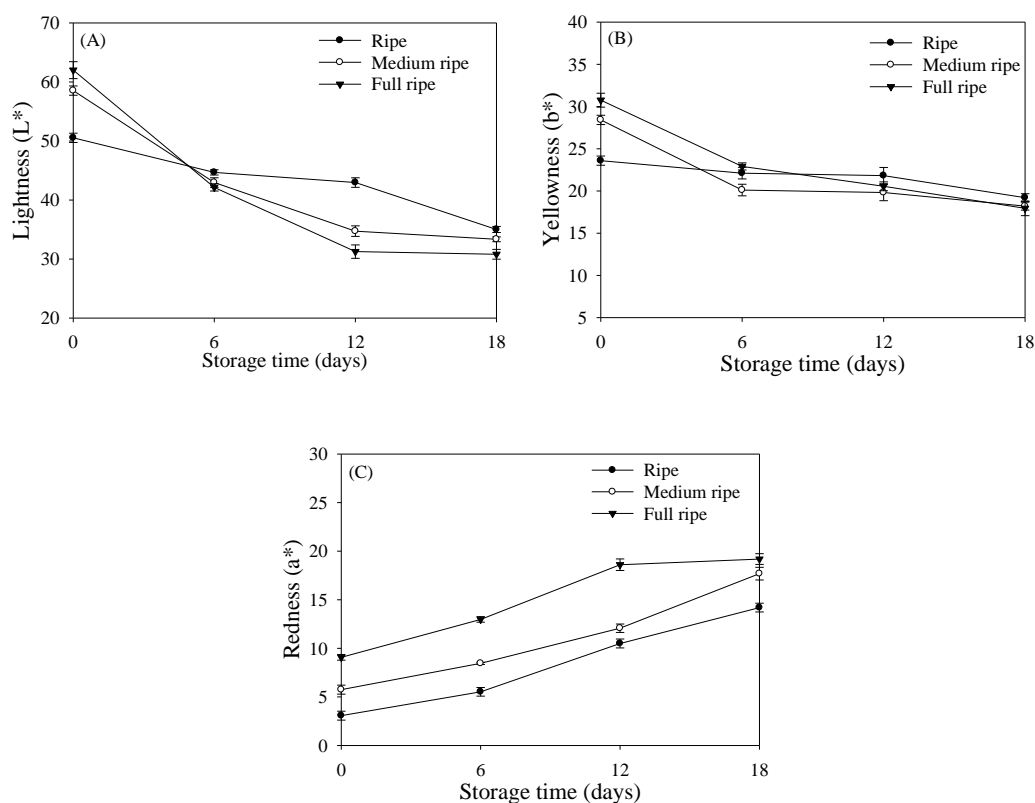
Figure 15 Lightness (A), yellowness (B) and redness (C) values of longkong at

different stages of on-tree maturation during storage under passive MAP

Note: Data are mean $\pm$ standard deviation (in ten replicates).

The decrease in C* value as from 29.14 to 23.79 (ripe), 29.97 to 25.49 (medium ripe) and 31.28 to 23.20 (full ripe) was observed (Figure 16B). The reduction of the angle of h° refers to fruit yellowness (h°, 90° = yellow) shifted to red skin color (h°, 0° = red-purple). The results showed a positive correlation with changes of CIE L*, a* and b* as mentioned in Figure 15.
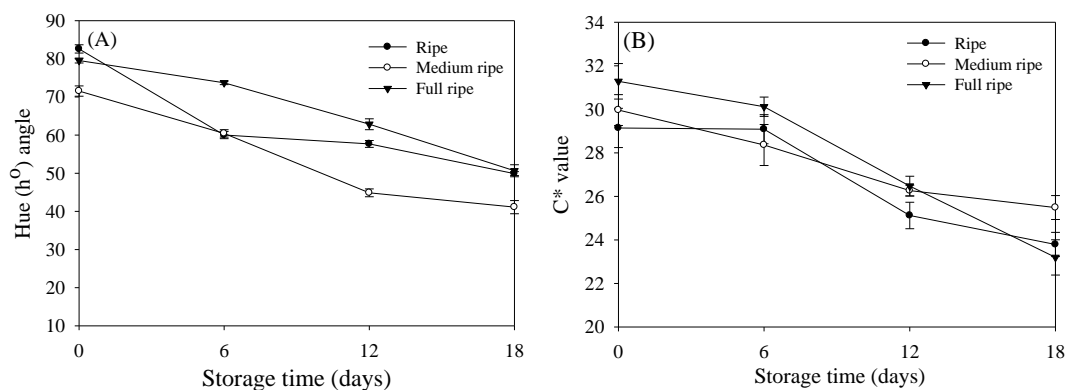
Figure 16 Hue (h°) angle and Chroma (C* values) of longkong at different stages of
on-tree maturation during storage under passive MAP

Note: Data are mean $\pm$ standard deviation (in ten replicates).

Hue (h°) angle = artangent b*/a*; Chroma (C* values) = $(a*^2+b*^2)^{1/2}$

Fruit firmness

Decrease in fruit firmness of longkong under passive MAP at 18°C was
found (p<0.05). The Figure 17 shows fruit firmness of longkong at different stages of
on-tree maturation during storage under passive MAP. Fruit firmness decreased from
1,952 to 1,762 g (ripe), 1,851 to 1,458 g (medium ripe) and 1,764 to 1,277 g (full
ripe).
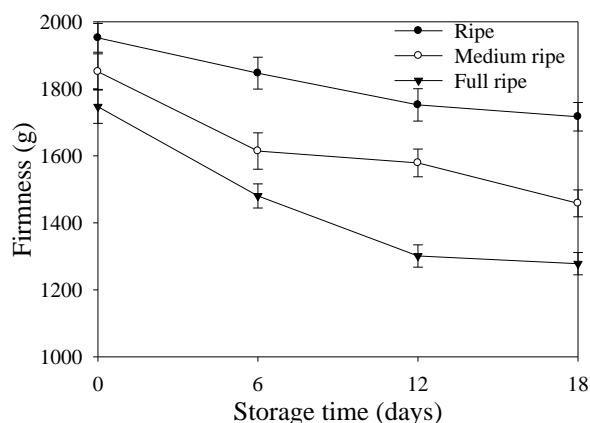
Figure 17 Fruit firmness of longkong at different stages of on-tree maturation during
storage under passive MAP

Note: Data are mean $\pm$ standard deviation (in ten replicates).

Under atmospheric condition, water loss and the resultant of turgor decline might contribute to the more rapid soften in fruits (Ali *et al*., 2004). It was found in longkong during storage under passive MAP (Figure 18). In addition, low O_2 or high CO_2 condition as passive MAP storage induces CO_2 stress. A fully mature fruit as full ripe longkong has a weakness tissue. It is easily to damage by stress condition. The CO_2 stress in a long-term of full ripe longkong storage is a main cause of loss on cell rigidity (Brummell and Harpster, 2001; White, 2002)

Weight loss

The increase in fruit weight loss overtime was noticed. Weight loss in longkong at different stages of on-tree maturation during storage under passive MAP is shown in Figure 18. It continuously increased until the end of storage to reach 1.41, 1.50 and 1.98 % for longkong at ripe, medium ripe and full ripe, respectively. Transpiration process exhibits loss of water, resulting in more weight loss. Loss of moisture content in fruit cell directly effects on fruit texture or firmness. It was in agreement with the decrease in longkong firmness overtime as previously described and demonstrated in Figure 17.
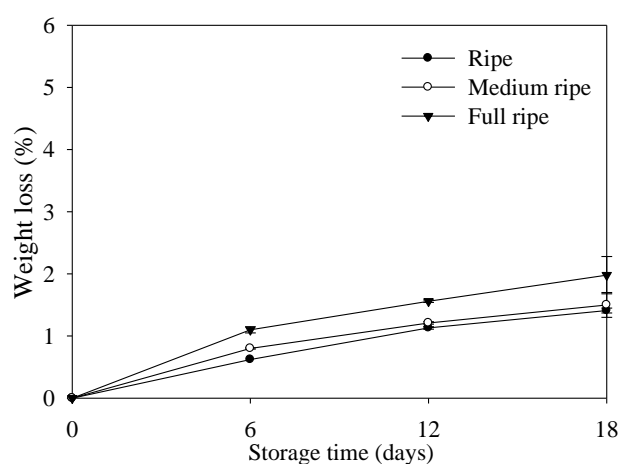
Figure 18 Weight loss of longkong at different stages of on-tree maturation during storage under passive MAP

Note: Data are mean $\pm$ standard deviation (in four replicates).

The highest weight loss was found in full ripe longkong during storage under passive MAP. The CO_2 stress occurred during long-term of passive MAP is more affects on full ripe longkong. More epidermal hair damage found in stressed longkong and it was a route for water loss in full ripe longkong (Venkatachalam, 2013).

5.4.2 Chemical quality

Changes in chemical qualities of longkong with storage time in all maturity stages was found ($p < 0.05$). Chemical quality changes during storage under passive MAP are reported in Table 10.

Total soluble solid (TSS), sugars, pH and acidity

Normally, sugar is a substrate for fruit respiration. The consumption of sugar in longkong associates with a reduction of sugar content in longkong under passive MAP storage. At the end of storage, the lowest of total sugar content in longkong was found in full ripe longkong. It might be due to high responsibility of metabolism in full ripe longkong under CO_2 stress. The CO_2 stress alerts high rate of respiration. Therefore, rate of sugar consumption also increased.

The slightly decrease in TA during 6 days was observed in all treatments ($p < 0.05$). After that, high acid content accumulated in longkong was detected. The acid accumulation in longkong was probably due to anaerobic or fermentative metabolism (Davies, 1980). The CO_2 accumulated inside a package always promotes during storage under passive MAP and induced anaerobic metabolism in a fruit cell. Under anaerobic metabolism, the Krebs cycle is shutdown. The oxidative enzymes are not active due to insufficient O_2.

Type and concentration of sugars

Changes in sugars concentration were monitored during storage under passive MAP. Changes in sucrose, glucose and fructose concentrations in longkong were significantly affected by stages of on-tree maturation and storage times ($p < 0.05$). Changes in sucrose, fructose and glucose were shown in Figure 19. During 6 days of storage under passive MAP, increase in fructose and glucose concentrations with decrease in sucrose concentration was noticed. It could be explained by inversion

reaction of sucrose (Ong *et al*., 2006). After that, the decreases in the concentrations of glucose and fructose until the end of storage were found. The fructose concentrations were 1.64% (ripe longkong), 0.62% (medium ripe) and 0.73% (full ripe longkong). The glucose concentrations were 1.45% in ripe longkong, 0.64% in medium ripe longkong and 0.78% in full ripe longkong. A reduction of sugar concentration during storage could be explained by respiration process (Ong *et al*., 2006). In addition, sucrose decreased overtime during passive MAP storage. It decreased from 7.99 to 1.03% (ripe longkong), 10.80 to 0.80% (medium ripe) and 7.99% to 0.34% (full ripe).
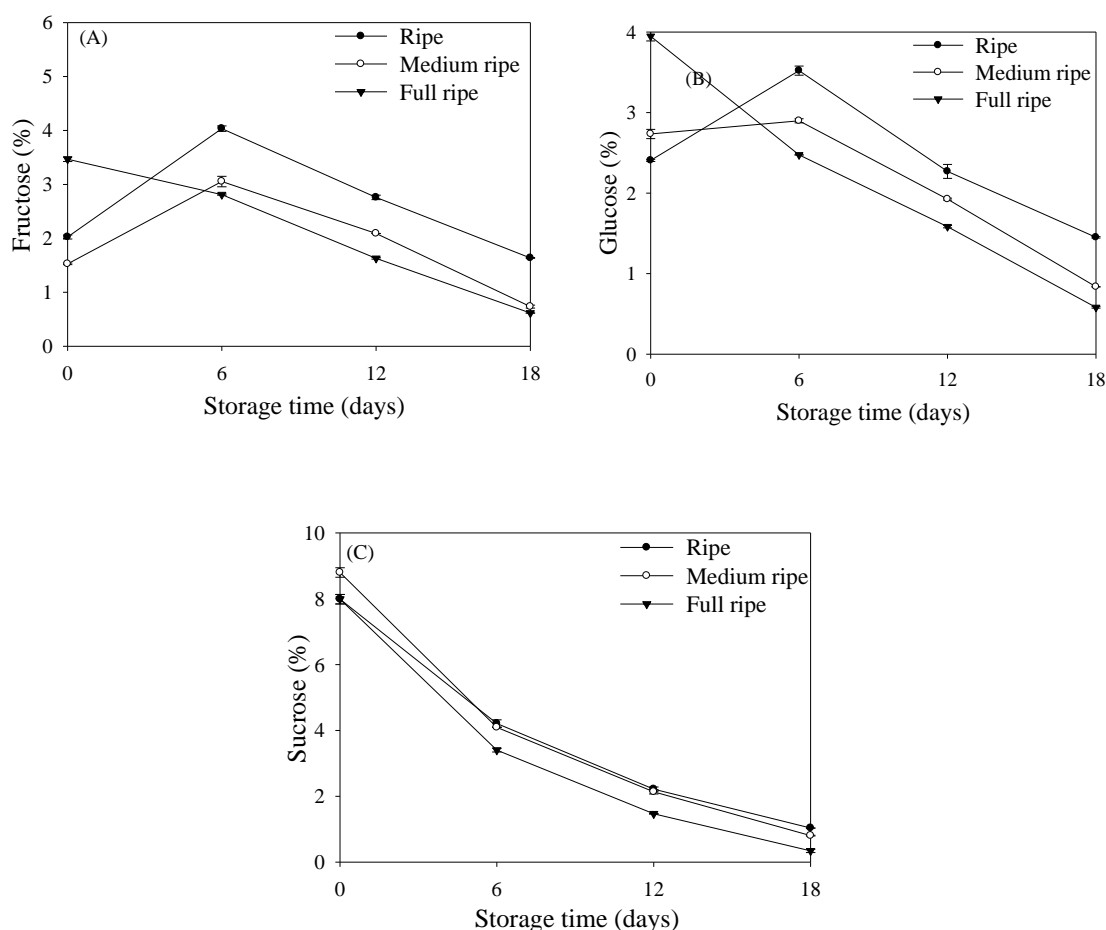
Figure 19 Types and concentrations of sugars content in longkong at different stages of on-tree maturation during storage under passive MAP

Note: Data are mean $\pm$ standard deviation (in four replicates)

Table 10 pH, titratable acidity (TA), total soluble solid (TSS), reducing sugar and total sugar of longkong at different stages of on-tree maturation during storage under passive MAP

Storage time (days)	Maturity stage	pH	TA (% as citric acid)	TSS (%)	Reducing sugar (%)	Total Sugar (%)
0	Ripe	3.98 ± 0.01^e	0.64 ± 0.01^b	14.1 ± 0.1^i	4.55 ± 0.00^i	12.20 ± 0.00^e
	Medium ripe	3.88 ± 0.02^f	0.61 ± 0.01^c	16.5 ± 0.1^d	5.39 ± 0.02^g	15.25 ± 0.01^b
	Full ripe	4.28 ± 0.02^a	0.58 ± 0.01^e	17.3 ± 0.1^a	5.70 ± 0.00^f	15.64 ± 0.05^a
6	Ripe	4.08 ± 0.00^c	0.58 ± 0.00^e	14.3 ± 0.1^h	6.43 ± 0.00^e	14.57 ± 0.00^c
	Medium ripe	4.04 ± 0.01^d	0.55 ± 0.01^g	17.0 ± 0.0^{bc}	7.58 ± 0.07^c	12.48 ± 0.02^d
	Full ripe	3.70 ± 0.01^g	0.58 ± 0.01^e	17.4 ± 0.0^a	9.31 ± 0.24^a	10.38 ± 0.02^g
12	Ripe	4.00 ± 0.00^e	0.60 ± 0.01^d	15.6 ± 0.1^g	7.62 ± 0.14^c	7.40 ± 0.02^k
	Medium ripe	4.15 ± 0.01^b	0.58 ± 0.01^e	16.9 ± 0.1^c	5.12 ± 0.10^h	9.75 ± 0.04^h
	Full ripe	3.60 ± 0.01^h	0.60 ± 0.01^d	16.6 ± 0.2^d	8.74 ± 0.06^b	10.52 ± 0.02^f
18	Ripe	3.99 ± 0.00^e	0.62 ± 0.01^c	15.9 ± 0.1^f	6.80 ± 0.00^d	6.80 ± 0.00^l
	Medium ripe	4.15 ± 0.01^b	0.63 ± 0.01^c	17.1 ± 0.1^b	4.56 ± 0.29^i	7.93 ± 0.00^i
	Full ripe	3.86 ± 0.01^f	0.67 ± 0.00^a	16.1 ± 0.1^e	5.10 ± 0.10^h	7.54 ± 0.02^j

Note: Data are mean $\pm$ standard deviation (in four replications).

Mean values in the same column with different superscript letters indicate that there are significant difference among stage of on-tree maturation ($p<0.05$).

Volatile flavor compounds

Volatile flavor compounds are an extremely important quality in fruit. Fruit volatile flavor profiles quite be changed by postharvest conditions. Volatile flavor compounds in longkong at different stage of on-tree maturation during storage under passive MAP were indentified (Tables 11, 12 and 13). The volatile flavor compounds namely, esters, alcohols, terpenes and their derivatives, acids, ketones and phenols were identified in longkong. The butyl butanoate, benzyl alcohol, 3-hydroxy-2-butanone and delta-germacrene were identified as abundances in longkong. They give fruity, sweet floral and herbaceous characteristics. However, their concentrations declined with storage time of longkong under passive MAP.

Normally, fruity esters and alcohols were produced through the beta-oxidation of fatty acid to form alcohol and convert to ester by alcohol acyltransferase (AAT) (Defilippi *et al*., 2005). But, insufficient O_2 might inhibit β- oxidation of fatty acid, yielding less of acetyl CoA for ester formation (Shalit *et al*., 2000. For a long-term of longkong in all maturity under passive MAP storage, the formation of acids and fermented alcohols were identified in the volatile flavor profiles. They were acetic acid, butanoic acid, hexanoic acid and iso-amylalcohol. They represent sour, sweat and fermented characteristics, respectively. In a summary, longkong in all treatments changed their fruity flavor profile to fermented flavor profile due to anaerobic or fermentation pathway which is induced by CO_2 stress (Defilippi *et al*., 2005).

Table 11 Volatile flavor compounds and their attributes identified in longkong at ripe stage during storage under passive MAP

Compounds	Rt[A]	RI[B]	Attributes[C]	Peak areas ($\times 10^4$) TIC[D]			
				Storage time (days)			
				0	6	12	18
Esters							
butyl butanoate	22.64	1841	fruity	-	11.03	31.88	21.11
Alcohols							
iso-amylalcohol	7.72	1218	fermented	2.80	10.52	38.43	64.44
benzyl alcohol	23.77	1895	floral, sweet	-	18.76	32.02	23.12
phenylethyl alcohol	24.45	2134	floral	-	11.03	39.90	21.09
Terpenes and their derivatives							
cis-linalool oxide	14.39	1479	floral, sweet	-	3.81	7.28	24.98
alpha-copaene	14.58	1486	woody	-	4.21	14.12	23.14
beta-bourbonene	15.22	1512	herbaceous	-	23.67	43.62	12.21
laevo-linalool	16.35	1559	fruity, sweet	-	6.84	10.28	49.61
delta-germacrene	19.75	1706	herbaceous	0.82	44.31	79.33	46.96
bicyclogermacrene	20.29	1731	woody	-	-	22.10	35.11
delta-cadinene	20.84	1756	herbaceous	-	-	26.20	30.02
epoxylinalool	21.21	1707	flower	-	5.82	15.32	24.53
spathuleol	28.37	2184	fruity, herbaceous	-	-	5.79	24.11
Acids							
acetic acid	14.21	1472	pungent, sour	0.50	24.21	39.88	59.00
butanoic acid	18.32	1643	sour	-	9.06	14.44	45.67
hexanoic acid	23.07	1862	sour, sweat	-	8.00	23.11	56.76
Ketones							
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	64.55	80.72	34.11
Phenol							
phenol	26.38	2027	phenol	2.89	34.44	65.45	48.65

Note: [A] Rt = Retention time (min);　[B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html,

http://www.flavornet.org/flavornet.html [D] TIC = total ion current; - = not detected

Table 12 Volatile flavor compounds and their attributes identified in longkong at medium ripe stage during storage under passive MAP

Compounds	Rt[A]	RI[B]	Attributes[C]	Peak areas ($\times 10^4$) TIC[D]			
				Storage time (days)			
				0	6	12	18
Esters							
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	-	13.47	15.22	44.58
butyl butanoate	22.64	1841	fruity	-	69.20	72.21	-
Alcohols							
2-methyl propanol	4.96	1107	fruity, sweet	-	2.13	5.95	-
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	5.61	18.11	-	-
benzyl alcohol	23.77	1895	floral, sweet	-	45.70	30.64	38.51
phenylethyl alcohol	24.45	2134	floral	-	79.15	38.67	52.10
Terpenes and their derivatives							
cis-linalool oxide	14.39	1479	floral, sweet	-	7.26	9.05	15.45
beta-bourbonene	15.22	1512	herbaceous	-	-	13.32	20.18
delta-germacrene	19.75	1706	herbaceous	15.68	35.84	32.69	31.85
delta-cadinene	20.84	1756	herbaceous	-	-	14.02	-
laevo-linalool	16.35	1559	fruity, sweet	-	12.20	25.11	-
epoxylinalool	21.21	1707	flower	-	29.38	17.06	26.28
spathuleol	28.37	2184	fruity, herbaceous	-	8.68	10.51	-
Acids							
acetic acid	14.21	1472	pungent, sour	-	33.14	88.94	110.34
butanoic acid	18.32	1643	sour	-	10.11	26.21	65.71
hexanoic acid	23.07	1862	sour, sweat	-	12.17	35.67	74.09
Ketones							
3-hydroxy-2-butanone	9.78	1298	sweet	14.84	35.44	32.95	28.00
Phenol							
phenol	26.38	2027	phenol	76.85	88.43	45.56	50.01

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html,

http://www.flavornet.org/flavornet.html

[D] TIC = total ion current; - = not detected

Table 13 Volatile flavor compounds and their attributes identified in longkong at full ripe stage during storage under passive MAP

Compounds	Rt[A]	RI[B]	Attributes[C]	Peak areas ($\times 10^4$) TIC[D]			
				Storage time (days)			
				0	6	12	18
Esters							
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	-	13.94	21.60	-
butyl butanoate	22.64	1841	fruity	-	45.51	60.36	-
Alcohols							
2-methyl propanol	4.96	1107	fruity, sweet	-	-	33.96	-
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	-	-	5.26	-
benzyl alcohol	23.77	1895	floral, sweet	1.53	37.23	31.82	-
phenylethyl alcohol	24.45	2134	floral	-	55.54	32.21	-
Terpenes and their derivatives							
cis-linalool oxide	14.39	1479	floral, sweet	9.55	29.96	32.60	-
delta-germacrene	19.75	1706	herbaceous	26.54	51.79	47.71	12.19
delta-cadinene	20.84	1756	herbaceous	2.97	10.76	9.50	-
laevo-linalool	16.35	1559	fruity, sweet	3.09	-	-	-
epoxylinalool	21.21	1707	flower	-	19.42	60.22	-
spathuleol	28.37	2184	fruity, herbaceous	-	8.53	17.58	-
Acids							
acetic acid	14.21	1472	pungent, sour	-	18.82	44.53	58.30
butanoic acid	18.32	1643	sour	-	-	41.10	65.11
hexanoic acid	23.07	1862	sour, sweat	-	-	45.58	67.24
Ketones							
3-hydroxy-2-butanone	9.78	1298	sweet	26.75	28.13	16.96	15.35
Phenol							
phenol	26.38	2027	phenol	74.40	88.51	62.78	44.21

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html,

http://www.flavornet.org/flavornet.html

[D] TIC = total ion current; - = not detected

Headspace gases composition

Changes in headspace oxygen (O_2) and carbon dioxide (CO_2) were monitored. The headspace O_2 significantly decreased overtime in all conditions ($p<0.05$). The concentration of headspace O_2 decreased from 21.35% to a range of 0.21-0.96% (Figure 20A). The headspace of CO_2 significantly increased with storage times in all conditions ($p<0.05$). The concentration of headspace CO_2 increased from 0.26% to a range of 76.96-80.55% (Figure 20B). According to fruit respiration, low headspace O_2 and/or high headspace CO_2 occurs. It is a role play for anaerobic metabolism.
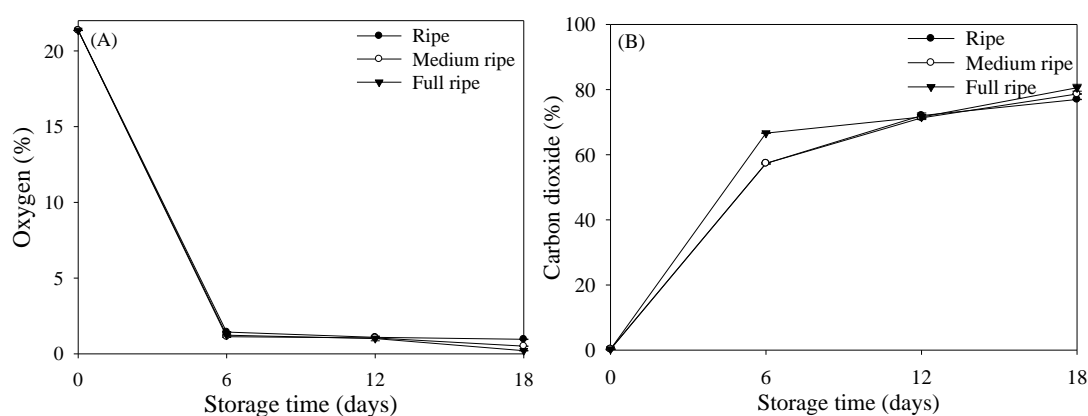
Figure 20 Headspace oxygen (A) and carbon dioxide (B) inside package of longkong at different stages of on-tree maturation during storage under passive MAP

Note: Data are mean $\pm$ standard deviation (in four replicates).

Ethanol concentration

Long-term under passive MAP condition, anaerobic metabolism occurs. It is induced by high CO_2 accumulation. The exposes for 18 days of longkong to passive MAP condition, a significantly increase in ethanol concentration was found ($p<0.05$). The concentration of ethanol in longkong is shown in Figure 21. Ethanol content in fresh longkong was 0.06 g/g fruit weight (g/g FW). At the end of storage, the ethanol concentration gradually increased to 0.15, 0.16 and 0.20 g/g FW in ripe, medium ripe and full ripe longkong, respectively.
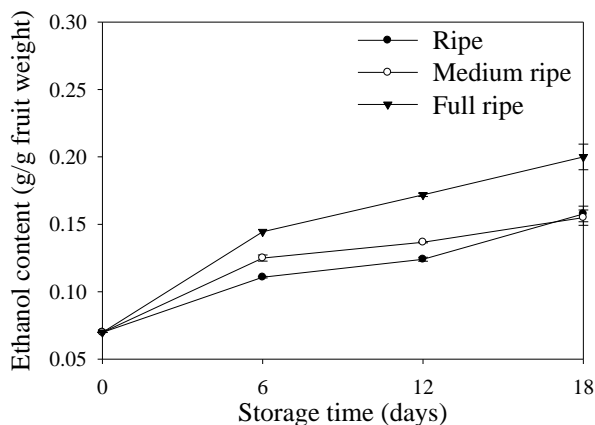
Figure 21 Ethanol content in longkong at different stages of on-tree maturation
during storage under passive MAP

Note: Data are mean $\pm$ standard deviation (in four replicates).

The highest of ethanol content was observed in full ripe longkong. It was in agreement with the lowest of headspace O_2 and the highest of headspace CO_2 inside a package (Figure 20). The large concentration of ethanol induced by very high CO_2 or very low O_2 concentrations. Under anaerobic pathway, Krebs cycle shut down and glycolysis is a predominant, yielding a pyruvate. Pyruvates undergo decarboxylation to produce acetaldehyde, which was reduced subsequently to ethanol (Ke and Kader, 1991; Ke *et al*., 1991; Mathooko, 1996; Ke *et al*., 1994).

Alcohol dehydrogenase (ADH) enzyme activity

Ethanol fermentation is produced by the reduction of acetaldehyde by ADH enzyme (Cossins, 1978; Ke *et al*., 1994). According to an increase in ethanol content in longkong is directly effect by the action of ADH enzyme. Therefore, the activity of ADH enzyme in longkong was monitored during storage under passive MAP. The activity of ADH enzyme was showed in Figure 22.
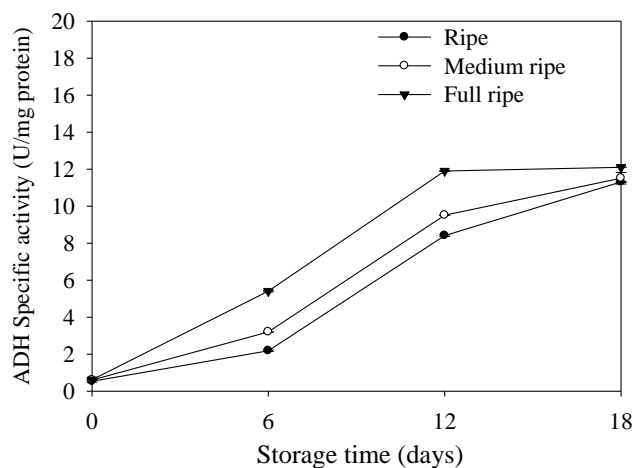
Figure 22 ADH specific activity of longkong at different stages of on-tree maturation
during storage under passive MAP

Note: Data are mean $\pm$ standard deviation (in four replicates).

The increase in ADH activity in longkong with storage time under passive MAP storage in all maturity stages was observed ($p<0.05$). The initial activity of ADH enzyme were 0.54, 0.59, 0.62 U/mg protein, for ripe, medium ripe and full ripe longkong, respectively. The ADH activity increased to 11.31, 11.51 and 12.00 U/mg protein for ripe, medium ripe and full ripe longkong, respectively (Figure 8). The highest activity of ADH enzyme was found in full ripe longkong. It was in agreement with the results of the highest of headspace CO_2 and the lowest of O_2 concentrations (Figure 20) as well as ethanol content (Figure 21). Fruit at the stage of full mature has its weakness cell membrane. It is quite easy damaged by stress condition and loss on its integrity and bad barrier for gas diffusion (Rajapakse *et al.*, 1990). Therefore, anaerobic metabolism might be taken place in full ripe longkong and giving in high accumulation of ethanol with high ADH activity.

Microbiological

Total viable count in longkong at the initial and the end of storage under passive MAP was presented in table 14. The initial load of total viable count was 1.50×10^3 CFU/g, 1.52×10^3 CFU/g and 1.55×10^3 CFU/g for ripe, medium ripe

and full ripe, respectively. The total viable count increased at the end of storage time. They were 6.41×10^5 CFU/g, 6.53×10^5 CFU/g and 6.68×10^5 CFU/g.

Table 14 Total viable count in longkong at different stages of on-tree maturation during storage under passive MAP

Longkong	Total viable count (CFU/g)	
	Storage time (days)	
	0 (Initial)	18 (End of storage)
Ripe	1.50×10^3	6.41×10^5
Medium ripe	1.52×10^3	6.53×10^5
Full ripe	1.55×10^3	6.68×10^5

Note: Data are mean $\pm$ standard deviation (in four replicates).

5.5 Conclusions

Longkong has its short shelf-life for 4-7 days at room temperature. The effective postharvest technology likes modified atmosphere need to be applied for prolonging its shelf-life. Passive MAP generally used to extend shelf-life of longkong. However, fruit maturity is the one important factor which enhances the effective of post harvest treatment. Longkong stored under passive MAP combined low temperature storage presented low ethanol content and low microbial population. Full ripe longkong seems to be not appropriate for storage under high CO_2 or low O_2 condition likes passive MAP. Caused of fruit at full ripe showed the most sensitive on stress high CO_2 or low O_2 condition, giving the disorder attributes. It was confirmed as the results of loss of bright yellow color, firmness, characteristic aroma rapidly. Longkong contained of high acidity, fermented and sour volatile compounds as well as ethanol content.

CHAPTER 6

CHANGES IN FRUIT QUALITY, VOLATILE FLAVOR COMPOUNDS AND POSSIBLY OFF-FLAVOR ACCUMALATION IN LONGKONG DURING STORAGE AT DIFFERENT TEMPERATURES UNDER PASSIVE MODIFIED ATMOSPHERE PACKAGING

6.1 Abstract

Passive modified atmosphere packaging (passive MAP) is an alternative way for fruit shelf-life extension. To enhance the effectiveness of passive MAP, the optimal temperature is required. Longkong was stored under passive MAP at three different storage temperatures at 4°C, 18°C and room temperature (~30°C). The quality of the longkong during storage was monitored at 3 day intervals. Longkong stored under passive MAP at 4°C for 36 days became more darken and soften (p<0.05). Fruit skin color is represented by L*, a* and b* values, were 25.57, 23.21 and 11.77, respectively. Fruit firmness decreased from 1849.30 to 1335.53 g. A slight increase in acidity was found. It increased from 0.59 to 0.73%. High CO_2 concentration inside package accelerated the activity of ADH enzymes to 11.34 U/mg protein. The ethanol content increased during storage to 0.16 g/g FW. The microbial population increased with storage time to 4.31×10^5 CFU/g.

6.2 Introduction

Passive modified atmosphere packaging (passive MAP) is atmospheric modification, by packaged natural fruit respiring by through consuming of O_2 and producing of CO_2 inside the package. The increased CO_2 inside a package has been shown to be effective on fruit quality maintenance (Kader *et al*., 1989). In addition, the storage temperature is one of the important factors. It needs to be set at the optimal level during storage under passive MAP conditions. Teerapawa and Premanode (1991) found that storage under passive MAP at low temperature could prolong longkong shelf-life. The longkong was wrapped with PVC plastic film and kept at ambient temperature, 15°C and 13°C, and the shelf-life was 7, 10 and 14 days, respectively. Bunsiri *et al*. (2006) reported that the storage of longkong in a

polystyrene tray covering with a plastic bag at 18°C was a suitable condition. It had a satisfactory quality for 4 weeks. It had less fruit drop with good taste. Similar to the study of Piyasaengthong *et al*. (1997) who reported that storage longkong at 18°C could extend the shelf-life for 21 days with 60% fruit drop. Longkong stored under passive MAP combined with a low temperature had a longer shelf-life than that kept under ambient temperatures. To date no scientific data has been reported on the possible of off-flavor accumulation during storage under passive MAP yet. Therefore, the objective of this study was to study the effect of storage temperature on longkong quality, volatile flavor compounds and possible off-flavor accumulation during storage.

6.3 Materials and Methods

Chemicals

The pyruvate decarboxylase and alcohol dehydrogenase enzymes were obtained from Fluka, Sigma Chemical Co. (St. Louis, MO), Aldrich Chemicals Co. (Milwaukee, WI) and Merck Co. (Darmstadt, Germany). D-glucose, D-fructose and sucrose were purchased from Fluka (Messerchmittstr, Switzerland). Acetonitrile and water (HPLC grade) were obtained from Prolabo (Paris, France). Sodium hydroxide, copper sulfate pentahydrate, trichloroacetic acid and sodium sulfite were obtained from Merck (Darmstadt, Germany). Potassium sodium tartrate, lead acetate and potassium oxalate were obtained from Riedel-deHaen (Seelze, Germany). Dichloromethane was obtained from LabScan Asia Co., Ltd (Bangkok, Thailand). Plate count agar (PCA) for the total viable counts was obtained from Merck Co. (Darmstadt, Germany).

Plant material and fruit preparation

Longkong was obtained from a contact garden in Natawee district (Songkhla province, Thailand). The maturity stage of 13 weeks after anthesis was chosen. The fruit was harvested in the morning and immediately transported on the same day to the Food Analysis laboratory, Faculty of Agro-Industry (approximately 2 hours). Fruits free from any apparent skin damage and uniform size (grade A about 3-3.5 cm in diameter, according to Tanyongmut market) were selected for analysis. The

fruit was prepared in an individual by cut of raceme. Individually fruit was cleaned with a brush and submerged in a mixed solution of 500 ppm benomyl and 1.5% citric acid for 5 min and dried at room temperature (~30°C) for 15 min. This was done to reduce the microbial load and anti-browning.

Passive modified atmosphere packaging and storage conditions

The 12 individual fruits were placed on a plastic tray. The tray was covered with 0.08 mm thickness nylon laminated with a linear Low Density Polyethylene (nylon/LLDPE) bag sized 7x11 inches with permeability to CO_2 and O_2 of 4.7 and 2.6 $cm^2/m^3/day$ at 25°C and 0% RH, respectively. It was immediately sealed by using a heat sealer. The sample bags were stored at 4°C, 18°C and room temperature (~30°C). The fruit quality was evaluated at 3 day intervals during storage.

Fruit quality evaluation

The physical qualities, such as fruit color, fruit firmness and weight loss, were measured in 10 fruits at 3 day intervals. The chemical qualities were also evaluated at 3-day intervals. These included total soluble solid (TSS), sugars (reducing sugars, total sugars and type and concentration of sugars), titratable acidity (TA), pH, alcohol dehydrogenase (ADH) activity and volatile flavor compounds. The homogenate prepared by flesh (de-seeded) blending was used for chemical quality analysis. This was prepared fresh before the analysis. The methods of physical and chemical analysis used are explained.

Fruit color

Two opposite sides of longkong fruit skin were quantified in terms of CIE lightness (L*), redness (a*) and yellowness (b*) values using a Color Flex, Hunter Lab colorimeter. CIE values were calculated in terms of Hue (h°) angle = artangent b*/a* and Chroma (C* value) = $(a*^2+b*^2)^{1/2}$ (Apai, 2010).

Fruit firmness

Two opposite sides of the fruit were measured by using a TA-XT2i Texture Analyzer (Stable Micro System, UK) equipped with a 2 mm diameter cylinder probe (P/2). The penetrometric method was applied. The results were expressed as gram force (Adapted from Sapii *et al*., 2000).

Fruit weight

The fruit weight was evaluated by weighing with a Sartorius BP310S analytical balance.

Total soluble solid (TSS)

The TSS was determined using an Atago 1E (Japan) hand refractometer at 25ºC and expressed as a percentage (Ong *et al*., 2006).

Total and reducing sugars

The 25 g of the longkong homogenate and 100 ml of distillated water were mixed and clarified by 45% neutral lead acetate (2 ml) and 22% potassium oxalate (2 ml). The sample volume was adjusted to 250 ml with distillated water and filtered through filter paper No 1. The total sugar and reducing sugar content was quantified by titration with Fehling's reagents according to Lane and Eynon (A.O.A.C., 2000).

Titratable acidity

The titratable acidity (TA) was quantified by titrating 10 ml of the homogenate to an end point of pH 8.2 with 0.1 N NaOH with 1% (v/v) phenolphthalein as an indicator. The result was calculated as a percentage of citric acid (Ong *et al*., 2006).

pH

pH was measured using a Sartorius PB-20 (Germany) digital pH meter (Adapted from Ong *et al*., 2006).

Types and concentrations of sugars

Types and concentrations of sugars (sucrose, fructose and glucose) were determined by HPLC (Shimadzu, CR 6A Chromatopac) with a Hypersil APS-2 column. For sample preparation, the homogenate was clarified by using refrigerated centrifugation (4ºC) at 10,000×g for 30 min. Clear solution of longkong juice was obtained. The clear solution was filtered through 0.45 µm pore size membrane filters and was kept at -20°C until analysis. The injection volume was 10 µl with an isocratic flow rate of 1 ml/min and a refractive index detector. The 90% acetonitrile was used as a mobile phase. Their concentrations were quantified by comparing the retention times and peak areas of the samples with sucrose, fructose and glucose standards (Adapted from Chyau *et al.*, 2003; Ong *et al.*, 2006; Soares *et al.*, 2007).

Volatile flavor compound isolation by direct solvent extraction (DSE) and identification by GC-MS

Volatile flavor compounds were extracted by direct solvent extraction and identified by GC-MS. The 100 ml clear solution of longkong juice was mixed with 100 ml of dichloromethane. Next, the mixture was gentle shaken for 90 min and left equilibrium for 30 min at room temperature (~30°C). The solvent phase was collected and re-extracted twice. The solvent phase was dried on anhydrous Na_2SO_4, kept overnight at -20°C, cold- filtered and concentrated using purging nitrogen gas. The concentrate was kept at -20°C prior to analysis. The 1 µl was directly injected into GC-MS (Adapted from Flores *et al.*, 2002; Elss *et al.*, 2005; Ong *et al.*, 2006).

The volatile flavor compounds were identified by GC-MS. A chromatograph, Hewlet-Packard 6890 (Palo Alto, CA, USA), was used with an HP-FFAP column (25 m×0.20 mm; film thickness 0.20 mm). The inlet temperature was set at 240°C and the column temperature was maintained at 40°C for 2 min, followed by an increase to 240°C at the rate of 5°C/min and held for 13 min. The carrier gas was applied by ultra high purity helium at a constant flow of 1.5 ml/min. A Hewlet-Packard 5973 A mass spectrometer with a quadrupole mass filter was coupled to the GC. A mass spectrum (MS) was obtained at 70 eV in the electronic impact (EI) and positive modes. The MS was scanned in the range m/z 40-350 at 1 s intervals (Adapted from Wanakhachornkrai and Lertsiri, 2003).

Headspace gas composition analysis

Changes in the headspace gas composition were monitored by gas chromatography (GC) coupling with a thermal conductivity detector (TCD-detector). The 1 ml of headspace gas was directly withdrawn from headspace inside the package and subsequently injected into Poropak N columns. Helium at flow rate of 50 ml/min was used as the carrier gas. The temperature oven and injection port was held at 60°C. The temperature TCD detector was held at 150°C. The internal package atmosphere was identified and quantified by comparison with external standard gas (Adapted from Tano *et al.*, 2007).

Ethanol isolation by headspace-solid phase microextraction (HS-SPME) and identification by GC-FID

The ethanol content in longkong was extracted by headspace solid phase microextraction (HS-SPME). The extraction was done as follows; the 100 g of the flesh of longkong was mixed with 100 ml of 20% NaCl (cold solution). It was blended at a constant speed using a blender for 3 min. It was then filtered through a stainless steel sieve. This process produced the homogenate. The 20 ml homogenate was placed into a 125 ml vial followed by the addition of 3 g of NaCl and fitted with a rubber septum. The vial was kept at 30ºC and left equilibrates for 60 min.

Equilibrated ethanol in the headspace was isolated by using 50/30 μm DVB/CAR/PDMS (divinylbenzene/Carboxen on poly (dimethylsiloxane) (Supelco, Bellafonte, PA, USA). The equilibrated ethanol in the headspace was adsorbed for 15 min at room temperature (~ 30°C) and desorbed for 5 min in GC-FID (PerkinElmer, Autosystem XL, USA) (Adapted from Lara *et al.*, 2006).

The ethanol was analyzed by GC-FID. The apparatus was equipped with an Rtx-5 (Restek) column (30 m×0.25 mm; film thickness 0.25 μm). The carrier gas was helium of ultra high purity helium at a constant flow of 1.5 ml/min. The injector was kept at 240°C and set for the splitless mode. The column temperature was set at 35°C for 3 min and then programmed to 230°C at the rate of 8°C/min and held for 2 min. The ethanol concentration was calculated using standard aqueous solutions of ethanol under the same conditions as were used for the samples (Adapted from Lara *et al.*, 2006).

ADH enzyme extraction and ADH activity evaluation

The 100 mg of longkong flesh was homogenized in 1 ml of extracted solution. The extract solution contained 85 mM 2-(N-mor-pholino) ethane-sulfonic acid (MES) buffer, with a pH 6.0, 5 mM dithiothreitol (DTT) and 1% (w/v) polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged at 25000×g for 15 min at 4°C to recover the supernatant. The supernatant was kept in ice as crude enzyme extract (Adapted from Lara *et al*., 2006).

The ADH activity was assayed by mixing 2.55 ml NADH solution (0.150 mM NADH in 85 mM MES, pH 6.0), 150 µl of acetaldehyde solution (80 mM acetaldehyde in 85 mM MES, with a pH 6.0), and 300 µl enzyme extract. The ADH activity was measured every 1 min by the spectrophotometer technique at 340 nm. The results were expressed as specific activity (U/mg protein) (Lara *et al*., 2006). The total protein in the extract was determined according to the method described by Bradford (1976).

Microbiological determination

The microbial was determined at the beginning and the end of storage. Homogenized longkong flesh (de-seed) 25g was combined with 225 ml of sterile 0.1% peptone water in a sterile polyethylene bag. The sample was pummeled with a stomacher (Seward Stomacher 400, UK) for 2 min using medium speed. The aliquot was used for the appropriate dilution.

The total viable counts were determined by pouring 1 ml of diluted sample onto plate count agar (PCA). The plates were incubated at 35°C for 48 hr, and the colonies were counted. The microbial counts were expressed in CFU/g of longkong (Koide and Shi, 2007).

Statistical analysis

The experiment was performed by a completely randomized design (CRD) with factorial treatment. A two-way factorial treatment (6 levels of storage times × 3 levels of storage temperatures) was performed. Significant differences between means were estimated by Duncan's new multiple range test (DMRT), with a level of significance of 0.05. Statistical analyses were performed by using the

Statistical Package for Social Science (SPSS for windows, SPSS Inc., Chicago, IL, USA).

6.4 Results and Discussion

The visible mold growth on the longkong skin was used as a marker concerning to reach the end of storage. The longkong stored under passive MAP at 4°C, 18°C and room temperature (~30ºC) for 36, 18 and 15 days, respectively. The interaction between the storage times and storage temperatures had a significant effect on the longkong qualities ($p<0.05$).

6.4.1 Physical quality

During storage under passive MAP, the quality lost was significantly different with the storage temperature ($p<0.05$). The fruit skin became brownish. The fruit firmness declined and there were increases in the percentage of weight loss.

Fruit color

The brownish fruit skin of longkong was increased with storage time under passive MAP at 4°C, 18°C and room temperature (~30°C). The interactions between storage times and storage temperatures showed significant decrease in the skin of the bright yellow fruit ($p<0.05$). The color was measured in CIE L*, a* and b* values. They represent lightness, redness and yellowness, respectively. Longkong lost its bright yellow skin. The initial lightness (L*) of fresh longkong was 64.42. It decreased up to the end of storage to 25.57, 26.00 and 23.28 for longkong stored at 4°C, 18°C and room temperature (~30°C), respectively. The b* value also decreased from 34.47 in fresh longkong to 11.77, 13.76 and 11.53 for longkong stored at 4°C, 18°C and room temperature (~30°C), respectively (Figures 23A and 23B). Conversely, an increase in the redness (a*) value in longkong over storage time was found. The a* value increased from 5.67 in fresh longkong to 23.21, 22.08 and 23.83 at the end of storage at 4°C, 18°C and room temperature (~30°C), respectively (Figure 24C). A sharp decrease in L* and b* values with an increase in a* value was found in longkong that was stored under passive MAP at room temperature (~30ºC). During storage at high temperature, such as room temperature, fruit has a high respiration rate. This high fruit respiration rate results in high CO_2 accumulation inside a package compared with low temperatures. High CO_2 can induce stress conditions which can

damage the integrity of fruit cells and cause enzymatic browning reaction (Castro *et al.*, 2007). Browning enzymes interacted with the substrate as phenolic compounds and caused the fruit skin to darken (Sacher, 1962; Azevedo *et al.*, 2008; Venkatachalam and Meenune, 2012).
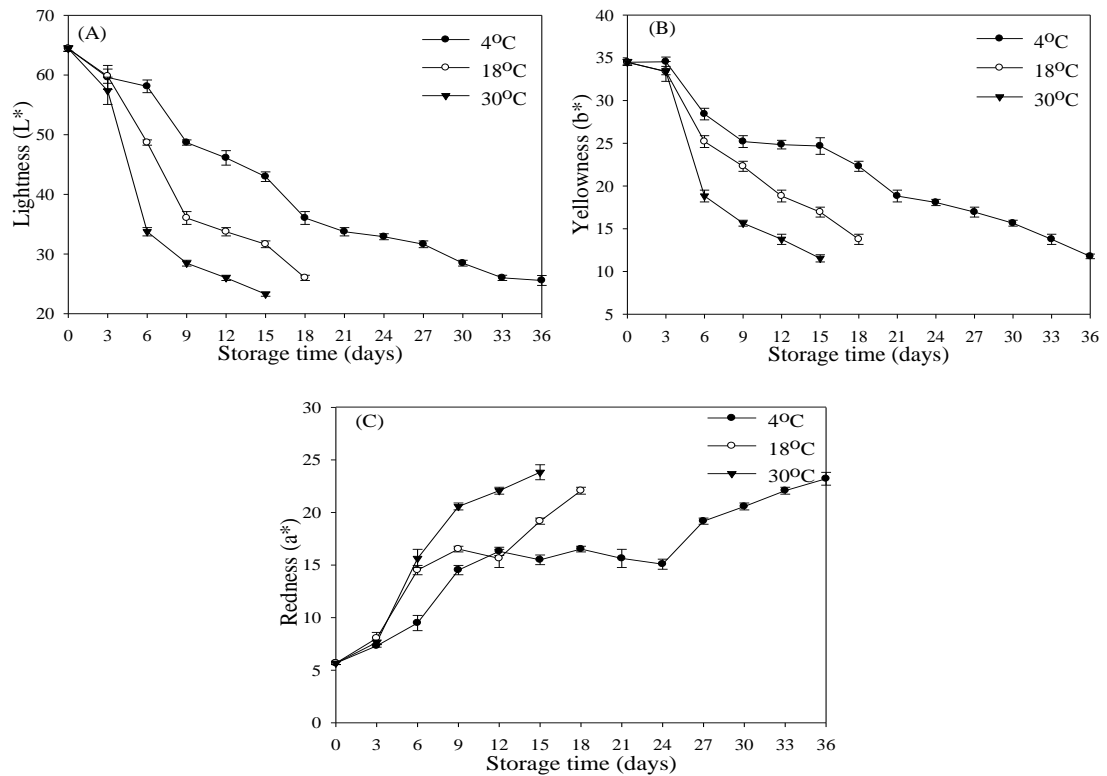
Figure 23 Lightness (A), yellowness (B) and redness (C) values of longkong during storage under passive MAP at different temperatures

Note: Data are mean $\pm$ standard deviation (in ten replicates).

The fruit skin color of longkong can be interpreted in terms of h° angle and C* value. The changes in the fruit skin color of longkong during storage under passive MAP at 4°C, 18°C and room temperature (~30°C) in terms of h° angle and C* value are shown in Figure 24. The interaction between storage times and storage temperatures were significantly affected by the h° angle and C* value ($p < 0.05$). A decrease in the h° angle was observed (Figure 24A). The h° angle of fresh longkong was 80.65. At the end of storage, the h° angle decreased to 26.88, 31.93 and 25.83 for longkong stored under passive MAP at 4°C, 18°C and room temperature (~30°C), respectively. The decrease in the h° angle refers to fruit yellowness (h° angle, 90° =

yellow) and the shifts to redness (h° angle, 0° = red-purple). Decreases in the h° angle showed the positive correlation with changes in CIE lightness (L*), redness (a*) and yellowness (b*).

The C* value indicates color intensity. In this study, C* values showed a significant decrease from 34.94 in fresh longkong to 23.54 (24 days at 4°C), 24.48 (12 days at 18°C) and 24.48 (6 days at 30°C). After that, C* values increased to 26.02, 26.02 and 26.50 for longkong stored under passive MAP at 4°C, 18°C and room temperature (~30°C), respectively. Decrease in C* values could be explained by the low intensity of yellowness in the longkong and this was due to the brownish color. On the other hand, increases in the C* values were obtained up to the end of storage. This was explained by the high intensity of the brownish color from the browning reaction.
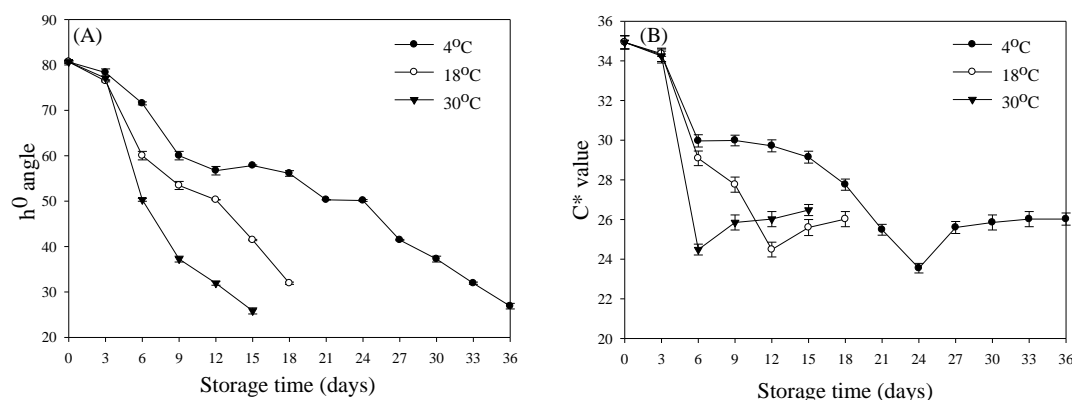
Figure 24 Hue (h°) angle (A) and Chroma (C*) value (B) of longkong during storage
under passive MAP at different temperatures

Note: Data are mean $\pm$ standard deviation (in ten replicates).

Hue (h°) angle = artangent b*/a*; Chroma (C* value) = $(a*^2 + b*^2)^{1/2}$

Fruit firmness

Soft flesh or the loss of fruit firmness of longkong occurred with storage time under passive MAP at 4°C, 18°C and room temperature (~30°C). The interaction between the storage times and storage temperatures was significantly decreased in terms of the firmness of the fruit (p<0.05). Changes in fruit firmness during storage under passive MAP are shown in Figure 25. The fruit firmness in fresh

longkong was 1,849.30 g. At the end of storage, it decreased to 1,335.53 g, 1,244.24 g and 1,155.95 g for longkong stored at 4°C, 18°C and room temperature (~30°C), respectively. The lowest fruit firmness was found in longkong which was stored under passive MAP at room temperature (~30°C). During storage at high temperature, such as room temperature, fruit has a high rate of respiration. Then high concentrations of CO_2 accumulated inside packages. Fruit stress is induced by high CO_2 and is called carbon dioxide injury. This carbon dioxide injury can damage the cell wall structure resulting in fruit softening (Ke and Kader, 1991). In addition, fruit softening is associated with cell integrity loss by carbon dioxide injury. This finding suggests that the pectolytic enzyme involved in the degradation of pectin structure was presented and degradation occurred (Rugkong *et al*., 2010).
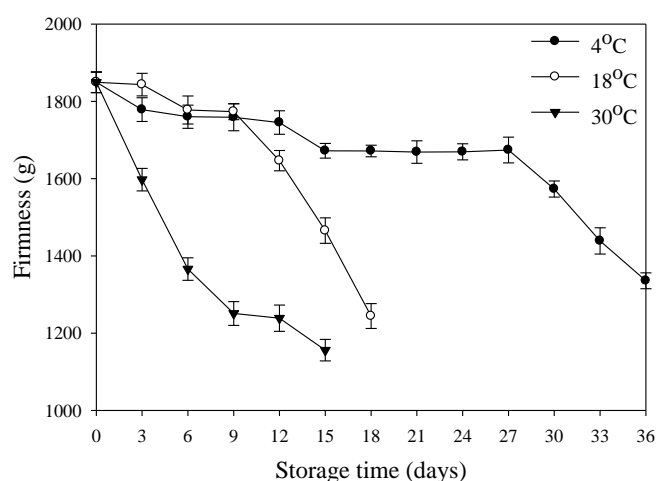
Figure 25 Fruit firmness of longkong during storage under passive MAP at different
temperatures

Note: Data are mean $\pm$ standard deviation (in ten replicates)

Weight loss

Weight loss in longkong took place with storage time under passive MAP at 4°C, 18°C and room temperature (~30°C). The interaction between storage times and storage temperatures significantly increased weight loss ($p < 0.05$). The percentage of weight loss in longkong is shown in Figure 26. At the end of storage the weight loss increased to 2.48%, 2.90% and 4.95% for the longkong stored at 4°C, 18°C and room temperature (~30°C), respectively. The rate of biological reaction

depends upon the temperature. A higher temperature accelerates a higher rate of fruit transpiration reaction. In this study, the highest rate of transpiration found in the longkong stored under passive MAP at room temperature (~30°C). This was indicated by the highest weight loss.
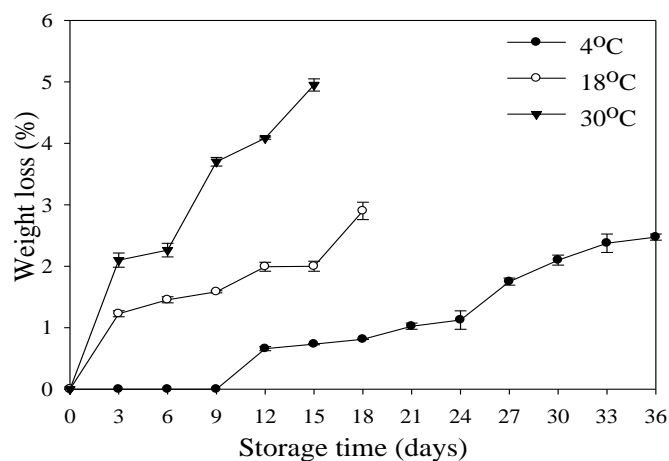
Figure 26 Weight loss of longkong during storage under passive MAP at different
       temperatures

Note: Data are mean $\pm$ standard deviation (in four replicates)

6.4.2 Chemical quality

        Changes in chemical quality during storage under passive MAP at 4°C, 18°C and room temperature (~30°C) were monitored. The interaction between storage times and storage temperatures had a significant effect on changes in quality (p<0.05). The results are shown in Table 15.

Total soluble solid (TSS), sugars, pH and TA

        Normally, sugars are used as a substrate for fruit respiration to produce fruit energy. In this study, the reduction of the TSS and total sugars in longkong during storage under passive MAP was observed (Table 15). The interaction between storage times and storage temperatures significantly decreased the TSS and total sugars (p<0.05). The decrease in TSS and total sugars in longkong throughout the storage times was significantly retarded by low temperature at 4°C (p<0.05). In contrast, longkong stored under passive MAP at room temperature (~30°C) had the lowest content of TSS and total sugars. The lowest rate of reduction in TSS and total

sugars could be explained by the slow rate of sugar consumption in fruit respiration under low temperatures (Kader, 2002).

Table 15 pH, titratable acidity (TA), total soluble solid (TSS), reducing sugar and total sugar of longkong during storage under passive MAP at different temperatures

Storage time (days)	Storage temp (ºC)	pH	TA (% as citric acid)	TSS (%)	Reducing sugar (%)	Total sugar (%)
0	4	3.98 ± 0.01^{bcde}	0.59 ± 0.00^{k}	17.7 ± 0.1^{a}	6.40 ± 0.01^{m}	15.33 ± 0.00^{a}
	18	3.98 ± 0.02^{bcde}	0.59 ± 0.00^{k}	17.7 ± 0.1^{a}	6.40 ± 0.02^{m}	15.33 ± 0.01^{a}
	30	3.98 ± 0.02^{bcde}	0.59 ± 0.00^{k}	17.7 ± 0.1^{a}	6.40 ± 0.01^{m}	15.33 ± 0.05^{a}
3	4	3.85 ± 0.00^{hi}	0.66 ± 0.00^{g}	16.5 ± 0.1^{b}	6.58 ± 0.00^{l}	15.06 ± 0.00^{c}
	18	4.00 ± 0.01^{bcd}	0.67 ± 0.01^{g}	16.6 ± 0.0^{b}	6.40 ± 0.07^{m}	15.33 ± 0.02^{a}
	30	3.98 ± 0.01^{bcde}	0.61 ± 0.01^{j}	16.6 ± 0.0^{b}	6.68 ± 0.20^{k}	15.19 ± 0.02^{b}
6	4	3.85 ± 0.00^{hi}	0.71 ± 0.00^{e}	16.2 ± 0.1^{d}	6.71 ± 0.00^{jk}	14.31 ± 0.02^{e}
	18	4.09 ± 0.01^{a}	0.63 ± 0.01^{hi}	16.1 ± 0.1^{d}	6.34 ± 0.07^{m}	14.57 ± 0.04^{d}
	30	4.05 ± 0.01^{ab}	0.83 ± 0.01^{a}	15.7 ± 0.2^{f}	5.03 ± 0.20^{o}	15.38 ± 0.02^{a}
9	4	3.92 ± 0.00^{efg}	0.70 ± 0.01^{e}	15.9 ± 0.1^{e}	6.33 ± 0.00^{m}	13.67 ± 0.00^{f}
	18	4.03 ± 0.01^{abc}	0.51 ± 0.01^{l}	15.7 ± 0.1^{f}	7.23 ± 0.20^{i}	13.62 ± 0.00^{f}
	30	4.08 ± 0.01^{a}	0.67 ± 0.01^{g}	14.7 ± 0.1^{k}	4.10 ± 0.10^{q}	12.20 ± 0.02^{g}
12	4	3.84 ± 0.00^{hij}	0.73 ± 0.01^{cd}	16.6 ± 0.1^{b}	5.40 ± 0.00^{n}	10.07 ± 0.00^{j}
	18	3.98 ± 0.01^{bcde}	0.62 ± 0.01^{ij}	15.5 ± 0.1^{g}	7.62 ± 0.21^{g}	7.41 ± 0.00^{o}
	30	3.90 ± 0.01^{fgh}	0.67 ± 0.01^{g}	14.1 ± 0.1^{mn}	7.51 ± 0.10^{h}	7.91 ± 0.02^{n}
15	4	3.78 ± 0.00^{jk}	0.64 ± 0.01^{h}	16.3 ± 0.1^{c}	4.86 ± 0.00^{p}	10.99 ± 0.00^{h}
	18	3.99 ± 0.01^{bcd}	0.75 ± 0.01^{c}	14.7 ± 0.1^{k}	7.60 ± 0.29^{gh}	7.87 ± 0.00^{n}
	30	3.83 ± 0.01^{ij}	0.79 ± 0.01^{b}	14.0 ± 0.1^{n}	7.21 ± 0.10^{i}	6.95 ± 0.02^{p}
18	4	3.80 ± 0.01^{ij}	0.64 ± 0.00^{h}	15.0 ± 0.1^{j}	9.20 ± 0.00^{b}	10.67 ± 0.00^{i}
	18	3.99 ± 0.02^{bcde}	0.83 ± 0.00^{a}	14.2 ± 0.1^{m}	6.80 ± 0.02^{j}	6.80 ± 0.01^{q}
	30	-	-	-	-	-
21	4	3.80 ± 0.00^{ij}	0.64 ± 0.00^{h}	15.3 ± 0.1^{hi}	8.82 ± 0.00^{e}	8.48 ± 0.00^{kl}
	18	-	-	-	-	-
	30	-	-	-	-	-
24	4	3.86 ± 0.00^{ghi}	0.67 ± 0.00^{g}	15.2 ± 0.1^{i}	10.06 ± 0.14^{a}	8.46 ± 0.02^{l}
	18	-	-	-	-	-
	30	-	-	-	-	-
27	4	3.94 ± 0.00^{def}	0.67 ± 0.01^{g}	15.4 ± 0.1^{gh}	8.86 ± 0.00^{de}	8.52 ± 0.00^{kl}
	18	-	-	-	-	-
	30	-	-	-	-	-
30	4	3.94 ± 0.00^{def}	0.67 ± 0.01^{g}	15.0 ± 0.1^{k}	9.00 ± 0.00^{cd}	8.61 ± 0.00^{k}
	18	-	-	-	-	-
	30	-	-	-	-	-
33	4	3.94 ± 0.00^{def}	0.68 ± 0.01^{f}	14.4 ± 0.1^{l}	8.98 ± 0.00^{c}	8.27 ± 0.00^{m}
	18	-	-	-	-	-
	30	-	-	-	-	-

Table 13 continue

Storage time (days)	Storage temp (°C)	pH	TA (% as citric acid)	TSS (%)	Reducing sugar (%)	Total sugar (%)
	4	3.72 ± 0.00^k	0.73 ± 0.00^d	14.2 ± 0.1^m	8.06 ± 0.00^f	8.56 ± 0.00^{kl}
36	18	-	-	-	-	-
	30	-	-	-	-	-

Note: - = not determine (due to fruit spoilage)

Data are mean $\pm$ standard deviation (in four replicates).

Mean values in the same column with different superscript letters indicate that there are significant different among storage temperatures (p<0.05).

Acids are also used as a substrate for fruit respiration. A slight decrease in TA in longkong stored under passive MAP in all storage temperatures was found. However, an extreme increase in acidity was found in longkong stored under passive MAP at room temperature (~30°C). The high concentration of CO_2 accumulated inside a package was generated by the high rate of respiration in longkong stored at room temperature (~30°C). High concentrations of CO_2, fruit metabolism might turn into anaerobic metabolism. Fermentation and/or the shutdown of Krebs cycle are major pathways to produce acids under anaerobic conditions (Davies, 1980). Therefore, a high acid content that indicated by high TA, was noticed in longkong stored under passive MAP at room temperature (~30°C).

Types and concentration of sugars

Changes in the sugars in longkong were monitored. The dominant sugars in longkong were sucrose, glucose and fructose. The interaction between storage times and storage temperatures significantly affected the concentration of sugars (p<0.05). Changes in sucrose, fructose and glucose concentrations are shown in Figure 27. Decreases in fructose, glucose and sucrose concentrations in the longkong were observed over 3 days under passive MAP storage. The decrease in sugar concentration was explained by sugar consumption from fruit respiration.
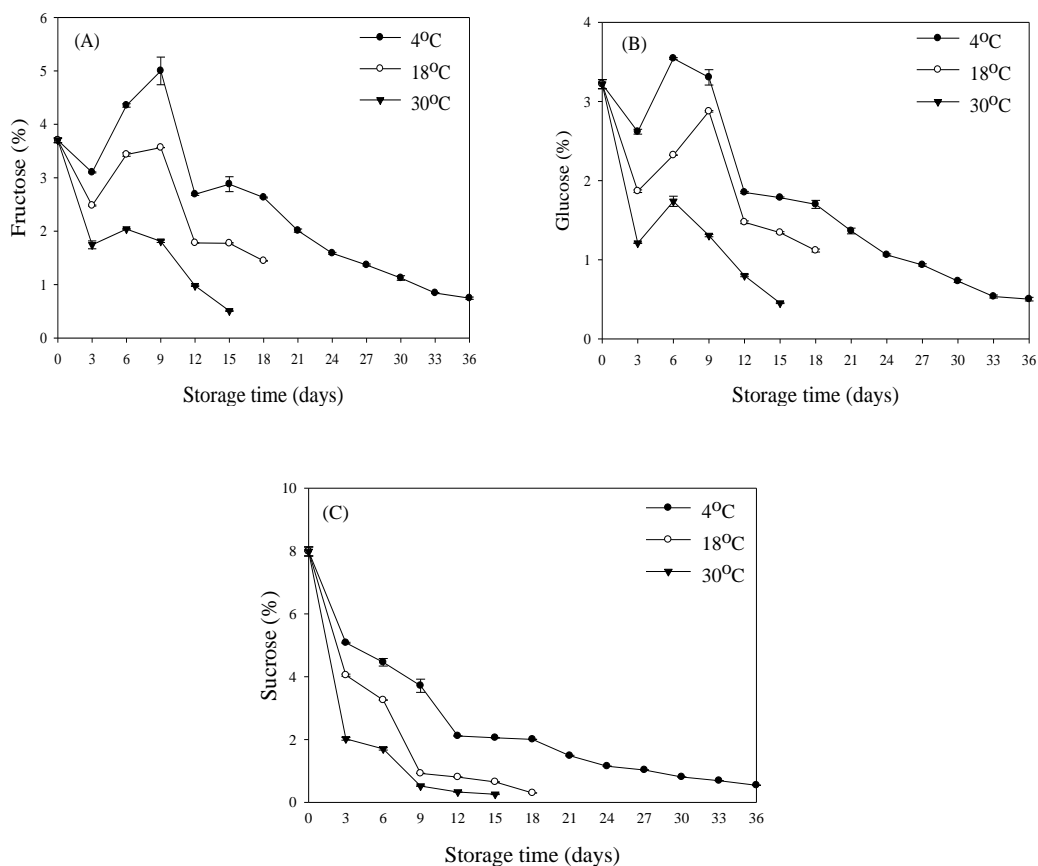
Figure 27 Types and concentrations of sugars content in longkong during storage
under passive MAP at different temperatures

Note: Data are mean $\pm$ standard deviation (in four replicates).

An increase in fructose and glucose and a decrease in sucrose concentrations were observed. The concentrations of fructose were 5.05% (9 days at 4°C), 3.60% (9 days at 18°C) and 2.14% (6 days at 30°C) (Figure 27A). The glucose concentrations were 3.50% (9 days at 4°C), 2.83% (6 days at 18°C) and 1.78% (6 days at 30°C) (Figure 27B). The concentrations of sucrose were 3.71% (9 days at 4°C), 1.15% (9 days at 18°C) and 0.56% (6 days at 30°C). These changes were explained by conversion reaction. After that a reduction was found in the fructose, glucose and sucrose concentrations until the end of storage. This could be explained by the consumption of sugars as a substrate for the fruit respiration process (Ong *et al.*, 2006).

Volatile flavor compounds

Volatile flavor compounds make up an extremely important quality in fruit. Changes in the fruit volatile flavor profiles depend on postharvest conditions. Volatile flavor compounds in longkong stored at different temperatures at 4°C, 18°C and room temperature (~30°C) under passive MAP were identified (Tables 16, 17 and 18). The volatile flavor compounds identified included esters, alcohols, terpenes and their derivatives, acids, ketones and phenols. The 3-hydroxy-2-butanone and delta-germacrene were identified in abundance. They present sweet and herbaceous characteristics, respectively. Their content declined with storage time. At the end of storage, acids and fermented compounds were detected in the volatile flavor profiles. These were acetic acid, butanoic acid, hexanoic acid and iso-amylalcohol. They show sour, sweat and fermented characteristics. The changes from fruity and sweet profiles to fermented and sour profiles are probably due to anaerobic or fermentation pathways (Defilippi *et al*., 2005). Longkong stored under passive MAP at 4°C presented the lowest content of fruity ester and alcohol. This was probably due to flavor precursor synthesis and/or the activity of related enzymes, for flavor synthesis is inhibited or retarded by low temperature (Kays, 1991). In addition, acid and fermented alcohol was produced in the lowest concentration in longkong stored under passive MAP at 4°C. Since the fruit respiration rate can be retarded, insufficient of O_2 surrounding the fruit cell is a causing of aerobic metabolism (Kays, 1991).

Table 16 Volatile flavor compounds and their attributes identified in longkong during storage under passive MAP at 4°C

Compounds	Rt[A]	RI[B]	Attributes[C]	Peak areas (×10⁴) TIC[D]												
				Storage time (days)												
				0	3	6	9	12	15	18	21	24	27	30	33	36
Esters																
ethyl butyrate	3.85	1049	fruity	1.50	3.17	3.29	3.94	3.98	4.08	6.71	5.02	4.24	1.80	1.26	1.25	-
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	0.12	1.02	1.25	1.50	2.10	4.89	9.25	13.8	13.3	10.41	5.01	2.31	1.88
Alcohols																
2-methyl propanol	4.96	1107	fruity, sweet	-	-	-	-	-	5.91	12.95	11.20	10.86	8.33	8.05	-	-
n-butanol	6.19	1157	fruity	1.25	1.80	2.00	2.20	2.50	3.40	7.00	6.32	5.86	5.01	4.17	3.66	-
Iso-amylalcohol	7.72	1218	fermented	0.80	5.12	5.80	6.82	14.80	17.47	28.00	29.80	45.96	47.41	48.24	51.95	53.47
n-hexanol	11.05	1347	green	1.25	2.21	2.30	2.58	3.11	4.25	19.06	26.95	9.48	9.06	1.82	-	-
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	1.58	1.72	1.81	1.85	2.22	4.03	4.27	4.44	2.00	1.65	1.44	-
benzyl alcohol	23.77	1895	floral, sweet	-	3.04	5.84	6.01	7.47	12.60	16.30	40.50	41.21	31.46	23.75	13.46	8.96
Terpenes and their derivatives																
cis-linalool oxide	14.39	1479	floral, sweet	-	-	-	-	-	1.52	2.19	5.66	7.00	10.15	5.55	1.09	0.88
laevo-linalool	16.35	1559	fruity, sweet	-	1.28	1.70	4.92	5.01	5.60	6.93	42.77	19.00	13.58	12.34	10.81	5.02
delta-germacrene	19.75	1706	herbaceous	0.82	5.84	9.85	10.24	11.08	13.66	16.52	6.80	4.28	4.21	3.96	3.27	-
epoxylinalool	21.21	1707	flower	-	-	-	-	1.46	1.65	6.32	8.01	6.03	5.11	3.42	-	-
spathuleol	28.37	2184	fruity, herbaceous	-	-	-	-	1.73	1.74	3.94	9.31	8.77	7.03	4.83	3.11	1.65

Table 16 continued

Compounds	Rt[A]	RI[B]	Attributes[C]	Peak areas (×10^4) TIC[D]												
				Storage time (days)												
				0	3	6	9	12	15	18	21	24	27	30	33	36
Terpenes and their derivatives																
alpha-cadinol	29.52	2199	herbaceous	-	-	-	-	-	-	-	4.91	4.12	3.85	2.72	-	-
alpha-cubebene	29.75	2208	herbaceous	-	-	-	-	-	-	-	2.48	2.32	2.00	1.67	-	-
epi-alpha-muurolol	30.34	2241	herbaceous	-	-	-	-	-	-	-	5.78	5.10	4.56	1.20	0.76	-
Acids																
acetic acid	14.21	1472	pungent, sour	0.50	1.36	1.52	1.57	1.74	1.86	2.67	4.56	5.63	8.93	14.38	15.58	28.52
butanoic acid	18.32	1643	sour	-	1.67	1.80	2.18	2.19	2.20	3.08	3.90	4.18	5.02	7.11	10.04	14.20
hexanoic acid	23.07	1862	sour, sweat	-	-	-	1.61	1.67	1.83	3.04	3.17	5.08	5.81	6.10	6.68	10.57
Ketones																
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	19.30	35.59	41.18	48.36	58.61	48.51	32.71	22.43	22.43	19.06	10.41	5.23
Phenol																
phenol	26.38	2027	phenol	2.89	3.88	4.99	28.97	34.25	48.88	55.86	64.91	66.82	17.50	5.78	5.12	2.01

Note: [A] Rt = Retention time (min);   [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = total ion current; - = not detected

Table 17 Volatile flavor compounds and their attributes identified in longkong during storage under passive MAP at 18°C

Compounds	Rt[A]	RI[B]	Attributes[C]	Peak areas ($\times 10^4$) TIC[D]						
				Storage time (days)						
				0	3	6	9	12	15	18
Esters										
ethyl butyrate	3.85	1049	fruity	-	11.78	4.26	2.28	1.78	-	-
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	-	13.94	24.20	34.60	36.17	41.81	-
Alcohols										
2-methyl propanol	4.96	1107	fruity, sweet	-	5.20	9.37	12.63	9.37	8.05	-
n-butanol	6.19	1157	fruity	1.25	1.33	3.05	3.40	5.96	-	-
iso-amylalcohol	7.72	1218	fermented	2.80	2.98	11.79	16.34	20.06	31.11	51.52
n-hexanol	11.05	1347	green	1.25	7.18	10.40	14.52	6.20	-	-
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	1.63	5.99	7.99	4.57	3.38	-
benzyl alcohol	23.77	1895	floral, sweet	-	25.2	44.03	57.84	41.56	38.75	-
Terpenes and their derivatives										
cis-linalool oxide	14.39	1479	floral, sweet	-	5.89	8.59	31.40	39.23	19.29	6.41
laevo-linalool	16.35	1559	fruity, sweet	-	6.83	10.27	13.71	34.15	20.16	12.81
delta-germacrene	19.75	1706	herbaceous	0.82	3.38	3.73	3.99	7.25	4.51	3.22
epoxylinalool	21.21	1707	flower	-	-	12.40	17.00	6.38	4.55	3.75
spathuleol	28.37	2184	fruity, herbaceous	-	-	-	2.55	6.35	19.26	3.02

Table 17 continued

Compounds	Rt[A]	RI[B]	Attributes[C]	Peak areas ($\times 10^4$) TIC[D]						
				Storage time (days)						
				0	3	6	9	12	15	18
Terpenes and their derivatives										
epi-alpha-muurolol	30.34	2241	herbaceous	-	-	-	-	5.31	4.01	2.27
Acids										
acetic acid	14.21	1472	pungent, sour	0.50	7.43	18.35	23.41	45.07	51.94	64.43
butanoic acid	18.32	1643	sour	-	4.67	5.37	5.45	5.51	12.31	45.70
hexanoic acid	23.07	1862	sour, sweat	-	-	25.30	38.75	41.56	44.02	57.84
Ketones										
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	23.83	87.97	92.78	44.36	43.68	8.34
Phenol										
phenol	26.38	2027	phenol	2.89	3.51	11.38	78.05	52.06	46.86	12.14

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = total ion current; - = not detected

Table 18 Volatile flavor compounds and their attributes identified in longkong during storage under passive MAP at room temperature (~30°C)

Compounds	Rt^A	RI^B	Attributes[C]	Peak areas ($\times 10^4$) TIC^D					
				Storage time (days)					
				0	3	6	9	12	15
Esters									
ethyl butyrate	3.85	1049	fruity	-	7.07	8.41	6.02	4.83	-
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	-	11.14	28.20	18.18	13.21	9.55
Alcohols									
2-methyl propanol	4.96	1107	fruity, sweet	-	18.14	22.11	29.15	7.10	5.64
n-butanol	6.19	1157	fruity	1.25	5.64	7.10	29.15	10.33	-
iso-amylalcohol	7.72	1218	fermented	2.80	3.43	9.07	16.63	18.19	31.58
n-hexanol	11.05	1347	green	1.25	3.11	9.43	6.44	3.06	2.74
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	2.48	4.20	3.88	1.92	-
benzyl alcohol	23.77	1895	floral, sweet	-	6.16	25.13	37.61	20.38	12.51
Terpenes and their derivatives									
cis-linalool oxide	14.39	1479	floral, sweet	-	1.14	7.87	34.6	14.72	12.64
laevo-linalool	16.35	1559	fruity, sweet	-	1.22	6.63	16.02	11.20	-
delta-germacrene	19.75	1706	herbaceous	0.82	2.32	8.97	5.28	4.40	-
epoxylinalool	21.21	1707	flower	-	1.92	5.53	1.78	-	
spathuleol	28.37	2184	fruity, herbaceous	-	-	4.00	1.46	-	

Table 18 continued

Compounds	Rt[A]	RI[B]	Attributes[C]	Peak areas ($\times 10^4$) TIC[D]					
				Storage time (days)					
				0	3	6	9	12	15
Acids									
acetic acid	14.21	1472	pungent, sour	0.50	5.31	7.66	16.93	19.30	56.13
butanoic acid	18.32	1643	sour	-	9.44	17.22	22.80	58.62	37.80
hexanoic acid	23.07	1862	sour, sweat	-	-	13.83	14.46	33.65	48.83
Ketones									
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	17.61	30.04	45.68	17.58	-
Phenol									
phenol	26.38	2027	phenol	2.89	9.87	24.80	2..22	1.27	-

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = total ion current; - = not detected

Headspace gases composition

Changes in the headspace oxygen (O_2) and carbon dioxide (CO_2) are associated with fruit respiration. In this study, the headspace O_2 and CO_2 inside the package were monitored. The interaction between storage times and storage temperatures significantly affected the increase in headspace O_2 while the headspace CO_2 decreased ($p<0.05$). The concentration of headspace O_2 decreased from 21.35%. to 2.04%, 1.74% and 1.08% for longkong stored under passive MAP at 4°C, 18°C and room temperature (~30°C), respectively (Figure 28A). In contrast, the concentration of headspace CO_2 sharply increased from 0.26% to 59.53, 69.18 and 71.02% for longkong stored under passive MAP at 4°C, 18°C and room temperature (~30°C), respectively (Figure 28B). These results were explained by the consumption of O_2 for fruit respiration. The highest headspace CO_2 and the lowest headspace O_2 was found in a package stored at room temperature (~30°C). This could be explained by the acceleration of the respiration rate with high temperatures (Cameron *et al*., 1995).
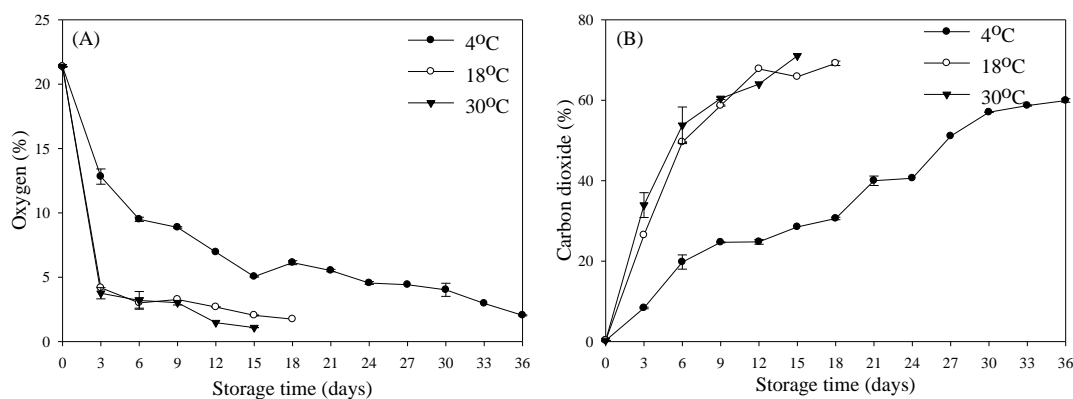
Figure 28 Headspace oxygen (A) and carbon dioxide (B) inside package of longkong during storage under passive MAP at different temperatures

Note: Data are mean $\pm$ standard deviation (in four replicates).

Ethanol concentration

Ethanol was presented in the longkong with storage time under passive MAP at 4°C, 18°C and room temperature (~30°C). The interaction between storage times and storage temperatures significantly increased the concentration of ethanol

(p<0.05). The ethanol concentration in longkong stored under passive MAP at different temperatures is shown in Figure 29. The ethanol content in fresh longkong was 0.06 g/g fruit weight (g/g FW). A gradual increase in ethanol was found over time during storage (p<0.05). At the end of storage, the ethanol concentration increased to 0.16 g/g FW, 0.22 g/g FW and 0.28 g/g FW for longkong stored under passive MAP at 4°C, 18°C and room temperature (~30°C), respectively. The highest ethanol content was detected in longkong stored at room temperature (p<0.05). This is in agreement with the lowest O_2 and the highest CO_2 in the headspace of a package stored at room temperature (Figure 28). The high ethanol accumulation was induced by high CO_2 production under an anaerobic pathway. Pyruvate was decarboxylated to produce acetaldehyde. The acetaldehyde was subsequently reduced to ethanol (Ke and Kader, 1991; Ke *et al*., 1991; Mathooko, 1996; Ke *et al*., 1994).
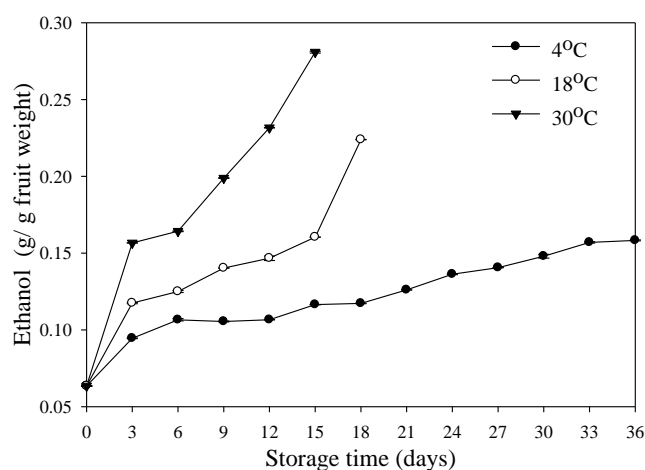
Figure 29 Ethanol content of longkong during storage under passive MAP at different
temperatures

Note: Data are mean $\pm$ standard deviation (in four replicates)

Alcohol dehydrogenase (ADH) enzyme activity

The activity of the ADH enzyme in longkong stored under passive MAP at different temperatures was monitored. The activity of the ADH enzyme directly related to the ethanol concentration. Ethanol was produced by the reduction of acetaldehyde by the alcohol dehydrogenase (ADH) enzyme (Cossins, 1978; Ke *et*

al., 1994). The interaction between storage times and storage temperatures significantly increased the activity of the ADH enzyme (p<0.05). The activity of the ADH enzyme in longkong is shown in Figure 30. The storage temperature significantly affected the ADH activity (p<0.05). The ADH activity in fresh longkong was 0.54 U/ mg protein. At the end of storage, the ADH activity increased to 11.35 U/ mg protein, 11.29 U/ mg protein and 11.81 U/ mg protein for longkong stored at 4°C, 18°C and room temperature (~30°C), respectively. The highest ADH enzyme activity was found in longkong stored under passive MAP at room temperature (~30°C). This was in agreement with the highest headspace CO_2 and the lowest O_2 concentrations (Figure 28) as well as the ethanol content (Figure 29). Fruit has a high respiration rate at room temperature (~30°C) compared with 4°C and 18°C. High rates of fruit respiration give the high accumulation of CO_2 inside a package which can induce the activity of the ADH enzyme (Kader, 2002).
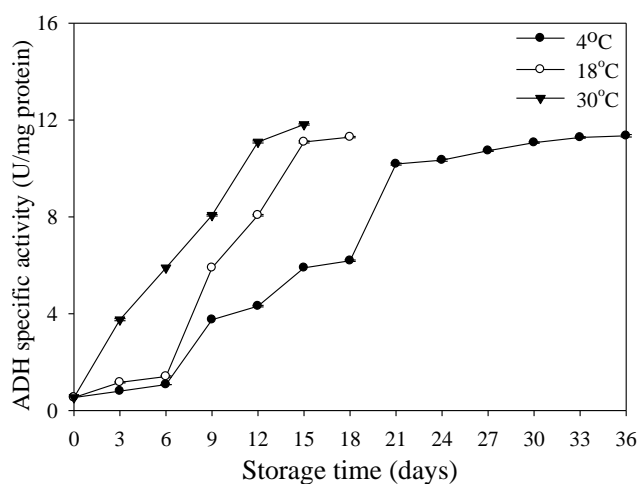
Figure 30 ADH specific activity of longkong during storage under passive MAP at different storage temperature

Note: Data are mean $\pm$ standard deviation (in four replicates)

Microbiological determination

Total viable count in longkong at the initial and the end of storage is presented in Table 19. Total viable count increased with storage time in all treatments. However, longkong stored under passive MAP at 4°C presented the lowest of total viable count.

Table 19 Total viable count in longkong at the initial and the end of storage under passive MAP at different storage temperature

Storage time (days)	Total viable count (CFU/g)
0	1.50×10^3
The end of storage	
36 (4ºC)	4.31×10^5
18 (18ºC)	7.53×10^5
15 (room temperature (~30ºC)	9.88×10^6

Note: Data are mean $\pm$ standard deviation (in four replicates).

6.5 Conclusions

Longkong has a short shelf-life of 4-7 days at room temperature. Effective postharvest technology such as modified atmosphere packaging needs to be applied to prolong its shelf-life. Passive MAP can be used as an alternative way to extend the shelf-life of longkong. Storage temperature is one important factor which enhances the effectiveness of passive MAP. The quality of longkong stored under passive MAP at lower temperature showed a better quality than that stored under higher temperatures such as room temperature (~30°C). This was indicated by low weight loss, TA and ethanol content as well as retaining a good fruit flavor. However, a high concentration of CO_2 during passive MAP storage can accelerate fruit stress due to CO_2 injury. The defective attributes were the rapid loss of bright yellow color, firmness, and characteristic aromas. The longkong also featured high acidity, fermented and sour volatile compounds, and ethanol content.

CHAPTER 7

CHANGES IN FRUIT QUALITY, VOLATILE FLAVOR COMPOUNDS AND POSSIBLY OFF-FLAVOR ACCUMALATION IN LONGKONG DURING STORAGE UNDER ACTIVE MODIFIED ATMOSPHERE PACKAGING

7.1 Abstract

Active modified atmosphere packaging (Active MAP) is an alternative tool for prolonging the shelf-life of fruit. To enhance the effectiveness of active MAP, an appropriate ratio of gases and an optimal temperature is required. Changes in longkong quality during storage under active MAP ($5\%CO_2$:$5\%O_2$, $5\%CO_2$:$10\%O_2$ and $10\%CO_2$:$5\%O_2$) at 18ºC and room temperature (~30ºC) were monitored. Throughout the storage time, the longkong lost its bright yellowness, fruit firmness of the fruit and fruit flavor. It also increased in acidity and perceived ethanolic-flavor. Longkong stored under active MAP ($5\%CO_2$:$5\%O_2$) at 18°C maintained fruit quality over the 24 days. At the end of storage, the longkong had lost its bright yellowness which was indicated by decreases in the L* and b* values. The total acidity increased from 0.59% to 0.72%. The increase in perceived ethanolic-flavor and a decrease in flavor were also in agreement with the high levels of ethanol content at 0.21 g/g FW at the end of storage. The activity of the ADH enzyme increased from 0.54 to 13.52 U/mg protein. The microbial population was 7.53×10^5 CFU/g.

7.2 Introduction

Fruit quality rapidly deteriorates after harvesting. Fruit needs to handle with appropriate post-harvest tools to extend its shelf-life. Active modified atmosphere packaging (active MAP) has been generally used for this. Active MAP is a technique that modifies the atmosphere inside the packaging by using adequate gases and maintains gas composition using fruit respiration and permeable packaging (Kader *et al*., 1989). Active MAP is an effective tool for extending fruit shelf-life. However, its efficiency needs to be enhanced by using optimal temperature. Storage under optimal low temperature can reduce the biological reactions. Biological

reactions normally increase 2 or 3 times with every 10°C increase in temperature (Mitchell, 1992). Therefore, providing an adequately low level of O_2 and a slightly higher level of CO_2 under low temperatures can reduce the biological reaction. Lichanporn (1999) studied the effect of modified atmosphere during storage on longkong shelf-life. It was found that longkong kept in a PVC box at 20°C had a longer shelf-life than kept under atmospheric conditions. Longkong shelf-life was 12 and 8 days, respectively. This is similar to the study undertaken by Meenune and Janthachum (2004) who reported that a longkong raceme which was kept under modified atmosphere packaging (MAP) has a longer shelf-life than one kept under atmospheric condition. Under active MAP conditions (5%CO_2:5%O_2), longkong raceme treated with 1.5% citric acid for 5 min prior to storage under MAP at 18°C had a shelf-life of 30 days. One of the major problems encountered in using active MAP is the accumulation of anaerobic metabolites in the packages. An anaerobic condition was built up and increased the production of off-flavor volatiles such as ethanol. An ethanolic-flavor adversely influences consumer acceptability with fresh fruit products (Shi, 2005). Studies of the effect of modified atmosphere condition on longkong shelf-life have been undertaken by many researchers. However, there have been no investigations or sensory test into quality in relation to how the flavors of fresh longkong are affected by active MAP. Therefore the objectives of this study were to study the effect of modified atmosphere conditions on the quality changes and possibly off-flavor accumulation in longkong during storage. Any correlation between instrumental analysis and sensory evaluation of longkong flavor was also investigated.

7.3 Materials and Methods
Chemicals

The pyruvate decarboxylase and alcohol dehydrogenase enzymes were obtained from Fluka, Sigma Chemical Co. (St. Louis, MO), Aldrich Chemicals Co. (Milwaukee, WI) and Merck Co. (Darmstadt, Germany). D-glucose, D-fructose, sucrose were purchased from Fluka (Messerchmittstr, Switzerland). Acetonitrile and water (HPLC grade) were obtained from Prolabo (Paris, France). Sodium hydroxide, copper sulfate pentahydrate, trichloroacetic acid and sodium sulfite were obtained

from Merck (Darmstadt, Germany). Potassium sodium tartrate, lead acetate and potassium oxalate were obtained from Riedel-deHaen (Seelze, Germany). Dichloromethane was obtained from LabScan Asia Co., Ltd (Bangkok, Thailand). Plate count agar (PCA) for total viable counts was obtained from Merck Co. (Darmstadt, Germany).

Plant material and fruit preparation

Longkong was obtained from a contact garden in Natawee district (Songkhla province, Thailand). The maturity stage of 13 weeks after anthesis was chosen. The fruit was harvested in the morning and immediately transported on the same day to the Food Analysis laboratory, Faculty of Agro-Industry (approximately 2 hours). Fruits free from any apparent skin damage and uniform size (grade A about 3-3.5 cm in diameter, according to Tanyongmut market) were selected for analysis. The fruit was prepared in an individual by cut of raceme. Individual fruit was cleaned with a brush and submerged in a mixed solution of 500 ppm benomyl and 1.5% citric acid for 5 min and dried at room temperature (~30°C) for 15 min. This was done to reduce the microbial load and anti-browning.

Active modified atmosphere packaging and storage conditions

The 12 individual fruits were placed in a plastic tray. The tray was covered with a bag of 0.08 mm thickness nylon laminated with linear Low Density Polyethylene (nylon/LLDPE). It was 7x11 inch in size and permeability to CO_2 and O_2 at the rate of 4.7 and 2.6 cm^2/m^3/day at 25°C and 0% RH, respectively. The atmosphere composition inside the longkong packages was modified by filling them with 3 different ratios, namely $5\%CO_2{:}5\%O_2$, $5\%CO_2{:}10\%O_2$ and $10\%CO_2{:}5\%O_2$ (balanced with N_2). After packing, the sample bags were stored at 18°C and room temperature (~30°C).

Evaluation of Fruit quality

The physical qualities such as fruit color, fruit firmness and weight loss, were measured in 10 fruits at 6-day intervals. The chemical qualities were also evaluated which included total soluble solid (TSS), sugars (reducing sugars, total

sugars and type and concentration of sugars), titratable acidity (TA), pH, alcohol dehydrogenase (ADH) activity and volatile flavor compounds. The homogenate prepared by flesh (de-seeded) blending was used for chemical quality analysis. This was prepared fresh before the analysis. The methods of physical and chemical analysis used are explained.

Fruit color

Two opposite sides of longkong fruit skin were quantified in terms of CIE lightness (L*), redness (a*) and yellowness (b*) values using a Color Flex, Hunter Lab colorimeter. CIE values were calculated in terms of Hue (h°) angle = artangent b*/a* and Chroma (C* value) = $(a^{*2}+b^{*2})^{1/2}$ (Apai, 2010).

Fruit firmness

Two opposite sides of the fruit were measured by using a TA-XT2i Texture Analyzer (Stable Micro System, UK) equipped with a 2 mm diameter cylinder probe (P/2). The penetrometric method was applied. The results were expressed as gram force (Adapted from Sapii *et al*., 2000).

Fruit weight

The fruit weight was evaluated by weighing with a Sartorius BP310S analytical balance.

Total soluble solid (TSS)

The TSS was determined using an Atago 1E (Japan) hand refractometer at 25ºC and expressed as a percentage (Ong *et al*., 2006).

Total and reducing sugars

The 25 g of the longkong homogenate and 100 ml of distillated water were mixed and clarified by 45% neutral lead acetate (2 ml) and 22% potassium oxalate (2 ml). The sample volume was adjusted to 250 ml with distillated water and filtered through filter paper No 1. The total sugar and reducing sugar content was

quantified by titration with Fehling's reagents according to Lane and Eynon (A.O.A.C., 2000).

Titratable acidity

The titratable acidity (TA) was quantified by titrating 10 ml of the homogenate to an end point of pH 8.2 with 0.1 N NaOH with 1% (v/v) phenolphthalein as an indicator. The result was calculated as a percentage of citric acid (Ong *et al*., 2006).

pH

pH was measured using a Sartorius PB-20 (Germany) digital pH meter (Adapted from Ong *et al*., 2006).

Types and concentrations of sugars

Types and concentrations of sugars (sucrose, fructose and glucose) were determined by HPLC (Shimadzu, CR 6A Chromatopac) with a Hypersil APS-2 column. For sample preparation, the homogenate was clarified by using refrigerated centrifugation (4ºC) at 10,000×g for 30 min. Clear solution of longkong juice was obtained. The clear solution was filtered through 0.45 µm pore size membrane filters and was kept at -20°C until analysis. The injection volume was 10 µl with an isocratic flow rate of 1 ml/min and a refractive index detector. The 90% acetonitrile was used as a mobile phase. Their concentrations were quantified by comparing the retention times and peak areas of the samples with sucrose, fructose and glucose standards (Adapted from Chyau *et al*., 2003; Ong *et al*., 2006; Soares *et al*., 2007).

Volatile flavor compound isolation by direct solvent extraction (DSE) and identification by Gas chromatography–mass spectrometry GC-MS

Volatile flavor compounds were extracted by direct solvent extraction and identified by GC-MS. The 100 ml clear solution of longkong juice was mixed with 100 ml of dichloromethane. Next, the mixture was gentle shaken for 90 min and left equilibrium for 30 min at room temperature (~30°C). The solvent phase was collected and re-extracted twice. The solvent phase was dried on anhydrous Na_2SO_4,

kept overnight at -20°C, cold- filtered and concentrated using purging nitrogen gas. The concentrate was kept at -20°C prior to analysis. The 1 μl was directly injected into GC-MS (Adapted from Flores *et al*., 2002; Elss *et al*., 2005; Ong *et al*., 2006).

The volatile flavor compounds were identified by GC-MS. A chromatograph, Hewlet-Packard 6890 (Palo Alto, CA, USA), was used with a HP-FFAP column (25 m×0.20 mm; film thickness 0.20 mm). The inlet temperature was set at 240°C and the column temperature was maintained at 40°C for 2 min, followed by an increase to 240°C at the rate of 5°C/min and held for 13 min. The carrier gas was applied by ultra high purity helium at a constant flow of 1.5 ml/min. A Hewlet-Packard 5973 A mass spectrometer with a quadrupole mass filter was coupled to the GC. A mass spectrum (MS) was obtained at 70 eV in the electronic impact (EI) and positive modes. The MS was scanned in the range m/z 40-350 at 1 s intervals (Adapted from Wanakhachornkrai and Lertsiri, 2003).

Headspace gas composition analysis

Changes in the headspace gas composition were monitored by gas chromatography (GC) coupling with a thermal conductivity detector (TCD-detector). The 1 ml of headspace gas was directly withdrawn from the headspace inside the package and subsequently injected into Poropak N columns. Helium at flow rate of 50 ml/min was used as the carrier gas. The temperature oven and injection port was held at 60°C. The temperature TCD detector was held at 150°C. The internal package atmosphere was identified and quantified by comparison with external standard gas (Adapted from Tano *et al*., 2007).

Ethanol isolation by headspace-solid phase microextraction (HS-SPME) and identification by GC-FID

The ethanol content in longkong was extracted by headspace-solid phase microextraction (HS-SPME). The extraction was done as follows; the 100 g of the flesh of longkong was mixed with 100 ml of 20% NaCl (cold solution). It was blended at a constant speed using a blender for 3 min. It was then filtered through a stainless steel sieve. This process produced the homogenate. The 20 ml homogenate

was placed into a 125 ml vial followed by the addition of 3 g of NaCl and fitted with a rubber septum. The vial was kept at 30ºC and left equilibrates for 60 min.

Equilibrated ethanol in the headspace was isolated by using 50/30 μm DVB/CAR/PDMS (divinylbenzene/Carboxen on poly (dimethylsiloxane) (Supelco, Bellafonte, PA, USA). The equilibrated ethanol in the headspace was adsorbed for 15 min at room temperature (~ 30°C) and desorbed for 5 min in GC-FID (PerkinElmer, Autosystem XL, USA) (Adapted from Lara *et al*., 2006).

The ethanol was analyzed by GC-FID. The apparatus was equipped with an Rtx-5 (Restek) column (30 m×0.25 mm; film thickness 0.25 μm). The carrier gas was ultra high purity helium at a constant flow of 1.5 ml/min. The injector was kept at 240°C and set for the splitless mode. The column temperature was set at 35°C for 3 min and then programmed to 230°C at the rate of 8°C/min and held for 2 min. The ethanol concentration was calculated using standard aqueous solutions of ethanol under the same conditions as were used for the samples (Adapted from Lara *et al*., 2006).

ADH enzyme extraction and ADH activity evaluation

The 100 mg of longkong flesh was homogenized in 1 ml of extracted solution. The extract solution contained 85 mM 2-(N-mor-pholino) ethane-sulfonic acid (MES) buffer, with a pH 6.0, 5 mM dithiothreitol (DTT) and 1% (w/v) polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged at 25000×g for 15 min at 4°C to recover the supernatant. The supernatant was kept in ice as crude enzyme extract (Adapted from Lara *et al*., 2006).

The ADH activity was assayed by mixing 2.55 ml NADH solution (0.150 mM NADH in 85 mM MES, pH 6.0), 150 μl of acetaldehyde solution (80 mM acetaldehyde in 85 mM MES, with a pH 6.0), and 300 μl enzyme extract. The ADH activity was measured every 1 min by the spectrophotometer technique at 340 nm. The results were expressed as specific activity (U/mg protein) (Lara *et al*., 2006). The total protein in the extract was determined according to the method described by Bradford (1976).

Sensory evaluation

The intensity of the ethanolic-flavor in longkong was rated by category line scale. The scale represented by 20-cm straight line (Appendix B) which was oriented by score and the description (0=imperceptible ethanolic-flavor, 50=moderate ethanolic-flavor and 100=strong ethanolic-flavor) (Adapted from Ke *et al.*, 1991; Lawless and Heymann, 2010). The training session was conducted to provide reliable and valid results. The panelists were trained for 12 hrs or more to get the precision. The training was performed by rating the intensity of the standard solution of ethanol at 0, 1, 1.25, 2.5, 5 and 10 % using a vertical line on the 0 to 100-category line scale. After that, the 12 precise-panelists were selected and the consensus in a group of selected panelist was analyzed by Generalized Procrustes analysis (GPA) as reported in Appendix C (Dijksterhuis, 1996). The intensity of the ethanolic-flavor in longkong during storage under active MAP was rated by 12 trained panelists, by placing a vertical line on the scale. Each panelist sat in an individual booth and was asked to score the intensity of the ethanolic-flavor. Three pieces of longkong in a closed container were served to each panelist in each session. The sensory evaluation was completely performed in 1 month after training session.

The 9-point hedonic scale ranged from "1=dislike extremely" to "9=like extremely" was used to determine the flavor acceptability. The 150 untrained panelists who liked to consume longkong regularly were selected. All samples were coded with a 3-digit random number and presented to the panelists in random order. The panelists were instructed to consume pieces of longkong and rinse their mouth with water at room temperature between evaluating each sample (Deng *et al.*, 2006).

Microbiological determination

The microbial was determined at the beginning and the end of storage. Homogenized longkong flesh (de-seed) 25g was combined with 225 ml of sterile 0.1% peptone water in a sterile polyethylene bag. The sample was pummeled with a stomacher (Seward Stomacher 400, UK) for 2 min using medium speed. The aliquot was used for the appropriate dilution.

The total viable counts were determined by pouring 1 ml of diluted sample onto plate count agar (PCA). The plates were incubated at 35°C for 48 hr, and

the colonies were counted. The microbial counts were expressed in CFU/g of longkong (Koide and Shi, 2007).

Statistical analysis

The experiment was performed using a completely randomized design (CRD) with a factorial treatment structure. A factorial treatment was performed with a structure (2 levels of storage temperatures $\times$ 3 levels of modified atmosphere conditions$\times$3 levels of storage times). Significant differences between the means were estimated by Duncan's new multiple range test (DMRT), with a level of significance of 0.05.The statistical analyses were performed by using the Statistical Package for Social Science (SPSS 11.0 for windows (SPSS Inc., Chicago, IL, USA).

7.4 Results and discussion

The visible mold growth on the longkong skin was used as a guide for reaching the end of storage. It was found that longkong shelf-life was 18 and 24 days under active MAP at 18ºC and room temperature (~30°C), respectively. The interaction between storage temperatures, active MAP conditions and storage times had a significant effect on the longkong quality (p<0.05). The results are discussed below.

7.4.1 Physical quality

During storage under active MAP, the quality loss was significantly different with different storage temperatures, active MAP conditions and storage times (p<0.05). The fruit skin became brownish. The firmness of the fruit declined with increases in the percentage of weight loss.

Fruit Color

Brownish longkong was found throughout storage in all conditions. The interaction between storage temperatures, active MAP conditions and storage times significantly decreased the bright yellow fruit skin (p<0.05). The color was measured in CIE L*, a* and b* values. They represent lightness, redness and yellowness, respectively. A significant decrease in L* and b* values was observed (p<0.05). At the end of storage of the longkong stored under active MAP at 18ºC (24 days), the L*values decreased from 64.42 in fresh longkong to 30.26 (5%CO_2:5%O_2), 30.52

($5\%CO_2$:$10\%O_2$) and 29.51 ($10\%CO_2$:$5\%O_2$) (Figure 31A). The b* values in fresh longkong were 34.47 and decreases to 10.25 ($5\%CO_2$:$5\%O_2$), 10.56 ($5\%CO_2$:$10\%O_2$) and 9.57 ($10\%CO_2$:$5\%O_2$) were observed (Figure 31C). A high storage temperature such as room temperature (~30^oC) seriously affected fruit skin color. Longkong stored under all conditions of active MAP at room temperature (~30^oC) had much darker skin. This was indicated by the lowest L* and b* values at the end of storage ($p<0.05$). At the end of storage under active MAP at room temperature (~30^oC) (18 days), the L* and b* values were 29.84 and 7.33 ($5\%CO_2$:$5\%O_2$), 28.67 and 9.33 ($5\%CO_2$:$10\%O_2$) and 27.37 and 6.33 ($10\%CO_2$:$5\%O_2$), respectively (Figures 31B and 31D). In contrast, the redness of the skin of longkong increased during storage under active MAP in all conditions. The a* values significantly increased from 5.70 in fresh longkong to 10.23 ($5\%CO_2$:$5\%O_2$), 9.55 ($5\%CO_2$:$10\%O_2$) and 10.61 ($10\%CO_2$:$5\%O_2$), at the end of storage under active MAP at 18^oC (24 days) (Figure 31E). Similarly, a higher a* value was observed in longkong stored under active MAP at room temperature (~30^oC). They were 10.51 ($5\%CO_2$:$5\%O_2$), 10.59 ($5\%CO_2$:$10\%O_2$) and 10.71 ($10\%CO_2$:$5\%O_2$) at the end of storage under active MAP at room temperature (~30^oC) (18 days) (Figure 31F).

Fruit skin color can be interpreted in terms of Hue (h^o) angle and Chroma (C* value). The h^o angle and C* values of longkong fruit skin during storage under active MAP at 18^oC and room temperature (~30^oC) are shown in Figure 2. The interaction between storage temperatures, active MAP conditions and storage times significantly affected the h^o angle and C* values ($p<0.05$). Decreases in the h^o angle in all atmospheric conditions and temperatures were observed (Figures 32A and 32B). Decreases in the h^o angle refers to fruit yellowness (h^o angle, 90° = yellow) and shifts to redness (h^o angle, 0° = red-purple). The h^o angle of fresh longkong was 80.65 and this decreased to 47.25 ($5\%CO_2$:$5\%O_2$), 45.79 ($5\%CO_2$:$10\%O_2$) and 42.58 ($10\%CO_2$:$5\%O_2$), at the end of storage under active MAP at 18^oC (24 days) (Figure 32A). The h^o angle was also found in longkong stored under active MAP at room temperatures (~30^oC). The h^o angle decreased from 80.65 to 41.39 ($5\%CO_2$:$5\%O_2$), 34.89 ($5\%CO_2$:$10\%O_2$) and 30.56 ($10\%CO_2$:$5\%O_2$) at the end of storage in longkong stored under active MAP at room temperature (~30^oC) (18 days) (Figure 32B).

The decreases in the h° angle showed the positive correlation with decreases in L* and b* values while there were increases in a* values. The chroma (C*) value indicates color intensity. In this study, C* values significantly decreased from 34.94 in fresh longkong to 14.14 ($5\%CO_2:5\%O_2$), 14.73 ($5\%CO_2:10\%O_2$) and 13.96 ($10\%CO_2:5\%O_2$), at the end of storage under active MAP at 18ºC (24 days) (Figure 32C). The decreases in C* values also was found in longkong stored under active MAP at room temperatures (~30ºC). The C* value decreased from 34.94 to 13.82 ($5\%CO_2:5\%O_2$), 14.11 ($5\%CO_2:10\%O_2$) and 12.45 ($10\%CO_2:5\%O_2$), at the end of storage under active MAP at room temperature (~30ºC) (18 days) (Figure 32D). The decreases in the h° angle showed the positive correlation with decreases in L* and b* values while there were increases in a* values. The chroma (C*) value indicates color intensity. In this study, C* values significantly decreased from 34.94 in fresh longkong to 14.14 ($5\%CO_2:5\%O_2$), 14.73 ($5\%CO_2:10\%O_2$) and 13.96 ($10\%CO_2:5\%O_2$), at the end of storage under active MAP at 18ºC (24 days) (Figure 32C). The decreasing in C* value also was found in longkong stored under active MAP at room temperatures (~30ºC). The C* value decreased from 34.94 to 13.82 ($5\%CO_2:5\%O_2$), 14.11 ($5\%CO_2:10\%O_2$) and 12.45 ($10\%CO_2:5\%O_2$), at the end of storage under active MAP at room temperature (~30ºC) (18 days) (Figure 32D). The decreases in C* values represent low yellowness intensity. The bright yellowness in longkong decreased and shifted to a brownish color during storage in all conditions. Longkong stored under $10\%CO_2:5\%O_2$ at room temperature (~30ºC) showed a rapid change in L*, a*, b* values, and h° angle and chroma (C*) values. This could be explained by enzymatic browning; namely polyphenol oxidase interacts with phenolic compounds and give a darker fruit skin (Sacher, 1962; Azevedo *et al*., 2008; Venkatachalam and Meenune, 2012). During storage under active MAP, a high CO_2 accumulation was noticeable. This showed fruit injury called high CO_2 injury. The high CO_2 accumulation was related to the high rate of fruit respiration during storage at high temperature, as well as to the highly modified CO_2 inside the package. High CO_2 can induce stress conditions which can damage the integrity of fruit cells. Therefore, enzymatic browning reaction took place (Castro *et al*., 2007).
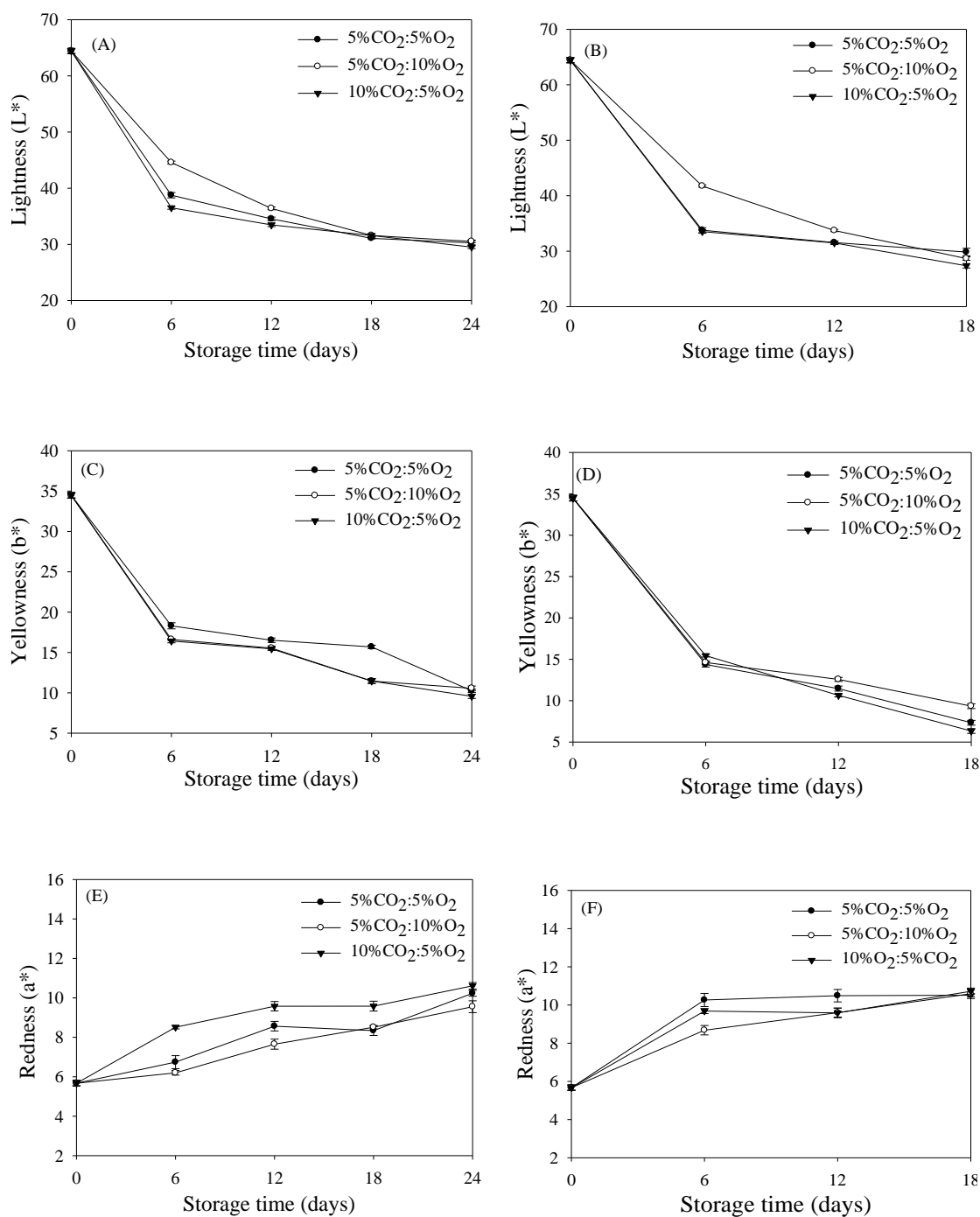
Figure 31 Lightness, yellowness and redness values of longkong during storage under
modified atmosphere packaging at different atmospheric conditions and
temperatures

Note: Storage at 18ºC (A, C, E) and room temperature (~30ºC) (B, D, F)

Data are mean $\pm$ standard deviation (in ten replicates).
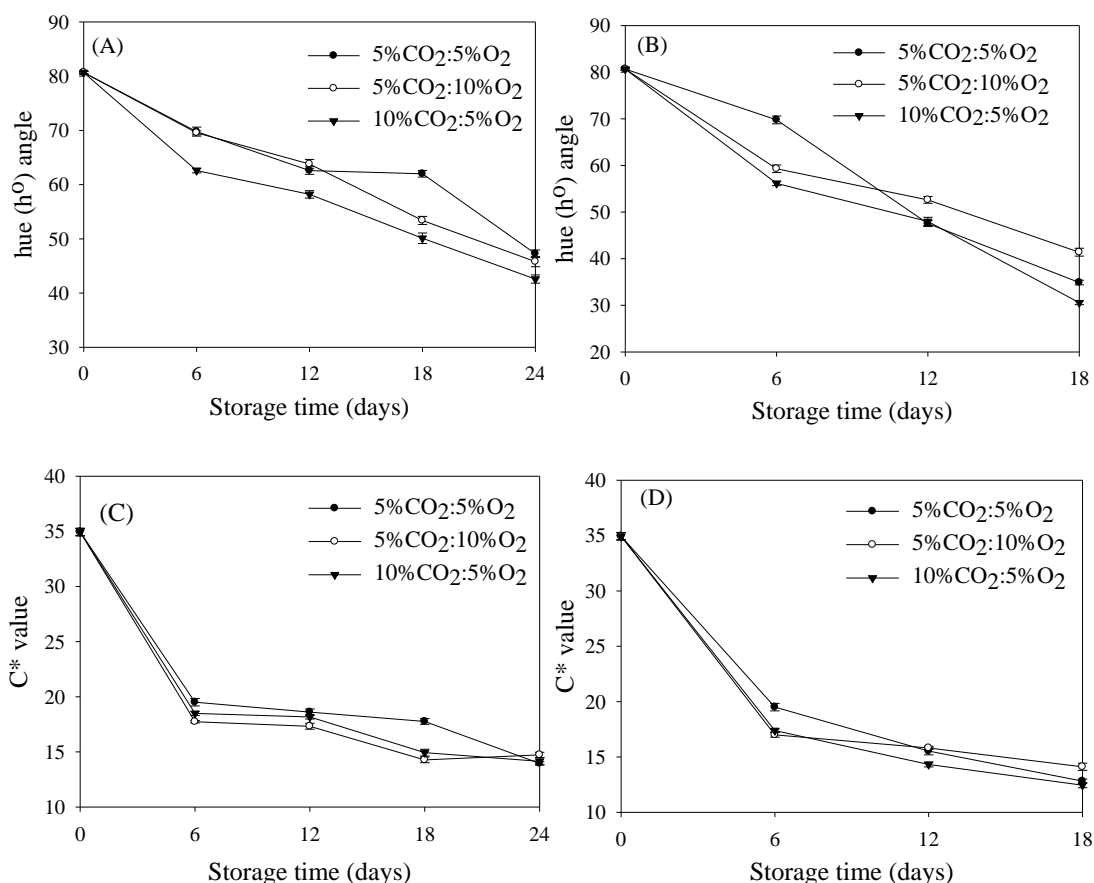
Figure 32 Hue (h°) angle and Chroma (C* value) of longkong during storage under
modified atmosphere packaging at different atmospheric conditions and
temperatures

Note: Storage at 18ºC (A and C) and room temperature (~30ºC) (B and D)

Data are mean $\pm$ standard deviation (in ten replicates).

Fruit firmness

Soft flesh or loss of fruit firmness in longkong occurred with storage
time under active MAP at 18°C and at room temperature (~30°C) were associated with
the loss of water content in longkong. The interaction between storage temperatures,
active MAP conditions and storage times significantly decreased the firmness of the
fruit ($p < 0.05$). Change in the fruit firmness during storage under active MAP is shown
in Figure 33. Higher firmness of the fruit was found in longkong stored at 18ºC than
that stored at room temperature (~30ºC) ($p < 0.05$). The fruit firmness of fresh longkong
was 1849.3 g. At the end of longkong storage under active MAP at 18ºC (24 days),

fruit firmness declined to 1,246.6 g ($5\%CO_2$:$5\%O_2$), 1,119.3 g ($5\%CO_2$:$10\%O_2$) and 1,638.3 g ($10\%CO_2$:$5\%O_2$) (Figure 33A). A similar reduction of fruit firmness took place in longkong stored under active MAP at room temperature (~30ºC) for 18 days. The firmness of the fruit decreased from 1,849.3 g to 1,915.9 g ($5\%CO_2$:$5\%O_2$), 1,059.0 g ($5\%CO_2$:$10\%O_2$) and 1,151.4 g ($10\%CO_2$:$5\%O_2$) (Figure 33B). The greatest firmness was found in fruit stored under the condition of $10\%CO_2$:$5\%O_2$ at 18ºC. High concentration of CO_2 and low temperature conditions give a lower rate of fruit transpiration. Therefore, fruit soften is retarded.
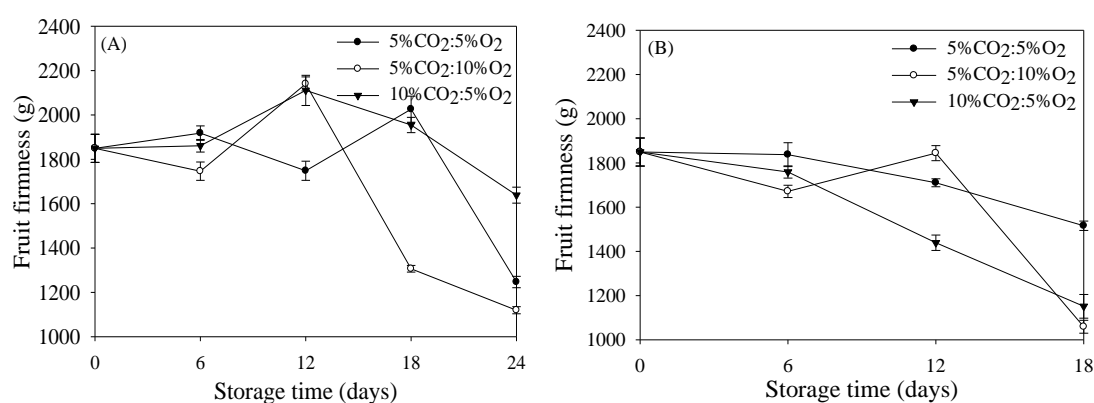
Figure 33 Firmness of longkong during storage under modified atmosphere

packaging at 18ºC (A) and room temperature (~30ºC) (B)

Note: Data are mean ± standard deviation (in ten replicates).

Weight loss

Weight loss was brought about in longkong with storage time under active MAP at 18°C and at room temperature (~30°C). The interaction between storage temperatures, active MAP conditions and storage times significantly increased fruit weight loss ($p < 0.05$). The percentage of weight loss in longkong during storage in all conditions of active MAP is shown in Figure 34. The weight losses of longkong stored under active MAP at 18ºC for 24 days were 0.75% ($5\%CO_2$:$5\%O_2$), 1.82% ($5\%CO_2$:$10\%O_2$) and 0.32% ($10\%CO_2$:$5\%O_2$) (Figure 34A). The weight losses in longkong at the end of storage under active MAP at room temperature (~30ºC) for 18 days were 2.00% ($5\%CO_2$:$5\%O_2$), 3.45% ($5\%CO_2$:$10\%O_2$) and 1.72% ($10\%CO_2$:$5\%O_2$) (Figure 34B). The lowest percentage of weight loss was found in

longkong stored under the condition of $10\%CO_2:5\%O_2$ at 18°C. This was in agreement with the study undertaken by Piyasaengthong *et al*. (1997). They reported that the fruit weight loss of longkong could be limited by storage under modified atmosphere packaging combined with low temperature at 18°C. The high levels of CO_2 inside package combined with low temperature can retard fruit transpiration.
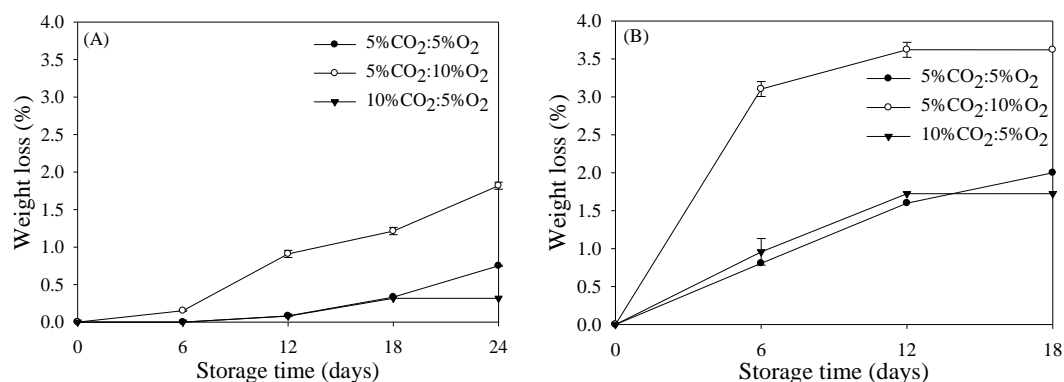
Figure 34 Weight loss of longkong during storage under modified atmosphere

packaging at different atmospheric conditions and temperatures

Note: Storage at 18°C (A) and at room temperature (~30°C) (B)

Data are mean $\pm$ standard deviation (in four replicates).

7.4.2 Chemical quality

The changes in chemical qualities in longkong were monitored during storage under active MAP ($5\%CO_2:5\%O_2$, $5\%CO_2:10\%O_2$ and $10\%CO_2:5\%O_2$) at 18°C and room temperature (~30°C). The interaction between storage temperatures, active MAP conditions and storage times significantly affected the changes in chemical qualities ($p<0.05$). The results are shown in Table 20.

Total soluble solid (TSS), sugars, pH and titratable acidity (TA)

The changes in TSS, sugar and TA in longkong during storage were observed. The interaction between storage temperatures, active MAP conditions and storage times significantly affected these changes ($p<0.05$). A continuous reduction in the TSS and sugar throughout the active MAP storage was detected. This was supported by the oxidation of sugar for the fruit respiration process (Ong *et al*., 2006). A high rate of sugar reduction was found in longkong stored at room temperature

(~30°C). This was probably due to the quick consumption of sugars at a high fruit respiration rate (Ong *et al*., 2006). The decrease in TA in longkong during the 12 days of storage was found. After that, the TA increased in all conditions of active MAP. This was probably due to the high acid accumulation in fruit under anaerobic metabolism (Ong *et al*., 2006). At the end of storage, the highest sugar concentrations and the lowest TA in longkong were found in a condition of $5\%CO_2:5\%O_2$ at 18°C. Conversely, the highest TA and the lowest sugar concentrations in longkong were found in a condition of $10\%CO_2:5\%O_2$ at room temperature (~30°C). This was probably due to the high temperature at room temperature (~30°C) generating a high fruit respiration rate. This resulted in high CO_2 accumulation which induces a stress condition in fruit called high CO_2 injury. Under the condition of high CO_2 injury, fruit metabolism became anaerobic which impaired the functions of Krebs cycle. Later the activity of oxidative enzymes was shut down and organic acids accumulated in fruit cells (Davies, 1980; Kay, 1991; Ong *et al*., 2006; Lara *et al*., 2006).

Types and concentrations of sugars

The sugars in longkong and changes in them were monitored throughout active MAP storage. The interaction between storage temperatures, active MAP conditions and storage times significantly affected the sugar concentrations ($p<0.05$). The dominant sugars in longkong were sucrose, glucose and fructose. The changes in sucrose, fructose and glucose concentrations are shown in Table 21. Over 6 days under active MAP storage, a decrease in sucrose with an increase in fructose and glucose in longkong were noticed. This was probably due to a conversion reaction in which sucrose was conversed to glucose and fructose (Ong *et al*., 2006). After that a decrease in all the sucrose, glucose and fructose concentrations in longkong were found. This could be explained by the consumption of sugar during fruit respiration. At the end of storage, longkong stored under the condition of $10\%CO_2:5\%O_2$ at 18°C remained the highest with concentrations of fructose and glucose. The results were supported by high CO_2 and low temperature retarding the rate of consumption of sugars (Ong *et al*., 2006).

Volatile flavor compounds

Volatile flavor compounds are an extremely important quality in fruit. Postharvest conditions such as ratio of gas or storage temperature directly affect the volatile flavor profile. The volatile flavor compounds in longkong stored under active MAP at 18°C and room temperature (~30°C) were identified and are presented in Tables 22-27. The volatile flavor compounds, namely esters, alcohols, terpenes, acids, ketones and phenols, were identified. Volatile flavor compounds, mainly fruity, sweet, floral and herbaceous compounds were identified as being in abundance. They were n-butanol, laevo-linalool, delta-germacrene and 3-hydroxy-2-butanone. The reduction of the main volatile flavor compound was noticed in all active MAP conditions whereby fermented compounds were formed. When anaerobic metabolism occurred, iso-amylalcohol, acetic acid, propanoic acid, butanoic acid and hexnoic acid were seen in flavor profiles of longkong. The loss of terpenes and phenol in longkong during storage under active MAP was observed, especially in longkong stored at room temperature (~30°C). This could be explained by the shutting down of Krebs cycle under anaerobic conditions. There was insufficient O_2 inside the longkong package and this corresponded to high O_2 consumption. This was caused by the high rate of fruit respiration during storage at room temperature (~30°C). Later on, the longkong metabolism turned to an anaerobic condition and Krebs cycle shut down. With the shutting down of Krebs cycle, Acetyl CoA for terpenes cannot be synthesized. In addition, the oxidation reaction of aromatic amino acids from the shikimic pathway needed to produce a phenol compound was shut down under the anaerobic conditions (Little and Croteau, 1999).

Table 20 pH, titratable acidity (TA), total soluble solid (TSS), reducing sugar and total sugar of longkong during storage under different atmospheric conditions and temperatures

Storage time (days)	Ratio of %CO_2:%O_2	Storage temp (°C)	pH	TA (% as citric acid)	TSS (%)	Sugars (%)	
						Reducing sugar	Total sugar
0	5:5	18	3.98 ± 0.00^g	0.591 ± 0.004^n	17.70 ± 0.12^a	6.40 ± 0.01^r	15.33 ± 0.00^b
		30	3.98 ± 0.00^g	0.591 ± 0.004^n	17.70 ± 0.12^a	6.40 ± 0.01^r	15.33 ± 0.00^b
	5:10	18	3.98 ± 0.00^g	0.591 ± 0.004^n	17.70 ± 0.12^a	6.40 ± 0.01^r	15.33 ± 0.00^b
		30	3.98 ± 0.00^g	0.591 ± 0.004^n	17.70 ± 0.12^a	6.40 ± 0.01^r	15.33 ± 0.00^b
	10:5	18	3.98 ± 0.00^g	0.591 ± 0.004^n	17.70 ± 0.12^a	6.40 ± 0.01^r	15.33 ± 0.00^b
		30	3.98 ± 0.00^g	0.591 ± 0.004^n	17.70 ± 0.12^a	6.40 ± 0.01^r	15.33 ± 0.00^b
6	5:5	18	4.01 ± 0.00^{de}	0.691 ± 0.001^f	14.70 ± 0.12^{fg}	6.37 ± 0.04^r	14.42 ± 0.00^e
		30	3.97 ± 0.00^l	0.674 ± 0.006^h	15.50 ± 0.12^d	7.15 ± 0.03^p	15.23 ± 0.00^c
	5:10	18	4.01 ± 0.00^e	0.678 ± 0.001^h	15.55 ± 0.10^d	6.30 ± 0.00^r	14.06 ± 0.10^f
		30	4.05 ± 0.00^b	0.667 ± 0.005^j	16.40 ± 0.40^c	17.16 ± 0.02^e	17.01 ± 0.06^a
	10:5	18	4.01 ± 0.00^{de}	0.682 ± 0.005^g	14.65 ± 0.10^{fg}	7.76 ± 0.00^o	14.56 ± 0.02^d
		30	4.02 ± 0.00^{de}	0.602 ± 0.001^m	16.65 ± 0.10^b	16.99 ± 0.18^f	13.98 ± 0.04^g
12	5:5	18	4.07 ± 0.00^a	0.589 ± 0.000^n	14.85 ± 0.10^f	6.91 ± 0.04^q	9.35 ± 0.01^n
		30	3.68 ± 0.00^m	0.666 ± 0.000^{ij}	15.05 ± 0.10^e	9.73 ± 0.04^j	10.64 ± 0.00^h
	5:10	18	4.04 ± 0.00^b	0.525 ± 0.000^o	14.25 ± 0.10^i	9.03 ± 0.00^m	9.69 ± 0.01^l
		30	4.04 ± 0.00^{bc}	0.475 ± 0.002^q	14.45 ± 0.10^h	20.37 ± 0.15^a	10.46 ± 0.00^i
	10:5	18	4.02 ± 0.00^{de}	0.513 ± 0.001^p	14.75 ± 0.10^{fg}	9.12 ± 0.07^m	8.67 ± 0.01^p
		30	4.03 ± 0.00^{cd}	0.514 ± 0.002^p	14.55 ± 0.10^{gh}	17.33 ± 0.10^d	7.20 ± 0.01^s

Table 20 continued

Storage time (days)	Ratio of %CO_2:%O_2	Storage temp (ºC)	pH	TA (% as citric acid)	TSS (%)	Sugars (%)	
						Reducing sugar	Total sugar
18	5:5	18	3.99 ± 0.00^f	0.654 ± 0.002^k	15.55 ± 0.10^d	9.57 ± 0.06^k	9.66 ± 0.00^l
		30	3.65 ± 0.00^n	$0.7520\pm.005^b$	14.15 ± 0.10^i	10.93 ± 0.06^g	6.59 ± 0.00^t
	5:10	18	3.85 ± 0.00^j	0.672 ± 0.000^l	14.55 ± 0.10^{gh}	8.83 ± 0.05^n	8.02 ± 0.01^q
		30	3.86 ± 0.00^i	0.667 ± 0.002^i	12.85 ± 0.10^j	18.34 ± 0.00^c	7.94 ± 0.00^r
	10:5	18	3.83 ± 0.00^k	0.736 ± 0.000^c	14.75 ± 0.01^{fg}	9.24 ± 0.00^l	8.71 ± 0.01^o
		30	3.75 ± 0.00^l	0.766 ± 0.004^a	14.45 ± 0.10^h	18.62 ± 0.11^b	5.93 ± 0.00^u
24	5:5	18	3.96 ± 0.00^h	0.716 ± 0.000^e	14.65 ± 0.10^{fg}	10.64 ± 0.05^h	10.24 ± 0.00^j
		30	-	-	-	-	-
	5:10	18	3.83 ± 0.00^k	0.731 ± 0.002^d	14.65 ± 0.10^{fg}	10.27 ± 0.05^i	9.73 ± 0.03^k
		30	-	-	-	-	-
	10:5	18	3.75 ± 0.00^l	0.726 ± 0.009^a	14.45 ± 0.10^h	10.60 ± 0.00^h	9.61 ± 0.00^m
		30	-	-	-	-	-

Note: 30ºC = room temperature; - = not determine (due to fruit spoilage at that date)

Data are mean $\pm$ standard deviation (in four replicates)

Mean values in the same column with different superscript letters indicate that

there are significant different among storage temperatures ($p<0.05$).

Table 21 Types and concentrations of sugars in longkong during storage during storage under modified atmosphere packaging at different atmospheric conditions and temperatures

Storage time (days)	Ratio of %CO$_2$:%O$_2$	Storage temp (ºC)	Sugars (%)		
			Fructose	Glucose	Sucrose
0	5:5	18	7.94±0.08^e	3.58±0.03^e	2.56±0.05^f
		30	7.94±0.08^e	3.58±0.03^e	2.56±0.05^f
	5:10	18	7.94±0.08^e	3.58±0.03^e	2.56±0.05^f
		30	7.94±0.08^e	3.58±0.03^e	2.56±0.05^f
	10:5	18	7.94±0.08^e	3.58±0.03^e	2.56±0.05^f
		30	7.94±0.08^e	3.58±0.03^e	2.56±0.05^f
6	5:5	18	7.40±0.02^g	3.36±0.05^f	3.71±0.04^b
		30	6.59±0.00^j	2.93±0.11^j	1.48±0.01^h
	5:10	18	6.79±0.05^i	3.10±0.00^h	0.73±0.00^l
		30	10.19±0.08^a	4.64±0.00^a	1.34±0.00^{ij}
	10:5	18	7.13±0.06^h	3.20±0.04^g	3.62±0.00^c
		30	7.20±0.15^h	3.17±0.04^g	3.99±0.05^a
12	5:5	18	8.83±0.11^c	4.02±0.12^c	3.42±0.01^d
		30	6.75±0.05^i	3.02±0.06^i	0.79±0.02^k
	5:10	18	4.71±0.02^{no}	2.14±0.01^o	1.59±0.01^g
		30	4.96±0.02^m	2.30±0.00^m	0.37±0.03^o
	10:5	18	9.48±0.10^b	4.25±0.08^b	3.10±0.01^e
		30	4.08±0.11^q	1.98±0.05^q	0.17±0.00^s

Table 21 continued

Storage time (days)	Ratio of %CO$_2$: %O$_2$	Storage temp (°C)	Sugars (%)		
			Fructose	Glucose	Sucrose
18	5:5	18	6.34±0.07^k	2.82±0.02^k	0.24±0.03^p
		30	6.38±0.12^k	2.86±0.04^k	0.45±0.00^n
	5:10	18	8.16±0.04^d	3.84±0.05^d	0.57±0.02^m
		30	4.50±0.05^o	2.11±0.03^o	0.08±0.00^r
	10:5	18	7.81±0.09^f	2.39±0.05^i	1.38±0.02^i
		30	4.37±0.06^p	1.89±0.04^p	0.01±0.00^q
24	5:5	18	4.62±0.01^o	2.07±0.01^o	0.24±0.00^p
		30	-	-	-
	5:10	18	4.75±0.05^n	2.20±0.04^n	0.01±0.00^s
		30	-	-	-
	10:5	18	7.81±0.06^m	3.57±0.01^l	1.33±0.00^j
		30	-	-	-

Note: 30°C = room temperature; - = not determine (due to fruit spoilage at that date)

Data are mean ± standard deviation (in four replicates)

Mean values in the same column with different superscript letters indicate that there are significant different among storage temperatures ($p<0.05$).

Table 22 Volatile flavor compounds and their attributes identified in longkong during storage under modified atmosphere packaging (the condition of $5\%CO_2:5\%O_2$) at 18ºC

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]				
				Storage time (days)				
				0	6	12	18	24
Esters								
ethyl butyrate	3.85	1049	fruity	-	-	-	12.71	20.55
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	-	15.17	19.95	13.57	7.33
Alcohols								
2-methyl propanol	4.96	1107	fruity, sweet	-	6.95	12.76	14.23	16.41
n-butanol	6.19	1157	fruity	1.25	17.31	31.83	32.84	55.58
iso-amylalcohol	7.72	1218	fermented	2.80	3.97	5.97	33.25	29.94
n-hexanol	11.05	1347	green	1.25	2.82	3.63	29.83	32.96
2-ethyl hexanol	14.89	1499	citrus,floral,sweet	1.28	2.17	15.70	26.45	8.73
benzyl alcohol	23.77	1895	floral,sweet	-	37.13	41.17	38.36	27.86
phenylethyl alcohol	24.45	2134	floral	-	6.96	35.27	51.47	40.80
Terpenes and their derivatives								
cis-linalool oxide	14.39	1479	floral,sweet	-	5.95	14.98	70.27	54.44
laevo-linalool	16.35	1559	fruity,sweet	-	13.04	14.33	95.00	54.41
delta-germacrene	19.75	1706	herbaceous	0.82	12.09	27.17	17.19	10.28
epoxylinalool	21.21	1707	flower	-	7.27	8.96	22.06	16.75

Table 22 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]				
				Storage time (days)				
				0	6	12	18	24
Acids								
acetic acid	14.21	1472	pungent, sour	0.50	-	17.14	36.51	78.40
propanoic acid	16.25	1555	pungent	-	-	12.42	28.48	37.78
butanoic acid	18.32	1643	sour	-	-	25.81	26.36	38.78
hexanoic acid	23.07	1862	sour, sweat	-	-	24.98	69.51	81.90
Ketones								
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	58.09	98.78	38.61	16.25
Phenol								
phenol	26.38	2027	phenol	2.89	13.05	62.72	41.26	14.80

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = total ion current; - = not detected

Table 23 Volatile flavor compounds and their attributes identified in longkong during storage under modified atmosphere packaging (the condition of $5\%CO_2:5\%O_2$) at room temperature (~30ºC)

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]			
				Storage time (days)			
				0	6	12	18
Esters							
ethyl butyrate	3.85	1049	fruity	-	-	-	10.87
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	-	9.20	24.87	18.04
Alcohols							
2-methyl propanol	4.96	1107	fruity, sweet	-	10.52	14.60	-
n-butanol	6.19	1157	fruity	1.25	4.41	27.11	-
iso-amylalcohol	7.72	1218	fermented	2.80	18.45	24.37	18.11
n-hexanol	11.05	1347	green	1.25	3.96	9.60	15.34
2-ethyl hexanol	14.89	1499	citrus,floral,sweet	1.28	2.97	10.18	7.17
benzyl alcohol	23.77	1895	floral,sweet	-	37.04	69.86	39.04
phenylethyl alcohol	24.45	2134	floral	-	3.85	32.82	16.34
Terpenes and their derivatives							
cis-linalool oxide	14.39	1479	floral,sweet	-	10.67	18.70	45.63
laevo-linalool	16.35	1559	fruity,sweet	-	16.83	23.73	45.95
delta-germacrene	19.75	1706	herbaceous	0.82	47.01	54.55	48.45
epoxylinalool	21.21	1707	flower	-	4.32	7.27	22.53

Table 23 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas (×10⁴) TIC[D]			
				Storage time (days)			
				0	6	12	18
Acids							
acetic acid	14.21	1472	pungent, sour	0.50	5.97	25.37	82.84
propanoic acid	16.25	1555	pungent	-	-	20.12	31.08
butanoic acid	18.32	1643	sour	-	21.51	27.93	33.64
hexanoic acid	23.07	1862	sour, sweat	-	7.08	27.93	75.38
Ketones							
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	27.54	46.38	13.84
Phenol							
phenol	26.38	2027	phenol	2.89	16.06	17.79	48.10

Note: [A] Rt = Retention time (min);   [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = total ion current; - = not detected

Table 24 Volatile flavor compounds and their attributes identified in longkong during storage under modified atmosphere packaging (the condition of 5%CO_2:10%O_2) at 18ºC

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]				
				Storage time (days)				
				0	6	12	18	24
Esters								
ethyl butyrate	3.85	1049	fruity	-	-	-	37.18	40.55
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	-	9.14	18.41	29.11	30.88
Alcohols								
2-methyl propanol	4.96	1107	fruity, sweet	-	-	3.91	48.20	95.34
n-butanol	6.19	1157	fruity	1.25	19.38	41.80	74.07	85.44
iso-amylalcohol	7.72	1218	fermented	2.80	8.53	12.41	30.87	39.67
n-hexanol	11.05	1347	green	1.25	2.82	8.63	25.24	32.96
2-ethyl hexanol	14.89	1499	citrus,floral,sweet	1.28	3.81	14.11	5.75	5.97
benzyl alcohol	23.77	1895	floral, sweet	-	21.74	77.54	45.67	33.73
phenylethyl alcohol	24.45	2134	floral	-	4.33	15.27	59.29	30.53
Terpenes and their derivatives								
cis-linalool oxide	14.39	1479	floral, sweet	-	-	-	32.80	60.89
laevo-linalool	16.35	1559	Fruity, sweet	-	2.21	3.15	96.77	52.07
delta-germacrene	19.75	1706	herbaceous	0.82	2.93	29.95	79.59	40.14
bicyclogermacrene	20.29	1731	woody	-	-	10.14	55.31	-

Table 24 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]				
				Storage time (days)				
				0	6	12	18	24
Terpenes and their derivatives								
delta-cadinene	20.84	1756	herbaceous	-	-	12.16	65.98	-
epoxylinalool	21.21	1707	flower	-	-	10.13	55.31	-
viridiflorol	27.52	2173	green, sweet	-	-	13.76	30.06	-
spathuleol	28.37	2184	fruity,herbaceous	-	-	24.47	9.03	-
alpha-cadinol	29.52	2199	herbaceous	-	-	42.06	7.69	-
Acids								
acetic acid	14.21	1472	pungent, sour	0.50	10.01	45.50	48.36	61.42
propanoic acid	16.25	1555	pungent	-	-	5.29	49.91	71.70
butanoic acid	18.32	1643	sour	-	-	18.56	74.53	89.52
hexanoic acid	23.07	1862	sour, sweat	-	-	-	17.13	34.75
Ketones								
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	21.88	39.91	26.42	13.02
Phenol								
phenol	26.38	2027	phenol	2.89	11.45	74.41	103.74	76.40

Note: [A] Rt = Retention time (min);　[B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = total ion current; - = not detected

Table 25 Volatile flavor compounds and their attributes identified in longkong during storage under modified atmosphere packaging (the condition of 5%CO_2:10%O_2) at room temperature (~30ºC)

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]			
				Storage time (days)			
				0	6	12	18
Esters							
ethyl butyrate	3.85	1049	fruity	-	-	18.84	41.02
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	-	9.96	32.23	22.35
Alcohols							
2-methyl propanol	4.96	1107	fruity, sweet	-	8.80	57.86	19.13
n-butanol	6.19	1157	fruity	1.25	7.41	10.82	38.59
iso-amylalcohol	7.72	1218	fermented	2.80	11.37	65.53	31.49
n-hexanol	11.05	1347	green	1.25	5.96	4.45	16.17
2-ethyl hexanol	14.89	1499	citrus,floral,sweet	1.28	13.25	16.14	-
benzyl alcohol	23.77	1895	floral,sweet	-	13.32	19.57	10.12
phenylethyl alcohol	24.45	2134	floral	-	17.04	53.33	23.34
Terpenes and their derivatives							
cis-linalool oxide	14.39	1479	floral,sweet	-	10.97	47.29	59.03
laevo-linalool	16.35	1559	fruity,sweet	-	8.86	12.66	45.56
delta-germacrene	19.75	1706	herbaceous	0.82	9.75	66.65	32.13
bicyclogermacrene	20.29	1731	woody	-	-	3.12	38.34

Table 25 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times10^4$) TIC[D]			
				Storage time (days)			
				0	6	12	18
Terpenes and their derivatives							
delta-cadinene	20.84	1756	herbaceous	-	-	6.70	28.54
epoxylinalool	21.21	1707	flower	-	4.66	7.11	22.23
alloaromadendrene	26.88		woody	-	-	3.12	12.49
viridiflorol	27.52	2173	green, sweet	-	-	10.04	38.07
spathuleol	28.37	2184	fruity,herbaceous	-	-	38.05	12.03
alpha-cadinol	29.52	2199	herbaceous	-	-	35.54	10.11
Acids							
acetic acid	14.21	1472	pungent, sour	0.50	24.71	37.85	99.11
propanoic acid	16.25	1555	pungent	-	-	13.32	74.69
butanoic acid	18.32	1643	sour	-	7.27	56.10	93.79
hexanoic acid	23.07	1862	sour, sweat	-	-	10.75	17.85
Ketones							
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	85.50	46.97	45.42
Phenol							
phenol	26.38	2027	phenol	2.89	14.06	38.96	73.79

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html; [D] TIC = total ion current; - = not detected

Table 26 Volatile flavor compounds and their attributes identified in longkong during storage under modified atmosphere packaging (the condition of 10%CO_2:5%O_2) at 18ºC

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]				
				Storage time (days)				
				0	6	12	18	24
Esters								
ethyl butyrate	3.85	1049	fruity	-	-	20.28	34.95	88.79
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	-	4.98	32.18	55.29	65.91
Alcohols								
2-methyl propanol	4.96	1107	fruity, sweet	-	-	14.87	50.20	72.42
n-butanol	6.19	1157	fruity	1.25	9.95	41.49	72.83	80.81
iso-amylalcohol	7.72	1218	fermented	2.80	8.53	44.23	51.72	66.46
n-hexanol	11.05	1347	green	1.25	1.02	4.00	21.13	31.09
2-ethyl hexanol	14.89	1499	citrus,floral,sweet	1.28	19.35	86.68	25.51	16.16
benzyl alcohol	23.77	1895	floral, sweet	-	30.36	67.85	31.51	29.48
phenylethyl alcohol	24.45	2134	floral	-	15.23	36.23	38.29	23.98
Terpenes and their derivatives								
cis-linalool oxide	14.39	1479	floral, sweet	-	5.71	38.83	40.86	80.18
laevo-linalool	16.35	1559	fruity, sweet	-	30.94	73.51	81.54	64.46
delta-germacrene	19.75	1706	herbaceous	-	59.09	97.48	99.09	20.62
bicyclogermacrene	20.29	1731	woody	-	-	7.21	7.91	11.88

Table 26 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]				
				Storage time (days)				
				0	6	12	18	24
Terpenes and their derivatives								
delta-cadinene	20.84	1756	herbaceous	-	-	4.89	15.00	52.72
epoxylinalool	21.21	1707	flower	-	-	20.28	36.65	7.52
viridiflorol	27.52	2173	green, sweet	-	-	-	10.03	30.31
spathuleol	28.37	2184	fruity, herbaceous	-	-	10.84	15.25	9.35
alpha-cadinol	29.52	2199	herbaceous	-	-	-	45.55	7.70
Acids								
acetic acid	14.21	1472	pungent, sour	0.50	7.12	26.40	99.09	59.32
propanoic acid	16.25	1555	pungent	-	-	-	72.90	67.99
butanoic acid	18.32	1643	sour	-	-	32.65	76.16	21.87
hexanoic acid	23.07	1862	sour, sweat	-	22.79	23.98	24.14	5.26
Ketones								
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	15.51	15.65	39.22	15.66
Phenol								
phenol	26.38	2027	phenol	2.89	11.83	32.86	103.62	51.50

Note: [A] Rt = Retention time (min);   [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = total ion current; - = not detected

Table 27 Volatile flavor compounds and their attributes identified in longkong during storage under modified atmosphere packaging (the condition of $10\% CO_2 : 5\% O_2$) at room temperature (~30ºC)

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]			
				Storage time (days)			
				0	6	12	18
Esters							
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	-	31.97	18.11	11.76
Alcohols							
n-butanol	6.19	1157	fruity	1.25	-	-	-
iso-amylalcohol	7.72	1218	fermented	2.80	8.15	1.84	-
n-hexanol	11.05	1347	green	1.25	-	-	-
2-ethyl hexanol	14.89	1499	citrus,floral,sweet	1.28	1.41	1.67	13.35
benzyl alcohol	23.77	1895	floral,sweet	-	14.95	31.20	40.11
phenylethyl alcohol	24.45	2134	floral	-	4.50	8.82	57.23
Terpenes and their derivatives							
cis-linalool oxide	14.39	1479	floral,sweet	-	10.08	41.05	67.43
laevo-linalool	16.35	1559	fruity,sweet	-	-	7.39	52.04
delta-germacrene	19.75	1706	herbaceous	0.82	12.10	51.78	28.65
delta-cadinene	20.84	1756	herbaceous	-	3.07	12.08	55.41

Table 27 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]			
				Storage time (days)			
				0	6	12	18
Terpenes and their derivatives							
epoxylinalool	21.21	1707	flower	-	4.53	4.73	14.79
viridiflorol	27.52	2173	green, sweet	-	-	12.11	48.78
spathuleol	28.37	2184	fruity,herbaceous	-	-	32.05	47.97
Acids							
acetic acid	14.21	1472	pungent, sour	0.50	20.35	31.11	71.08
butanoic acid	18.32	1643	sour	-	9.58	56.93	90.33
hexanoic acid	23.07	1862	sour, sweat	-	1.40	6.75	15.22
Ketones							
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	19.95	55.77	26.98
Phenol							
phenol	26.38	2027	phenol	2.89	17.76	29.07	80.59

Note: [A] Rt = Retention time (min);   [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = total ion current; - = not detected

Headspace gases composition

Changes in the headspace oxygen (O_2) and carbon dioxide (CO_2) inside the longkong package were monitored during storage under active MAP. The interaction between the storage temperatures, active MAP conditions and storage times significantly affected on the headspace gases composition ($p<0.05$). A decrease in the headspace O_2 and an increase in headspace CO_2 are shown in Figure 35. At the end of longkong storage under active MAP at 18°C for 24 days the headspace O_2 inside the packages was 0.76% (5%CO_2:5%O_2), 0.41% (5%CO_2:10%O_2) and 1.01% (10%CO_2:5%O_2). The headspace O_2 inside the package of longkong stored under active MAP at room temperature (~30ºC) for 18 days were 0.51% (5%CO_2:5%O_2), 0.99% (5%CO_2:10%O_2) and 0.72% (10%CO_2:5%O_2) (Figures 35A and 35B). A sharp increase in the headspace CO_2 inside the packages of longkong during 6 days of active MAP storage was observed. The headspace CO_2 inside the longkong package stored under active MAP at 18°C increased to 55.67% (5%CO_2:5%O_2), 56.49% (5%CO_2:10%O_2) and 52.26% (10%CO_2:5%O_2). An increase in the headspace CO_2 inside longkong packages was also found in all packages stored at room temperature (~30ºC). The amounts were 57.99% (5%CO_2:5%O_2), 59.41% (5%CO_2:10%O_2) and 53.46% (10%CO_2:5%O_2). After that, the headspace CO_2 inside longkong packages gradually increased until the end of storage. The headspace CO_2 inside longkong packages stored under active MAP at 18°C for 24 days was 79.96% (5%CO_2:5%O_2), 88.25% (5%CO_2:10%O_2) and 86.97% (10%CO_2:5%O_2). The headspace CO_2 inside longkong packages stored under active MAP at room temperature (~30°C) for 18 days were 75.36% (5%CO_2:5%O_2), 86.22% (5%CO_2:10%O_2) and 87.47% (10%CO_2:5%O_2) (Figures 35C and 35D).

Changes in the headspace CO_2 and O_2 inside longkong packages could be explained by fruit respiration. Fruit utilizes O_2 from the surrounding environment and releases CO_2 (Kader *et al*., 1989). The rate of fruit respiration needed to consume O_2 and release CO_2 depends upon temperature. High temperatures cause a high fruit respiration rate (Cameron *et al*., 1995). Therefore, lower O_2 and a higher accumulation of CO_2 were found in longkong packages stored under room temperature (~30°C) than in those stored at 18°C. In addition, the ratio of gases used inside the packages was associated with the rates of O_2 consumption and releases of

CO_2. The condition $10\%CO_2:5\%O_2$ at $18°C$ showed the lowest changing rate of composition of headspace gases ($p<0.05$). Meanwhile, the condition $5\%CO_2:10\%O_2$ at room temperature ($\sim30°C$) showed the highest CO_2 inside packages.
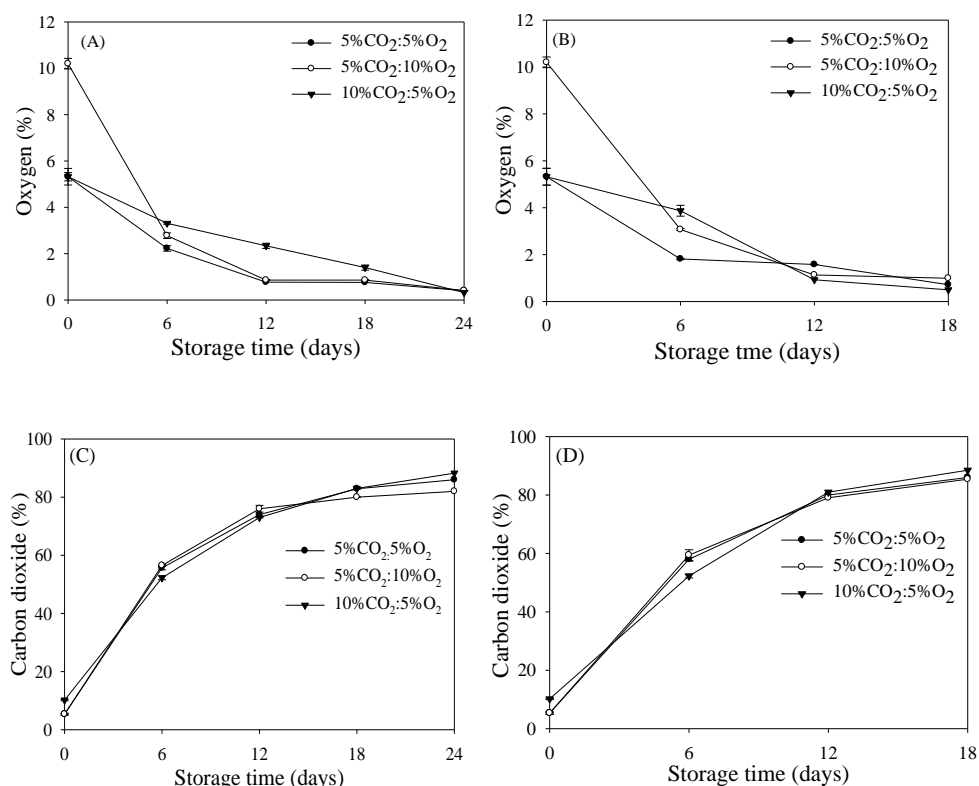
Figure 35 Headspace oxygen (A and B) and carbon dioxide (C and D) inside package of longkong during storage under modified atmosphere packaging at different atmospheric conditions and temperatures

Note: Storage at $18°C$ (A and C) and at room temperature ($\sim30°C$) (B and D)

Data are mean $\pm$ standard deviation (in four replicate)

Ethanol concentration

The ethanol concentration in longkong was monitored during storage under active MAP. The interaction between storage temperatures, active MAP conditions and storage times significantly affected ethanol concentration ($p<0.05$). An increase in ethanol concentration in lonkong is shown in Figure 36. It was found that the ethanol concentration in longkong significantly increased until the end of storage ($p<0.05$). At the end of longkong storage under active MAP at $18°C$ for 24 days the ethanol concentration increased from 0.06 g/g FW in fresh longkong to 0.21 g/g FW

(5%CO_2:5%O_2), 0.20 g/g FW (5%CO_2:10%O_2) and 0.25 g/g FW (10%CO_2:5%O_2). An increase in ethanol concentration in longkong at the end of longkong storage under active MAP at room temperature (~30ºC) was also observed. The ethanol concentrations were 0.24 g/g FW (5%CO_2:5%O_2), 0.23 g/g FW (5%CO_2:10%O_2) and 0.27 g/g FW (10%CO_2:5%O_2). The increase in ethanol concentration in longkong during storage under active MAP was associated with high CO_2 accumulation and/or insufficient O_2. Under high CO_2 accumulation and/or insufficient O_2, the longkong metabolism might become anaerobic and produce high concentrations of ethanol (Kader *et al*., 1989). In addition, a higher ethanol concentration was found in longkong stored under active MAP at room temperature (~30°C). This was probably because the longkong metabolism was rapidly shifted to anaerobic at high temperatures (Haugaard, 1968). Therefore, a high ethanol concentration was present during storage over a longer time.
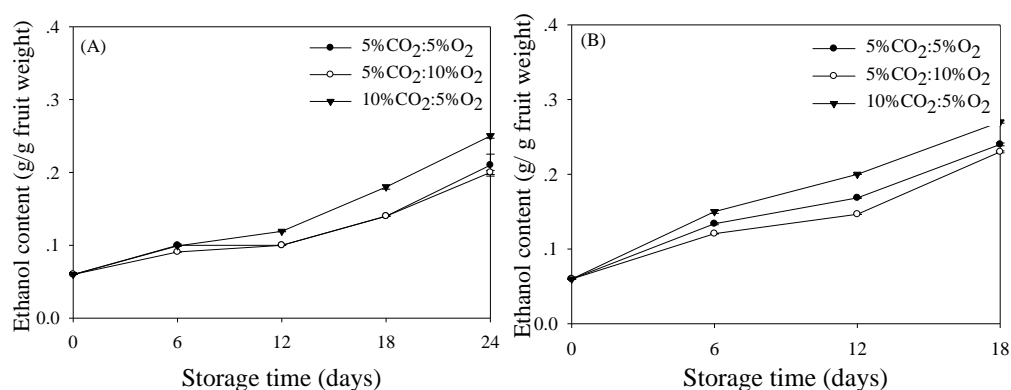
Figure 36 Ethanol content in longkong during storage under modified atmosphere packaging at different atmospheric conditions and temperatures

Note: Storage at 18ºC (A) and at room temperature (~30ºC) (B)

          Data are mean $\pm$ standard deviation (in four replicates).

Sensory evaluation

          Sensory evaluation was used to estimate the correlation between human perception and the ethanol concentrations that increased in longkong during storage under active MAP. The positive correlation between perceived ethanolic-flavor intensity rating and ethanol content in longkong was detected as can be seen in

Appendix D. The perception of ethanol in longkong was indicated by the high rating for the ethanolic-flavor and the reduction in fruit flavor scores (Figures 37 and 38). The ethanolic-flavor was rated at 4.5 in fresh longkong. It was rated highly at 61.75 ($5\%CO_2$:$5\%O_2$), 56.50 ($5\%CO_2$:$10\%O_2$) and 68.17 ($10\%CO_2$:$5\%O_2$) (Figure 37A) in longkong stored under active MAP at 18°C for 24 days. At the end of longkong storage under active MAP at room temperature (~30°C) for 18 days, the ethanolic-flavor was rated at 63.58 ($5\%CO_2$:$5\%O_2$), 59.33 ($5\%CO_2$:$10\%O_2$) and 70.42 ($10\%CO_2$:$5\%O_2$) (Figure 37B).
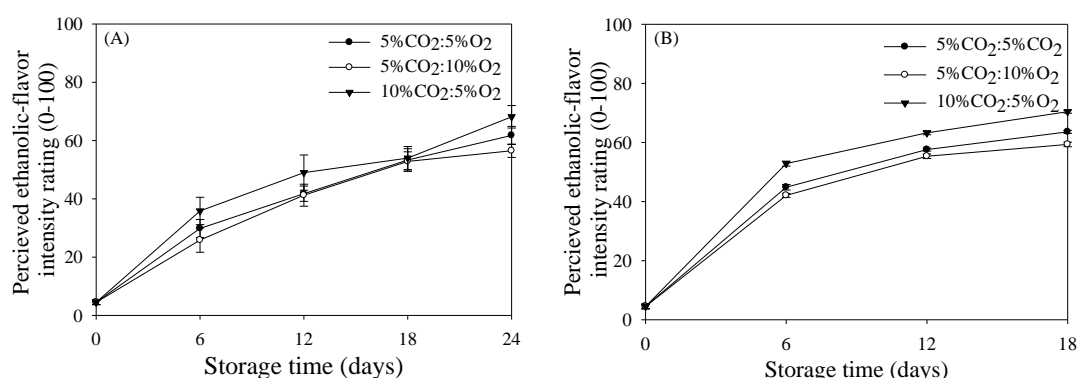
Figure 37 Intensity rating for ethanolic-flavor in longkong during storage under modified atmosphere packaging at different atmospheric conditions and temperatures

Note: Storage at 18°C (A) and at room temperature (~30°C) (B)

Data are mean $\pm$ standard deviation (in twelve replicates).

In addition, consumer acceptability of the longkong flavor during storage under active MAP was determined in terms of flavor scores. Decreases in the flavor score corresponding to high ethanol concentrations were observed. The flavor score in fresh longkong was 8.3 and this decreased to 3.1 ($5\%CO_2$:$5\%O_2$), 2.2 ($5\%CO_2$:$10\%O_2$) and 1.3 ($10\%CO_2$:$5\%O_2$) (Figure 38A), in longkong stored under active MAP at 18°C for 24 days. A decrease in the fruit flavor score was also found in longkong stored under active MAP at room temperature (~30°C). The flavor scores decreased to 2.8 ($5\%CO_2$:$5\%O_2$), 2.1 ($5\%CO_2$:$10\%O_2$) and 1.3 ($10\%CO_2$:$5\%O_2$) (Figure 38B). The increase in the ethanolic-flavor rating positively correlated with the

decrease in the flavor score. Both of these were in agreement with the ethanol concentration.
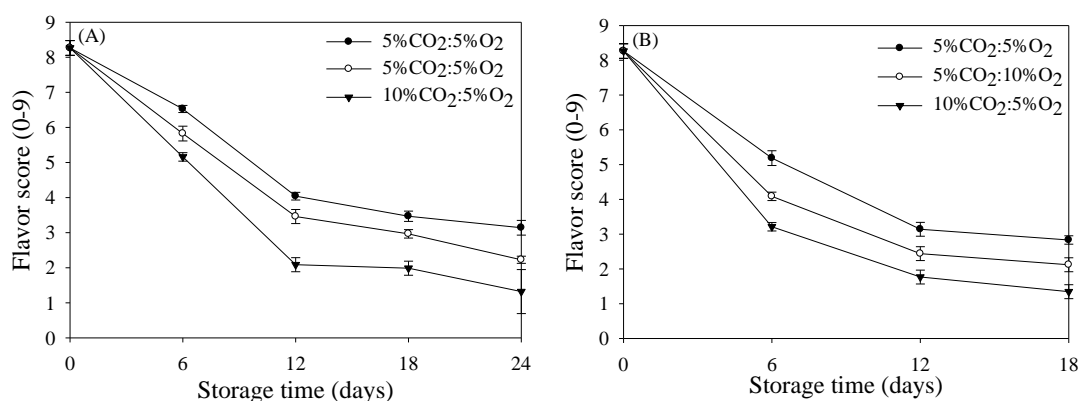
Figure 38 Flavor score of longkong during storage under modified atmosphere
packaging at different atmospheric conditions and temperatures

Note: Storage at 18ºC (A) and at room temperature (~30ºC) (B)

Data are mean $\pm$ standard deviation (in one hundred and fifty replicates).

Alcohol dehydrogenase (ADH) enzyme activity

The activity of the ADH enzyme, played an important role in ethanol formation in longkong during storage under active MAP, was monitored. The interaction between storage temperatures, active MAP conditions and storage times significantly increased the activity of the ADH enzyme ($p<0.05$). The changes in the ADH activity in lonkong during storage under active MAP are shown in Figure 39. The activity of the ADH enzyme in fresh longkong was 0.54 U/ mg protein. This increased until the end of the longkong storage under active MAP at 18°C for 24 days. They were 13.52 U/mg protein ($5\%CO_2:5\%O_2$), 12.46 U/mg protein ($5\%CO_2:10\%O_2$) and 15.58 U/mg protein ($10\%CO_2:5\%O_2$) (Figure 39A). The increase in the activity of the ADH enzyme in longkong up to the end of longkong storage under active MAP at room temperature (~30°C) was also observed. They were 15.41 U/ mg protein ($5\%CO_2:5\%O_2$), 13.33 U/ mg protein ($5\%CO_2:10\%O_2$) and 15.61 U/ mg protein ($10\%CO_2:5\%O_2$) (Figure 39B). The high activity of the ADH enzyme showed a positive correlation with high levels of CO_2 that accumulated inside package as well as the high ethanol concentration in longkong.
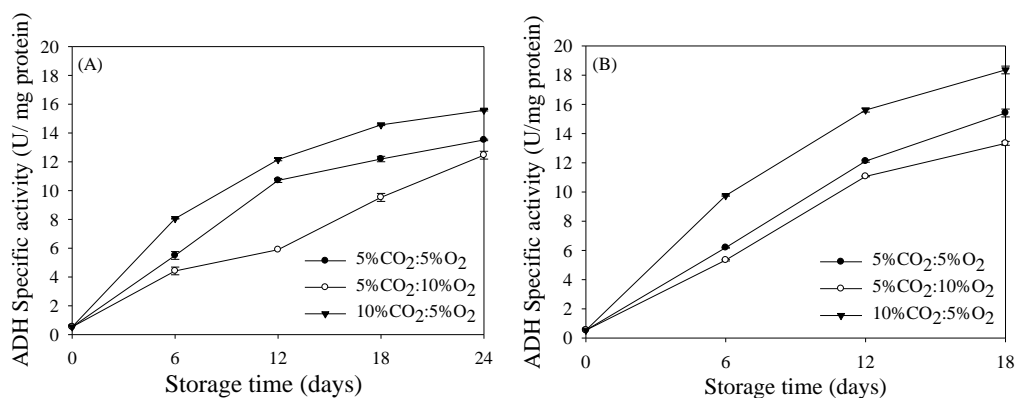
Figure 39 ADH specific activity of longkong during storage under modified
atmosphere packaging at different atmospheric conditions and temperatures

Note: Storage at 18ºC (A) and at room temperature (~30ºC) (B)

Data are mean $\pm$ standard deviation (in four replicates)

Microbiological determination

Total viable count in longkong at the initial and the end of storage is
shown in Table 28. They increased with storage time in all treatments.

Table 28 Total viable counts in longkong at the initial and the end of storage under
modified atmosphere packaging at different atmospheric conditions and
temperatures

Ratio of %CO$_2$:%O$_2$	Storage time (days)	Storage temp (ºC)	Total viable counts (CFU/g)
	0		1.50×10^3
	The end of storage		
5:5	24	18	7.53×10^5
	18	Room temp (~30 ºC)	8.86×10^6
5:10	24	18	7.58×10^5
	18	Room temp (~30 ºC)	8.88×10^6
10:5	24	18	7.65×10^5
	18	Room temp (~30 ºC)	8.86×10^6

Note: Data are mean $\pm$ standard deviation (in four replicates).

7.5 Conclusion

Effective postharvest treatments, such as active MAP, are needed to prolong the shelf-life of longkong. An appropriate ratio of high CO_2 and/or low O_2 combined with an optimal temperature directly affects fruit quality. Longkong stored under the optimal ratio of modified gases 5%CO_2:5%O_2 at 18°C resulted in good quality longkong that still had its unique flavor characteristics. However, high concentrations of CO_2 and/or insufficient O_2 concentrations inside packages occurred during active MAP storage. This can accelerate fruit stress through the forming of CO_2 that causes injury to the fruit. This results in poor quality fruit as it rapidly loses its bright yellow skin, its firmness and its fruity flavor. In addition, the fermented ethanol brought about the high acid content. This was positively correlated with the highly intense ethanolic flavor that was perceived as well as the reduction in the fruit flavor score of the longkong.

CHAPTER 8

CHANGES IN FRUIT QUALITY, VOLATILE FLAVOR COMPOUNDS AND POSIBLY OFF-FLAVOR ACCUMULATION IN LONGKONG DURING STORAGE UNDER ACTIVE MODIFEIED ATMOSPHERE PACKAGING WITH INTERMITTENT WARMING TREATMENT

8.1 Abstract

During long-term storage under active MAP condition, longkong shows undesirable attributes such as brownish color, loss of fruit firmness and high ethanol accumulation resulting in ethanolic-flavor. To reduce those attributes, the intermittent warming (IW) treatment has been introduced. The IW treatment was subjected to longkong in a different time during storage. Interestingly, longkong treated with a cycle of IW treatment to ambient condition in every 2 days showed a good quality with 0.73% weight loss. Under this condition, longkong had the lowest rate of fruit skin color changing. The L* and b* values on the 12th day were 40.14 and 27.10, respectively. They were correlated with high h° angle and C* values. The amount of 3-hydroxy-2-butanone, laevo-linalool and delta-germacrene were maintained by IW treatment. In addition, low concentration of ethanol at 0.21 g/g FW with the highest longkong-like flavor score was noticed.

8.2 Introduction

Fruit is rapidly changed in its quality after harvest. Therefore, optimal storage atmosphere and temperature are required for fruit shelf-life prolonging. Highly perishable fruits need to have an effective tool such as modified atmosphere storage to keep their fresh-like (Bunsiri *et al*., 2006; Meenune and Janthachum, 2004; Teerapawa and Premanode, 1991; Pantastico *et al*., 1968). Even atmospheric modification packaging (MAP) and/or storage under low temperature can be used as an effective tool for prolonging longkong shelf-life, but longkong becomes stress during storage under these conditions for a longer time. Under a stress condition, longkong presents undesirable attributes such as brownish color, off-flavor volatiles (ethanol) that is directly influenced on consumer acceptability (Shi, 2005). Therefore,

to retard longkong from a stress condition, intermittent warming (IW) treatment is introduced. The IW treatment has been applied to alleviate disorder attributes in many fruits (Akbudak and Eris, 2004; Schirra and Cohen, 1999; Artés *et al*., 1998; Fernádez-Trujillo and Artés, 1998). It involves with keeping fruits under low temperature and then transfers them to an ambient condition. However, the potential for reversal varies with length of time under the stress condition. In addition, to re-open the modified atmosphere packaging in order to return the fruit to an ambient condition for a while is considered as an alternative way to reduce anaerobic metabolism. Many studies were focused on longkong shelf-life prolonging by modified atmosphere under low temperature during storage. But, no one has not been solved the problem such as ethanolic-flavor encouraged from modified atmosphere conditions during storage for a longer time. Therefore, the objective of this study was to introduce IW treatment to longkong during storage under active MAP and monitor its quality and possibly off-flavor accumulation.

8.3 Material and Methods

Chemicals

The pyruvate decarboxylase and alcohol dehydrogenase enzymes were obtained from Fluka, Sigma Chemical Co. (St. Louis, MO), Aldrich Chemicals Co. (Milwaukee, WI) and Merck Co. (Darmstadt, Germany). D-glucose, D-fructose, sucrose were purchased from Fluka (Messerchmittstr, Switzerland). Acetonitrile and water (HPLC grade) were obtained from Prolabo (Paris, France). Sodium hydroxide, copper sulfate pentahydrate, trichloroacetic acid and sodium sulfite were obtained from Merck (Darmstadt, Germany). Potassium sodium tartrate, lead acetate and potassium oxalate were obtained from Riedel-deHaen (Seelze, Germany). Dichloromethane was obtained from LabScan Asia Co., Ltd (Bangkok, Thailand). Plate count agar (PCA) for total viable counts was obtained from Merck Co. (Darmstadt, Germany).

Plant material and fruit preparation

Longkong was obtained from a contact garden in Natawee district (Songkhla province, Thailand). The maturity stage of 13 weeks after anthesis was

choosen. Fruit was harvested in the morning and immediately transported on the same day to the Food Analysis laboratory, Faculty of Agro-Industry (approximately 2 hours). Fruits free from any apparent skin damage and uniform size (grade A about 3-3.5 cm in diameter, according to Tanyongmut market) were selected for analysis. Each fruit was individually prepared by cut of raceme, cleaned with brush and submerged into mixed solution of 500 ppm benomyl and 1.5% citric acid for 5 min to reduce microbial load and anti-browning, drying at room temperature (~30°C) for 15 min.

Active modified atmosphere packaging and storage conditions

The 12 individual fruits were placed into a plastic tray. A tray was covered with 0.08 mm thickness nylon laminated with linear Low Density Polyethylene (nylon/LLDPE) bag; size 7x11 inch with permeability to CO_2 and O_2 of 4.7 and 2.6 cm^2/m^3/day at 25°C and 0% RH, respectively. Atmosphere composition inside longkong packages were modified by filling up with ratios of 5%CO_2:5%O_2, (balanced with N_2). After that, all sample bags were stored at 18°C for 24 days.

Intermittent warming (IW) treatment

The IW treatments were introduced to longkong in a different day during storage. The IW treatments were (1) a cycle of IW treatment to ambient condition every 2 days, (2) IW treatment on the 2nd day, (3) IW treatment on the 5th day, (4) IW treatment on the 8th day, (5) IW treatment on the 11th day, (6) IW treatment on the 14th day, (7) IW treatment on the 17th day, (8) IW treatment on the 20th day and (9) IW treatment on the 23rd day. The sample without IW treatment was used as a control (Figure 40). The IW treatment was performed by shifting longkong from modified atmosphere condition to an ambient condition for 15 min. After that, headspace atmospheric was modified to a condition of 5%CO_2:5%O_2 (balanced with N_2) and stored at 18°C for 24 days.
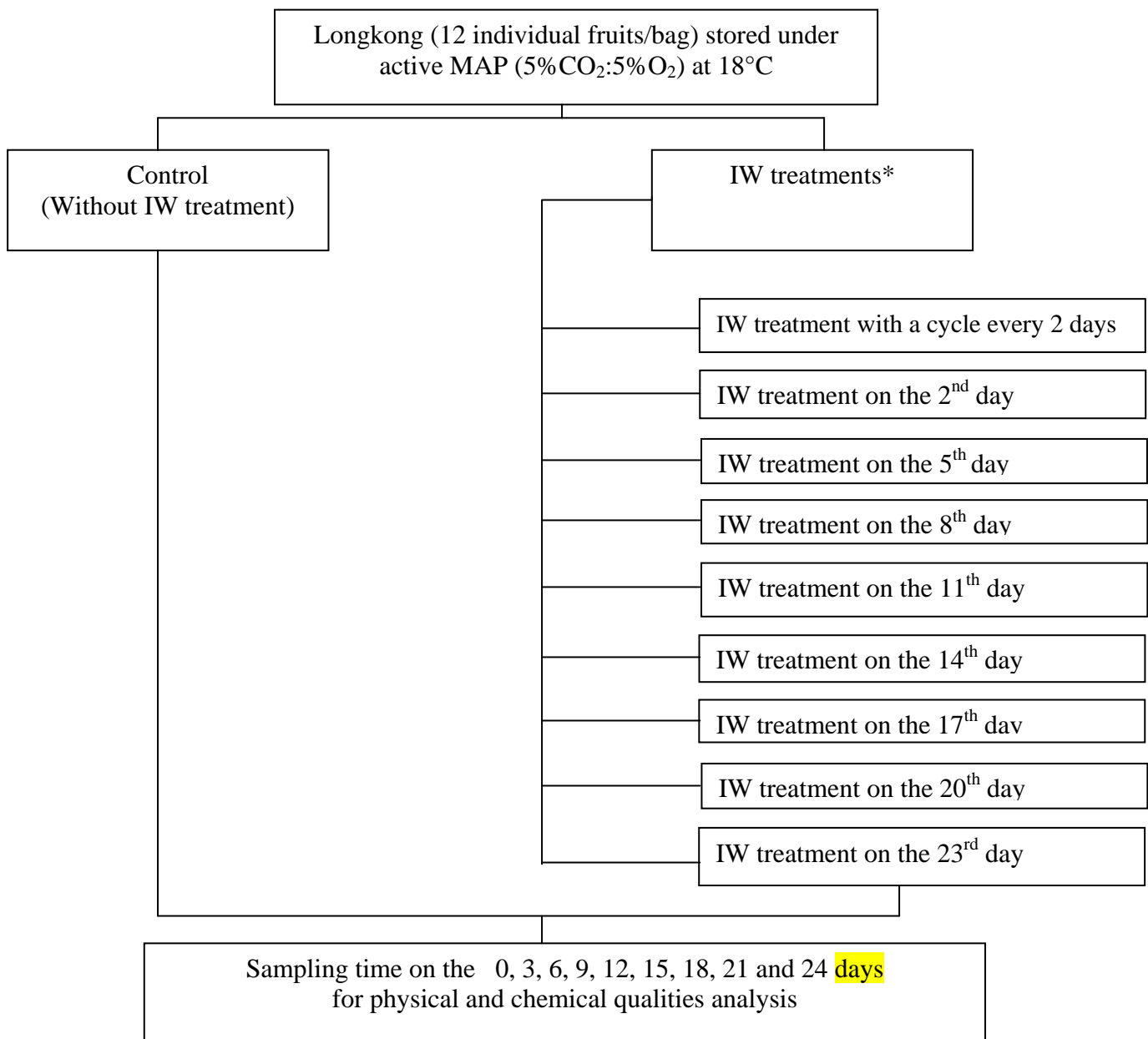
Figure 40 Schematic chart of IW treatment introducing to longkong during storage
under active modified atmosphere packaging on a different day

Note: * The IW treatment was performed by shifting longkong from modified
atmosphere condition to an ambient condition for 15 min. After that,
headspace atmospheric was modified to a condition of $5\%CO_2:5\%O_2$
(balanced with N_2) and stored at 18°C for 24 days.

Fruit quality evaluation

The physical qualities as fruit color, fruit firmness and weight loss were measured in 10 replicates (10 fruits) at 3 day intervals. The chemical qualities such as total soluble solid (TSS), sugars (reducing sugars, total sugars and type and concentration of sugars), titratable acidity (TA), pH, alcohol dehydrogenase (ADH) activity, volatile flavor compounds and ethanol concentration were also evaluated at 3 day intervals.

The homogenate was prepared for chemical analysis. It was done by flesh blending at a constant speed, using a blender for 3 min at 4°C. After that, it was filtered through the stainless steel sieve, yielding the homogenate. The homogenate was fresh prepared and immediately used for chemical analysis. Physical and chemical analysis methods were explained in details as follows.

Fruit color

Two opposite sides of longkong fruit skin were quantified in terms of CIE Lightness (L*), Redness (a*) and Yellowness (b*) values using a Color Flex, Hunter Lab colorimeter. The CIE values were calculated in terms of Hue (h°) angle = artangent b*/a* and Chroma (C* values) = $(a*^2+b*^2)^{1/2}$ (Apai, 2010).

Fruit firmness

Two opposite sides of each fruit was measured by using a TA-XT2i Texture Analyzer (Stable Micro System, UK) equipped with a 2 mm diameter cylinder probe (P/2). The penetrometric method was applied. The results were expressed as gram force (Adapted from Sapii *et al*., 2000).

Fruit weight

The fruit weight was evaluated by weighing with a Sartorius BP310S analytical balance.

Total soluble solid (TSS)

The TSS was determined using an Atago 1E (Japan) hand refractometer at 25°C and expressed as a percentage (Ong *et al*., 2006).

Total and reducing sugars

The 25 g of the longkong homogenate and 100 ml of distillated water were mixed and clarified by 45% neutral lead acetate (2 ml) and 22% potassium oxalate (2 ml). The sample volume was adjusted to 250 ml with distillated water and filtered through filter paper No 1. The total sugar and reducing sugar content was quantified by titration with Fehling's reagents according to Lane and Eynon (A.O.A.C., 2000).

Titratable acidity

The titratable acidity (TA) was quantified by titrating 10 ml of the homogenate to an end point of pH 8.2 with 0.1 N NaOH with 1% (v/v) phenolphthalein as an indicator. The result was calculated as a percentage of citric acid (Ong *et al*., 2006).

pH

pH was measured using a Sartorius PB-20 (Germany) digital pH meter (Adapted from Ong *et al*., 2006).

Types and concentrations of sugars

Types and concentrations of sugars (sucrose, fructose and glucose) were determined by HPLC (Shimadzu, CR 6A Chromatopac) with a Hypersil APS-2 column. For sample preparation, the homogenate was clarified by using refrigerated centrifugation (4ºC) at 10,000×g for 30 min. Clear solution of longkong juice was obtained. The clear solution was filtered through 0.45 μm pore size membrane filters and was kept at -20°C until analysis. The injection volume was 10 μl with an isocratic flow rate of 1 ml/min and a refractive index detector. The 90% acetonitrile was used as a mobile phase. Their concentrations were quantified by comparing the retention times and peak areas of the samples with sucrose, fructose and glucose standards (Adapted from Chyau *et al*., 2003; Ong *et al*., 2006; Soares *et al*., 2007).

Volatile flavor compound isolation by direct solvent extraction (DSE) and identification by GC-MS

Volatile flavor compounds were extracted by direct solvent extraction and identified by GC-MS. The 100 ml clear solution of longkong juice was mixed with 100 ml of dichloromethane. Next, the mixture was gentle shaken for 90 min and left equilibrium for 30 min at room temperature (~30°C). The solvent phase was collected and re-extracted twice. The solvent phase was dried on anhydrous Na_2SO_4, kept overnight at -20°C, cold- filtered and concentrated using purging nitrogen gas. The concentrate was kept at -20°C prior to analysis. The 1 μl was directly injected into GC-MS (Adapted from Flores *et al.*, 2002; Elss *et al.*, 2005; Ong *et al.*, 2006).

The volatile flavor compounds were identified by GC-MS. A chromatograph, Hewlet-Packard 6890 (Palo Alto, CA, USA), was used with a HP-FFAP column (25 m×0.20 mm; film thickness 0.20 mm). The inlet temperature was set at 240°C and the column temperature was maintained at 40°C for 2 min, followed by an increase to 240°C at the rate of 5°C/min and held for 13 min. The carrier gas was applied by ultra high purity helium at a constant flow of 1.5 ml/min. A Hewlet-Packard 5973 A mass spectrometer with a quadrupole mass filter was coupled to the GC. A mass spectrum (MS) was obtained at 70 eV in the electronic impact (EI) and positive modes. The MS was scanned in the range m/z 40-350 at 1 s intervals (Adapted from Wanakhachornkrai and Lertsiri, 2003).

Headspace gas composition analysis

Changes in the headspace gas composition were monitored by gas chromatography (GC) coupling with a thermal conductivity detector (TCD-detector). The 1 ml of headspace gas was directly withdrawn from the headspace inside the package and subsequently injected into Poropak N columns. Helium at flow rate of 50 ml/min was used as the carrier gas. The temperature oven and injection port was held at 60°C. The temperature TCD detector was held at 150°C. The internal package atmosphere was identified and quantified by comparison with external standard gas (Adapted from Tano *et al.*, 2007).

Ethanol isolation by headspace-solid phase microextraction (HS-SPME) and identification by GC-FID

The ethanol content in longkong was extracted by headspace-solid phase microextraction (HS-SPME). The extraction was done as follows; the 100 g of the flesh of longkong was mixed with 100 ml of 20% NaCl (cold solution). It was blended at a constant speed using a blender for 3 min. It was then filtered through a stainless steel sieve. This process produced the homogenate. The 20 ml homogenate was placed into a 125 ml vial followed by the addition of 3 g of NaCl and fitted with a rubber septum. The vial was kept at 30ºC and left equilibrates for 60 min.

Equilibrated ethanol in the headspace was isolated by using 50/30 μm DVB/CAR/PDMS (divinylbenzene/Carboxen on poly (dimethylsiloxane) (Supelco, Bellafonte, PA, USA). The equilibrated ethanol in the headspace was adsorbed for 15 min at room temperature (~ 30°C) and desorbed for 5 min in GC-FID (PerkinElmer, Autosystem XL, USA) (Adapted from Lara *et al*., 2006).

The ethanol was analyzed by GC-FID. The apparatus was equipped with an Rtx-5 (Restek) column (30 m×0.25 mm; film thickness 0.25 μm). The carrier gas was ultra high purity helium at a constant flow of 1.5 ml/min. The injector was kept at 240°C and set for the splitless mode. The column temperature was set at 35°C for 3 min and then programmed to 230°C at the rate of 8°C/min and held for 2 min. The ethanol concentration was calculated using standard aqueous solutions of ethanol under the same conditions as were used for the samples (Adapted from Lara *et al*., 2006).

ADH enzyme extraction and ADH activity evaluation

The 100 mg of longkong flesh was homogenized in 1 ml of extracted solution. The extract solution contained 85 mM 2-(N-mor-pholino) ethane-sulfonic acid (MES) buffer, with a pH 6.0, 5 mM dithiothreitol (DTT) and 1% (w/v) polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged at 25000×g for 15 min at 4°C to recover the supernatant. The supernatant was kept in ice as crude enzyme extract (Adapted from Lara *et al*., 2006).

The ADH activity was assayed by mixing 2.55 ml NADH solution (0.150 mM NADH in 85 mM MES, pH 6.0), 150 μl of acetaldehyde solution (80 mM

acetaldehyde in 85 mM MES, with a pH 6.0), and 300 µl enzyme extract. The ADH activity was measured every 1 min by the spectrophotometer technique at 340 nm. The results were expressed as specific activity (U/mg protein) (Lara *et al*., 2006). The total protein in the extract was determined according to the method described by Bradford (1976).

Sensory evaluation

Longkong-like flavor was evaluated by using a numeric scale nine point (0=no longkong-like flavor and 9=intense longkong-like flavor). The scale was estimated by 15 experienced panelists who like to consume longkong. Samples were coded with 3-digit random number and presented to panelist in random order. Panelists were instructed to consume pieces of longkong and rinsed mouth with water between sample evaluations (Adapted from Pelayo *et al*., 2003).

Microbiological determination

The microbial was determined at the beginning and the end of storage. Homogenized longkong flesh (de-seed) 25g was combined with 225 ml of sterile 0.1% peptone water in a sterile polyethylene bag. The sample was pummeled with a stomacher (Seward Stomacher 400, UK) for 2 min using medium speed. The aliquot was used for the appropriate dilution.

The total viable counts were determined by pouring 1 ml of diluted sample onto plate count agar (PCA). The plates were incubated at 35°C for 48 hr, and the colonies were counted. The microbial counts were expressed in CFU/g of longkong (Koide and Shi, 2007).

Statistical analysis

The experiment was performed using a completely randomized design (CRD) with a factorial treatment structure. A factorial treatment was performed with a structure (10 levels of a different day to treat with IW treatment × 8 levels of storage times). Significant differences between the means were estimated by Duncan's new multiple range test (DMRT), with a level of significance of 0.05. The statistical

analyses were performed by using the Statistical Package for Social Science (SPSS 11.0 for windows (SPSS Inc., Chicago, IL, USA).

8.4 Results and Discussion

Visible mold growth on longkong fruit skin was used as a judgment for ending storage. Longkong stored under active MAP+IW treatment at 18°C was 24 days. The interaction between a different day of IW treatment and storage time was significantly affected on longkong quality (p<0.05). The IW treatment was directly affected on quality decay. Longkong treated with IW treatment with a cycle to ambient condition in every 2 days showed a slowly changing in fruit skin color, maintained unique longkong-like flavor as well as produced low concentration of ethanol accumulation. The almost constant of headspace of O_2 and CO_2 was associated with a cycle of IW treatment to ambient condition in every 2 days. Under this condition, high concentration of headspace CO_2 was eliminated. It had better not accumulate too much of CO_2 concentration. In addition, IW treatment by explosion longkong to an ambient condition for 15 min left the core temperature of longkong equilibrates to room temperature (~30°C). The results were reported and discussed as follows.

8.4.1 Physical quality

Lost on physical quality of longkong was noticed throughout storage time. Fruit skin color became brownish, fruit firmness also declined with increases in fruit weight loss in all conditions. However, changes in physical quality of longkong showed a significantly different with a different day of IW treatment and storage time (p<0.05).

Fruit Color

Fruit color is one of the most important visual attributes in longkong. It was measured in CIE L*, a* and b* values. They represent for lightness, redness and yellowness, respectively. In addition, fruit color was interpreted and presented in terms of Hue (h°) angle and Chroma (C* value). Figures 41 and 42 show the changes in fruit color throughout storage time. The results showed that, longkong lost on its bright yellowness and became to brownish which was indicated by decreases in L*

and b* values while increases in a* value. The L* value significantly decreased from 62.00 to a range of 31.59-32.87 at the end of storage longkong under active MAP+IW treatment for 24 days (Figure 41A). At the end of storage, the highest L* value (L* value=32.88) was found in longkong treated with a cycle of IW treatment to ambient condition in every 2 days. But, it was a non-significant with control (longkong without IW treatment (L* value=32.87) (p≥0.05). Longkong treated with a cycle of IW treatment to ambient condition in every 2 days shows the lowest rate to decrease in L* value as can be seen in Figure 41A. Lost on longkong yellowness and more brownish becomes indicated by a significantly decreases in b* with increases in a* values (p<0.05). The b* value significantly decreased from 30.75 to a range of 15.71-18.07 (Figure 41B). In addition, the significantly increases in a* value from 5.75 to a range of 15.09-19.18 was observed (p<0.05) (Figure 41C). At the end of storage, the highest b* value (b* value=16.97) and the lowest a* value (a* value=16.80) was found in longkong treated with a cycle of IW treatment to ambient condition in every 2 days. The control had a significant higher b* value (b* value=18.07) and a lower a* value (a* value=15.09) than that longkong under a cycle of IW treatment to ambient condition in every 2 days.

However, longkong treated with a cycle of IW treatment to ambient condition in every 2 days shows the lowest changing rate of b* and a* values (Figures 2B and C). In addition, longkong skin color was interpreted in terms of Hue (h°) angle and Chroma (C* value). Figure 42 shows the decrease in both of h° angle and C* values in longkong during storage under active MAP+IW treatment for 24 days. Decreases in h° angle and C* values represents fruit skin color shifts from yellowness to redness (Apai, 2010). A decrease in h° angle and C* values in longkong showed a positive correlation with L*, a* and b* values. The h° angle continuously decreased from 79.41 to a range of 40.98-45.30 at the end of storage longkong under active MAP+IW treatment for 24 days (Figure 42A). The C* values also decreased until the end of storage (p<0.05). They significantly decreased from 31.28 to a range of 21.95-26.00 at the end of storage longkong under active MAP+IW treatment for 24 days (Figure 42B). The lowest rates of h° angle and C* values changes were noticed in longkong treated with a cycle of IW treatment to ambient condition in every 2 days.

To eliminate high CO_2 accumulation, IW treatment was introduced in this study. A cycle of IW treatment to ambient condition in every 2 days was an effective condition to release CO_2 stress in longkong compare to other treatments. Changes in brownish longkong were retarded. Brownish longkong is associated by enzymatic browning, namely, polyphenol oxidase. It interacts with phenolic compounds and represents a darken fruit skin (Sacher, 1962; Azevedo *et al.*, 2008; Venkatachalam and Meenune, 2012).
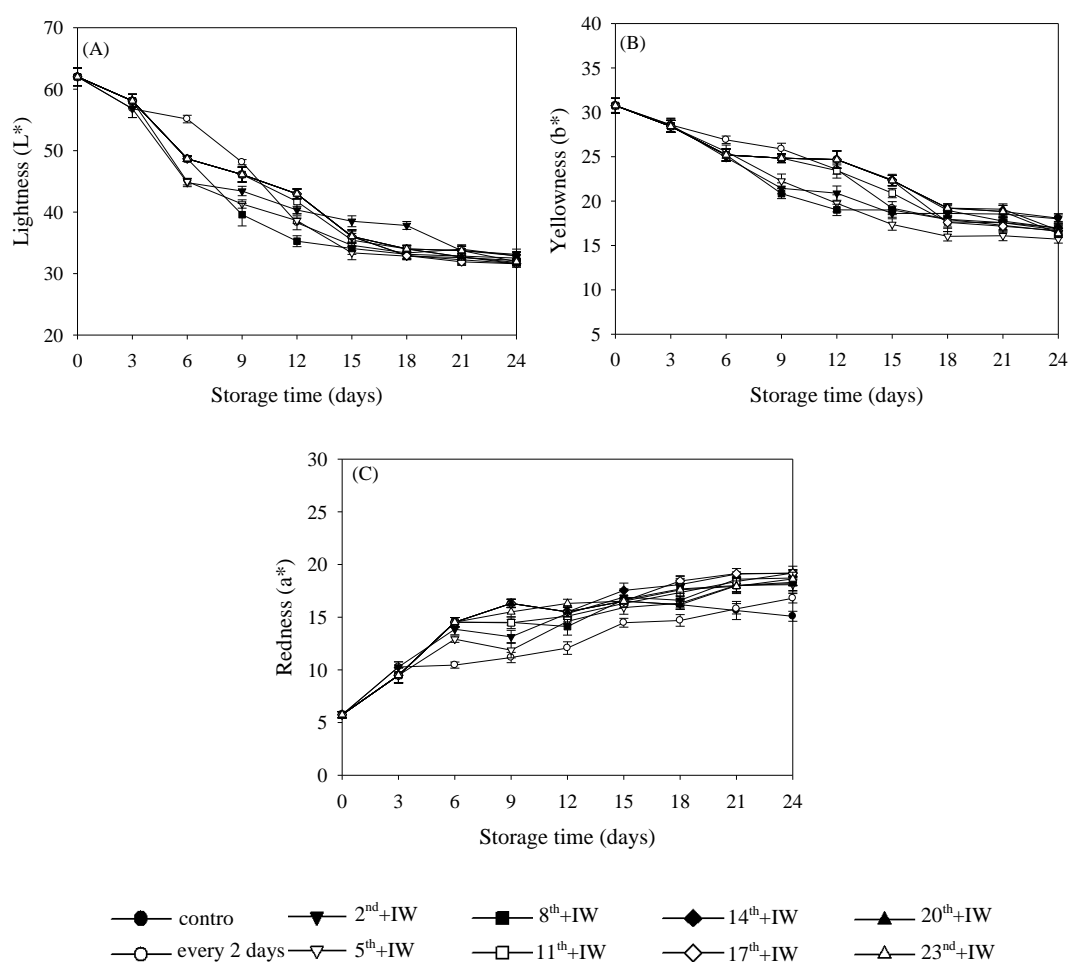
Figure 41 Lightness (A), yellowness (B) and redness (C) values of longkong during
storage under active modified atmosphere packaging on a different day of
intermittent warming treatment

Note: Data are mean $\pm$ standard deviation (in ten replicates)
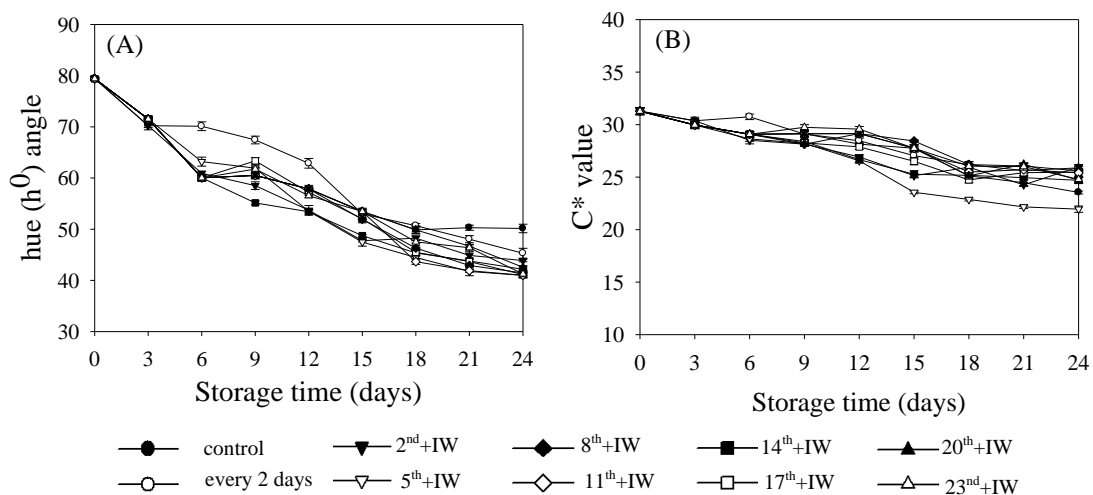
Figure 42 Hue (h°) angle and Chroma (C* value) of longkong during storage under active modified atmosphere packaging on a different day of intermittent warming treatment

Note: Data are mean $\pm$ standard deviation (in ten replicates)

The enzymatic browning is normally alerted by sufficient O_2 under atmospheric condition. To inhibit the enzymatic browning activity, atmospheric modification by low O_2 and/or high CO_2, was introduced. However, long-term storage under atmospheric modification leaded to CO_2 stress in fruits damaged cell membrane. The leakage of cell membrane could potentially leads to an oxidative reaction between enzyme and substrate, resulting in fruit darkens (Castro *et al*., 2008).

Fruit firmness

Lost in fruit firmness of longkong during storage continuously occurred as can be seen in Figure 43. The interaction between a different day of IW treatment and storage time had a significant effect on fruit firmness ($p<0.05$). A slightly significant different of fruit firmness among IW treatments was observed. It decreased from 1,952 g to a range of 1,413-1,458 g at the end of storage longkong under active MAP+IW treatment for 24 days. At the end of storage, the highest fruit firmness was 1,458 g which was found in longkong treated with a cycle of IW

treatment to an ambient condition in every 2 days. It was probably due to a releasing of an excessively high CO_2 accumulation by IW treatment in every 2 days.

Normally, water loss and the resultant of turgor decline might contribute to the more rapid soft on the stored fruits (Ali et al., 2004). It was in agreement with water loss which was found in longkong during storage under active MAP+IW treatments. In addition, long-term storage under high CO_2 leads to high CO_2 stress in fruit cell, resulting in fruit softening. The softening involves the compositional changes by the action of hydrolytic enzyme (Fischer and Bannett, 1991). High CO_2 stress damages cell membrane. The leakage of cell membrane could potentially lead to a reaction between hydrolytic enzyme and complex carbohydrates in cell wall structure (Ali et al., 2004).
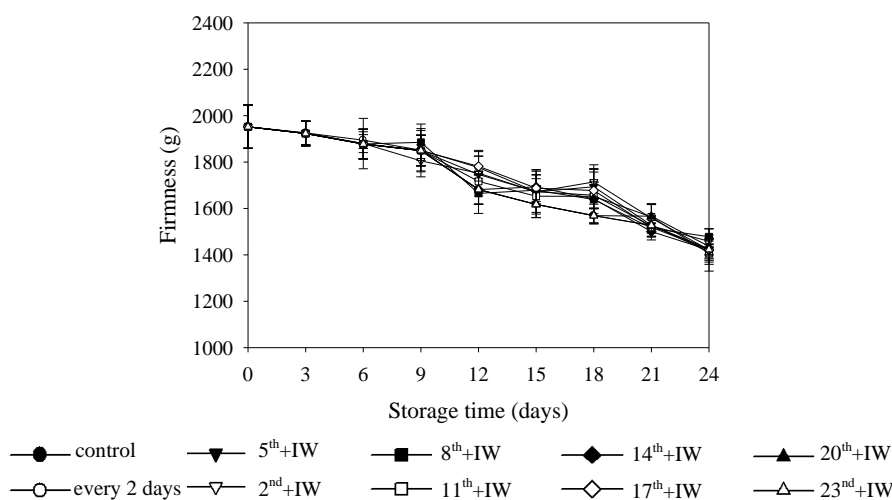
Figure 43 Fruit firmness of longkong during storage under active modified
atmosphere packaging on a different day of intermittent warming treatment

Note: Data are mean $\pm$ standard deviation (in ten replicates).

Weight loss

Weight loss increased throughout storage time ($p < 0.05$). In this study, weight loss in longkong promoted on the 9th day onwards in all conditions (Figure 44). Weight loss continuously increased until the end of storage to reach a range of 0.64-0.73%. Transpiration process exhibits loss of water, resulting in weight loss. Water in fruit might be lost through the stomata, the cracks and the surface damage

(Cohen *et al*., 1994). According to the study on pericarp ultrastructural changes undertaken by Venkatachalam (2013) showed that longkong had much more epidermal hair changes during storage under passive MAP at 18°C. It was an evidence to support epidermis damage appeared. It might be the route for water loss in longkong. The highest weight loss was noticed in longkong treated with a cycle of IW treatment to an ambient condition in every 2 days. It was probably due to a lot of water being lost when fruit expose to an ambient condition.

Figure 44 Weight loss of longkong during storage under active modified atmosphere packaging on a different day of intermittent warming treatment

Note: Data are mean $\pm$ standard deviation (in four replicates).

8.4.2 Chemical quality

During storage under active MAP+IW treatment for 24 days, changes in chemical qualities in longkong were monitored. The interaction between a different day of IW treatment and storage time was significantly affected on chemical quality changes ($p < 0.05$). The results were reported and discussed as follows.

Total soluble solid (TSS) and sugars

During storage under active MAP+IW treatment for 24 days, the sugar and total soluble solid (TSS) in longkong was decreased (Figure 45). The interaction between a different day of IW treatment and storage time was significantly affected

on the sugar and TSS changes (p<0.05). During 12 days of storage, the reducing sugar significantly increased from 3.15% to a range of 3.80-4.82%, after that its concentration decreased until the end of storage (Figure 45A). At the end of storage longkong under active MAP+IW treatment for 24 days, the reducing sugar remained in a range of 2.00-2.88% (Figure 45A). The increases in reducing sugar could be explained by inversion reaction of sucrose. Sucrose, a disaccharide composed of a glucose and fructose molecule, is a typically source of carbon for glycolytic pathway. Sucrose is a non-reducing sugar that less reactive molecule than the reducing sugars (glucose and fructose), thus, the inversion reaction to form glucose and fructose by invertase enzyme occurs (Knee, 2002). Finally, sugars (non-reducing and reducing sugar) act as a carbon source for fruit respiration process (Ong *et al*., 2006). The total sugar in longkong was decreased until the end of storage in all conditions. The total sugar significantly decreased from 17.17% to a range of 9.00-10.43% (Figure 45B).

The TSS increased positively correlate with the increase in reducing sugar during 12 days of storage in all conditions. After that, the TSS significantly declined to a range of 12.30-13.25% at the end of storage longkong under active MAP+IW treatment for 24 days. The TSS decreased probably due to a reduction of sugar for fruit respiration (Ong *et al*, 2006; Sharaf and El-Saadery, 1996). The highest concentration of sugar and TSS were found in longkong treated with a cycle of IW treatment to ambient condition in every 2 days. It could be explained by a frequent day for doing IW treatment to an ambient condition that induced normal aerobic respiration. Therefore, an appropriated concentration of sugars was consumed for aerobic respiration process. Whereas, under CO_2 stress or anaerobic condition, the pyruvic acid cannot enter to TCA cycle and be oxidized. Therefore, ethanol fermentation was occurred. The ethanol fermentation gives a net yield of 2ATP. It is only one-eighteenth fold that derived when glucose is fully oxidized. Therefore much more sugars must be oxidized under high CO_2 stress (Knee, 2002).

pH and titratable acidity (TA)

Changes in pH and TA in longkong during storage under active MAP+IW treatment for 24 days were monitored. The interaction between a different day of IW treatment and storage time was significantly affected on pH and TA

changes (p<0.05). Changes in pH and TA in longkong are presented in Figure 46. During 12 days of storage in all conditions, slightly increase in pH from 3.95 to a range of 4.10-4.13 was observed (Figure 46A). The TA in longkong significantly decreased during 12 days of storage from 0.78% to a rage of 0.54-0.60% (Figure 46B). As can be seen in Figure 46B, the lowest rate of acid consumption for fruit respiration was found in longkong treated with a cycle of IW treatment to an ambient condition in every 2 days. The TA decreased was probably due to acids were used as a substrate for fruit respiration (Ong *et al*., 2006). Normally, acid consumption for fruit respiration is often found in late-mature fruit or storage fruit under a condition that inhibit glucose-oxidized process (Ong *et al*., 2006). Therefore, a frequent turning longkong to an ambient condition by a cycle of IW treatment in every 2 days induces aerobic respiration (glucose-oxidized process), resulting in highly appropriate concentration of acid in longkong (Figure 46B). The 12th day onwards, TA increased, while pH decreased in longkong in all conditions. The TA significantly increased to a range of 0.62-0.72% at the end of storage longkong under active MAP+IW treatment for 24 days. These phenomenal might be explained by a CO_2-induced drop in pH of cell sap through its accumulation of carbonic acid. In addition, high CO_2 and/or limitation of O_2 inhibit some oxidative enzymes in Krebs cycle such as isocitrate dehydrogenase and succinate dehydrogenase. Therefore, citric acid and succinic acid also accumulated in fruit cell, resulting in increases in TA with pH drop (Knee, 2002).

Types and concentrations of sugars

The dominant sugars in longkong were sucrose, glucose and fructose. Their changes were monitored throughout storage time. The interaction between a different day of IW treatment and storage time was significantly affected on sugar concentrations (p<0.05). Figure 47 shows changes of sugar concentrations in longkong throughout storage time. The initial concentrations of fructose, glucose and sucrose were 2.76%, 3.76% and 9.87%, respectively. The concentration of fructose and glucose sharpen increased during 3 days of storage in all conditions. The results were in agreement with an increase in reducing sugar as previously discussed and demonstrated in Figure 45A. Later on, all sugar concentrations significantly decreased until the end of storage. At the end of storage of longkong storage under active

MAP+IW treatment for 24 days, concentrations of fructose were 1.63-2.69% (Figure 47A), glucose were 0.30-0.50% (Figure 47B) and sucrose were 0.25-0.84% (Figure 47C). The increases in glucose and fructose in early storage time could be explained by the inversion reaction of sucrose. Sucrose, a disaccharide composed of a glucose and fructose molecule, is a typically source of carbon for glycolytic pathway. Sucrose is a non-reducing sugar that has less reactive molecules than the reducing sugars (glucose and fructose). Therefore, the hydrolysis reaction to form glucose and fructose by invetase enzyme occurs (Knee, 2002). Finally, sugars (sucrose, glucose and fructose) act as a carbon source for fruit respiration process (Ong $et\ al.$, 2006). The highest concentration of all sugars was noticed in longkong treated with a cycle of IW treatment to ambient condition in every 2 days. It could be supported by frequent turning longkong to an ambient condition induces normal aerobic respiration and appropriated concentration of sugars are consumed for aerobic respiration process. Whereas, high CO_2 stress or anaerobic condition, the pyruvic acid cannot enter to TCA cycle and be oxidized. Therefore, the reaction requires energy is provided by ethanol fermentation. The ethanol fermentation gives a net yield of 2ATP. It is only one eighteen that derived when glucose is fully oxidized. Therefore much more sugars must be oxidized to meet the energy requirement of fruit cell under high CO_2 stress (Knee, 2002).

Volatile flavor compounds

Volatile flavor compounds are an extremely important quality in longkong. Their changes were depended on postharvest conditions. Normally, fermentation products such as ethanol and acids were induced by high CO_2 accumulation. The CO_2 stress was normally found in a fruit kept under active MAP for a long-term. To improve and maintain good unique volatile flavor compounds in longkong, IW treatment was introduced. Volatile flavor compounds in longkong during storage under active MAP+IW treatment for 24 days are presented in Tables 29-38. Volatile flavor compounds in longkong included of esters, alcohols, terpenes and their derivatives, acids, ketones and phenols. In early time during storage under active MAP+IW treatment, most of fruity, sweet, floral and herbaceous compounds such as laevo-linalool, 3-hydroxy-2-butanone and delta-germacrene were identified.

After that, these compounds declined with storage time. It might be lacking of acetyl-CoA which was a primary compound to form esters and terpenes under insufficient O_2 condition (Little and Croteau, 1999; Lara *et al*., 2006). In addition, fermented alcohols and acids from anaerobic metabolism were formed. To maintain unique volatile flavor in longkong and elimination of fermented alcohols and acids, the IW treatment was introduced. It was found that, longkong which was treated with a cycle of IW treatment to ambient condition in every 2 days still had many types of volatile flavor compound compare with the longkong stored under active MAP without IW treatment (control). It was probably due to a frequent turning longkong to adequate O_2 in atmospheric condition retain longkong-like volatile flavor compounds in longkong.

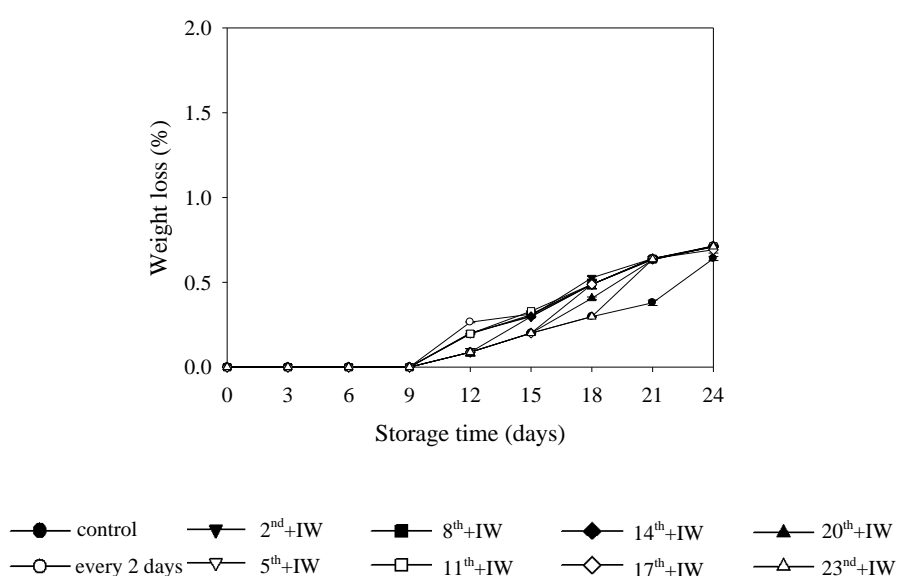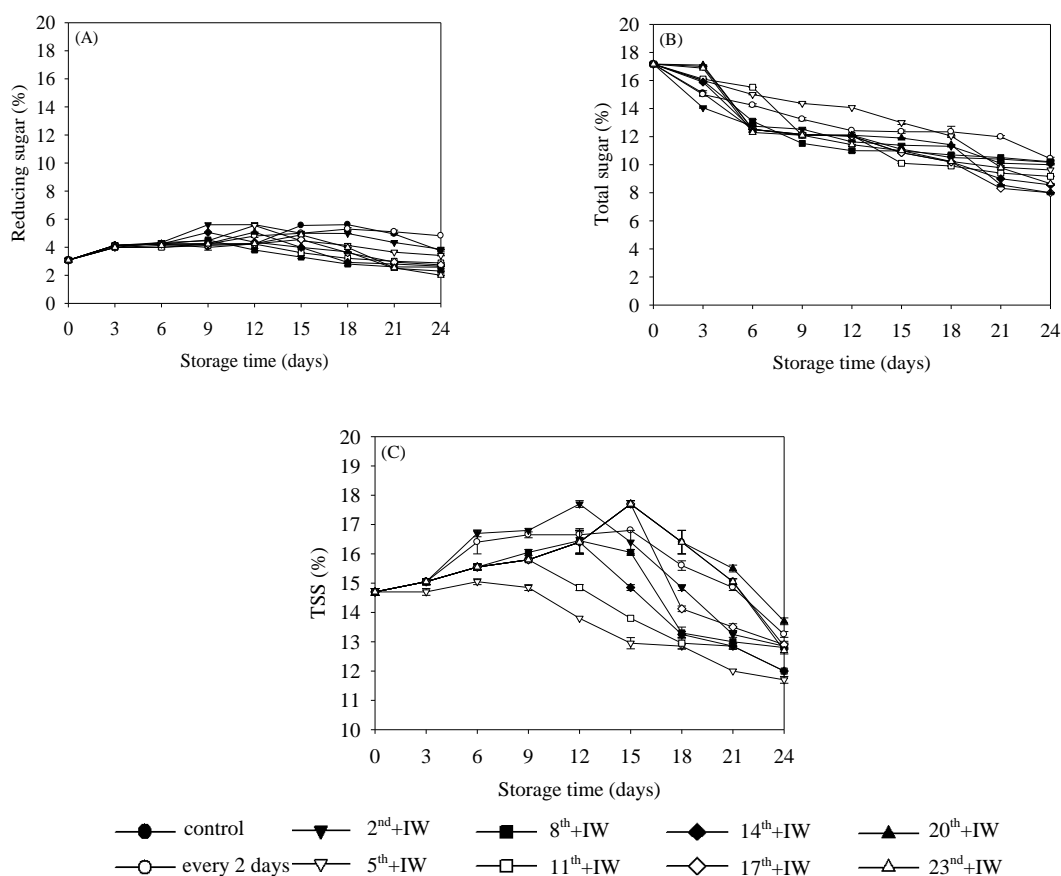**Figure 45** Reducing sugar (A), total sugar (B) and total soluble solid (TSS) (C), of longkong during storage under active modified atmosphere packaging on different day of intermittent warming treatment

Note: Data are mean $\pm$ standard deviation (in four replicates).
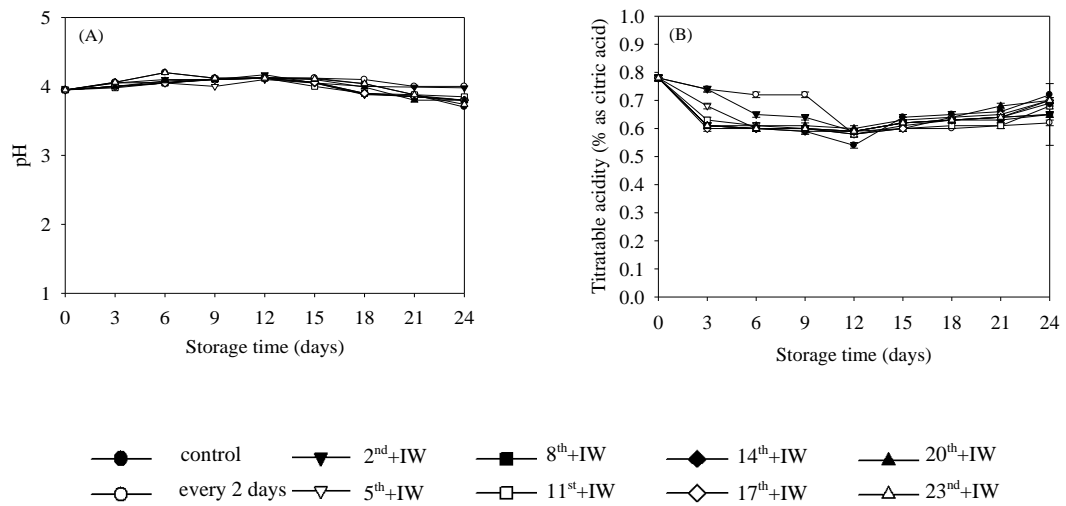
Figure 46 pH (A) and titratable acidity (TA) (B) of longkong during storage under
modified atmosphere packaging with different day of intermittent warming
treatment

Note: Data are mean $\pm$ standard deviation (in four replicates).
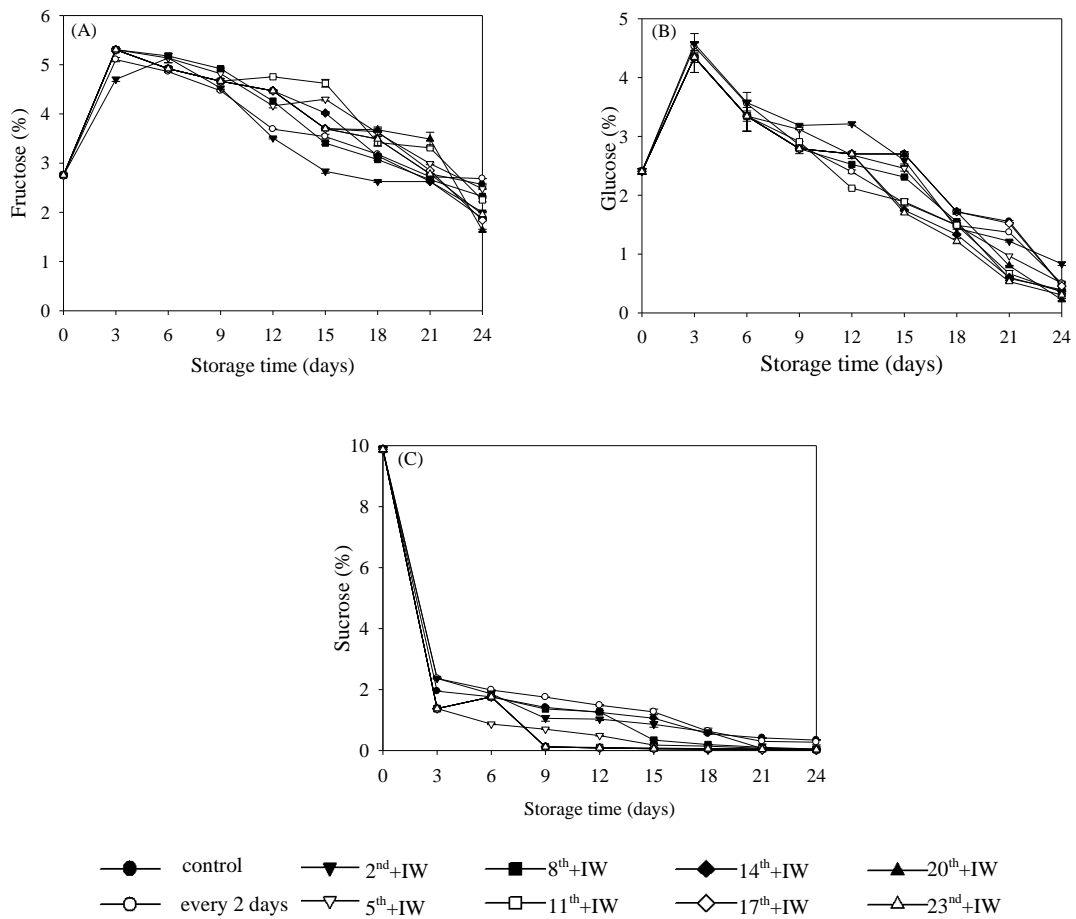
Figure 47 Types and concentrations of sugars content in longkong during storage
under active modified atmosphere packaging on a different day of
intermittent warming treatment

Note: Data are mean $\pm$ standard deviation (in four replicates).

Table 29 Volatile flavor compounds and their attributes in longkong during storage under active modified atmosphere packaging without intermittent warming treatment (control)

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Esters												
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	2.80	13.24	15.17	17.11	19.95	15.83	13.57	9.87	7.33
Alcohols												
2-methyl propanol	4.96	1107	fruity, sweet	nd	nd	6.95	8.14	12.76	15.16	14.23	10.50	6.41
n-butanol	6.19	1157	fruity	1.25	10.11	17.31	27.41	31.83	32.00	32.84	33.08	55.58
iso-amylalcohol	7.72	1218	fermented	0.00	3.24	3.97	4.56	5.97	29.00	33.25	49.94	55.99
n-hexanol	11.05	1347	green	1.25	1.86	2.82	3.00	3.63	17.92	29.83	18.21	12.96
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	1.95	2.17	8.11	15.70	18.14	26.45	9.21	8.73
benzyl alcohol	23.77	1895	floral, sweet	nd	35.25	37.13	39.23	41.17	87.83	38.36	27.86	11.87
phenylethyl alcohol	24.45	2134	floral	nd	4.52	6.96	27.72	35.27	48.00	51.47	40.80	18.15
Terpenes and their derivatives												
cis-linalool oxide	14.39	1479	floral, sweet	nd	nd	5.95	8.01	14.98	54.76	70.27	54.44	42.48
laevo-linalool	16.35	1559	fruity, sweet	nd	nd	13.04	13.65	14.33	66.79	95.00	60.54	54.41
delta-germacrene	19.75	1706	herbaceous	0.82	10.03	12.09	26.81	27.17	71.17	17.19	22.32	10.28
epoxylinalool	21.21	1707	flower	nd	6.77	7.27	8.14	8.96	72.67	22.06	19.03	16.75

Table 29 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Acids												
acetic acid	14.21	1472	pungent, sour	0.50	2.14	7.14	13.56	17.14	36.51	47.20	69.74	78.40
propanoic acid	16.25	1555	pungent	nd	nd	nd	9.11	12.42	15.87	28.48	34.05	37.78
butanoic acid	18.32	1643	sour	nd	5.06	12.11	20.81	25.81	32.70	36.36	40.24	46.36
hexanoic acid	23.07	1862	sour, sweat	nd	nd	nd	nd	24.98	45.83	69.51	74.44	81.90
Ketones												
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	50.33	58.09	61.01	98.78	74.06	38.61	71.60	26.25
Phenol												
phenol	26.38	2027	phenol	2.89	5.34	13.05	43.81	62.72	54.12	41.26	23.00	14.80

Note: [A] Rt = Retention time (min);   [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = Total ion current; nd = Not detected

Table 30 Volatile flavor compounds and their attributes in longkong during storage under active modified atmosphere packaging with intermittent warming treatment in every 2 days (every 2 days)

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D] Storage time (days)								
				0	3	6	9	12	15	18	21	24
Esters												
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	2.80	28.87	27.89	27.66	24.98	22.82	21.87	14.47	13.71
Alcohols												
2-methyl propanol	4.96	1107	fruity, sweet	nd	15.46	18.39	23.14	16.61	14.42	10.10	nd	nd
n-butanol	6.19	1157	fruity	1.25	5.67	6.61	10.22	17.82	16.95	nd	nd	nd
iso-amylalcohol	7.72	1218	fermented	0.00	9.24	28.31	29.11	42.40	51.81	39.53	20.00	3.20
n-hexanol	11.05	1347	green	1.25	3.11	5.10	7.45	7.22	18.65	18.10	nd	nd
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	2.09	8.96	12.98	16.22	22.00	nd	nd	nd
benzyl alcohol	23.77	1895	floral, sweet	nd	25.86	35.56	38.41	56.31	32.56	18.16	18.50	10.85
phenylethyl alcohol	24.45	2134	floral	nd	23.87	26.41	28.56	78.85	20.99	28.06	14.48	12.99
Terpenes and their derivatives												
cis-linalool oxide	14.39	1479	floral, sweet	nd	14.37	22.19	26.76	20.06	14.32	5.66	4.95	1.73
alpha-copene	14.58	1486	woody	nd	nd	16.71	19.65	28.76	29.50	29.99	nd	
beta-bourbonene	15.22	1512	herbaceous	nd	nd	9.94	10.22	20.11	12.65	7.23	nd	nd
laevo-linalool	16.35	1559	fruity, sweet	nd	7.17	15.21	18.12	27.05	21.02	12.42	nd	nd
delta-germacrene	19.75	1706	herbaceous	0.82	22.89	34.75	52.11	67.11	31.09	24.27	24.52	21.10
bicyclogermacrene	20.29	1731	woody	nd	nd	7.31	9.04	14.35	15.03	15.10	nd	nd
delta-cadinene	20.84	1756	herbaceous	nd	nd	8.24	15.92	20.00	26.75	29.20	21.68	3.22

Table 30 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Terpenes and their derivatives												
epoxylinalool	21.21	1707	flower	nd	nd	17.69	19.21	24.53	30.57	15.09	8.37	5.49
spathulenol	28.37	2184	herbaceous, fruity	nd	nd	5.15	10.01	14.12	18.11	7.77	7.08	4.30
Acids												
acetic acid	14.21	1472	pungent, sour	0.50	12.09	14.09	35.45	38.54	45.34	61.99	68.29	77.30
propanoic acid	16.25	1555	pungent	nd	nd	nd	nd	nd	14.44	20.01	20.54	nd
butanoic acid	18.32	1643	sour	nd	nd	nd	nd	8.80	16.32	46.26	54.49	64.74
hexanoic acid	23.07	1862	sour, sweat	nd	nd	nd	nd	nd	23.75	29.02	32.22	41.46
Ketones												
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	27.79	69.14	66.51	64.20	71.64	63.85	25.32	6.90
Phenol												
phenol	26.38	2027	phenol	2.89	12.45	20.72	28.45	34.21	40.01	52.33	21.01	10/00

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = Total ion current; nd = Not detected

Table 31 Volatile flavor compounds and their attributes in longkong during storage under active modified atmosphere packaging with intermittent warming treatment on the day 2nd (2nd+IW)

Compounds	Rt^A	RI^B	$Attribute^C$	Peak areas ($\times 10^4$) TIC^D								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Esters												
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	2.80	28.87	30.11	33.28	41.28	59.95	81.18	76.96	48.36
Alcohols												
2-methyl propanol	4.96	1107	fruity, sweet	nd	15.46	16.18	26.81	31.05	21.54	14.76	10.58	3.67
n-butanol	6.19	1157	fruity	1.25	5.67	6.00	7.93	18.14	22.34	30.33	16.56	7.61
iso-amylalcohol	7.72	1218	fermented	0.00	6.24	7.48	8.19	23.00	28.77	21.58	17.37	5.54
n-hexanol	11.05	1347	green	1.25	3.11	5.01	9.43	12.48	32.86	15.31	7.09	5.27
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	2.09	15.68	21.31	33.00	56.14	12.17	7.41	4.44
benzyl alcohol	23.77	1895	floral, sweet	nd	25.86	38.05	50.83	62.00	89.99	96.63	67.73	64.60
phenylethyl alcohol	24.45	2134	floral	nd	23.87	40.09	58.94	59.00	59.98	95.01	65.00	52.38
Terpenes and their derivatives												
cis-linalool oxide	14.39	1479	floral, sweet	nd	14.37	18.21	18.93	24.93	26.35	10.99	4.62	nd
alpha-copene	14.58	1486	woody	nd	nd	10.37	15.97	14.29	11.75	10.27	5.85	nd
beta-bourbonene	15.22	1512	herbaceous	nd	nd	nd	nd	21.41	32.85	60.06	35.00	10.79
laevo-linalool	16.35	1559	fruity, sweet	nd	7.17	10.00	12.69	16.73	38.24	31.13	13.12	2.11
delta-germacrene	19.75	1706	herbaceous	0.82	22.89	36.21	50.88	62.67	78.50	31.98	21.53	12.19
bicyclogermacrene	20.29	1731	woody	nd	nd	nd	12.67	21.05	8.70	7.92	7.21	6.98
delta-cadinene	20.84	1756	herbaceous	nd	nd	2.12	5.27	12.45	7.14	5.49	3.42	2.39

Table 31 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Terpenes and their derivatives												
epoxylinalool	21.21	1707	flower	nd	nd	8.45	17.45	45.55	15.11	6.21	5.87	4.53
spathulenol	28.37	2184	herbaceous, fruity	nd	nd	nd	2.06	10.47	16.02	19.62	12.00	7.01
Acids												
acetic acid	14.21	1472	pungent, sour	0.50	12.09	27.75	28.06	54.47	55.42	82.99	90.91	96.48
propanoic acid	16.25	1555	pungent	nd	nd	nd	nd	nd	20.08	23.01	28.14	-
butanoic acid	18.32	1643	sour	nd	nd	8.03	12.65	32.04	43.06	46.03	56.11	64.44
hexanoic acid	23.07	1862	sour, sweat	nd	nd	nd	11.32	18.11	25.15	29.02	36.82	51.46
Ketones												
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	27.79	31.45	37.17	48.61	59.77	34.86	22.93	15.58
Phenol												
phenol	26.38	2027	phenol	2.89	12.45	20.72	28.45	31.21	35.01	42.13	20.01	12.00

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = Total ion current; nd = Not detected

Table 32 Volatile flavor compounds and their attributes in longkong during storage under active modified atmosphere packaging with intermittent warming treatment on the day 5th (5th+IW)

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times10^4$) TIC[D] Storage time (days)								
				0	3	6	9	12	15	18	21	24
Esters												
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	2.80	28.87	24.49	25.25	34.84	41.28	81.18	76.96	48.36
Alcohols												
2-methyl propanol	4.96	1107	fruity, sweet	nd	15.46	22.85	31.00	23.93	14.66	14.76	10.58	3.67
n-butanol	6.19	1157	fruity	1.25	5.67	10.81	15.21	24.22	6.28	30.33	16.56	7.61
iso-amylalcohol	7.72	1218	fermented	0.00	6.24	35.49	48.32	57.83	34.04	21.58	17.37	5.54
n-hexanol	11.05	1347	green	1.25	3.11	4.32	5.01	10.32	12.06	15.31	7.09	5.27
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	2.09	4.56	15.99	19.65	22.76	12.17	7.41	4.44
benzyl alcohol	23.77	1895	floral, sweet	nd	25.86	40.86	54.50	58.00	64.50	96.63	67.73	64.60
phenylethyl alcohol	24.45	2134	floral	nd	23.87	44.19	65.31	68.51	73.28	95.01	65.00	52.38
Terpenes and their derivatives												
cis-linalool oxide	14.39	1479	floral, sweet	nd	14.37	15.55	19.66	28.53	9.68	10.99	4.62	nd
alpha-copene	14.58	1486	woody	nd	nd	8.47	9.63	10.83	13.21	10.27	5.85	nd
beta-bourbonene	15.22	1512	herbaceous	nd	nd	5.98	11.67	38.57	49.99	60.06	35.00	10.79
laevo-linalool	16.35	1559	fruity, sweet	nd	7.17	9.03	14.21	18.88	19.35	31.13	13.12	2.11
delta-germacrene	19.75	1706	herbaceous	0.82	22.89	41.05	57.38	58.01	43.96	31.98	21.53	12.19
bicyclogermacrene	20.29	1731	woody	nd	nd	11.39	20.31	18.84	9.12	7.92	7.21	6.98
delta-cadinene	20.84	1756	herbaceous	nd	nd	18.31	21.08	30.09	8.93	5.49	3.42	2.39

Table 32 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Terpenes and their derivatives												
epoxylinalool	21.21	1707	flower	nd	nd	22.67	24.00	32.26	11.04	6.21	5.87	4.53
spathulenol	28.37	2184	herbaceous, fruity	nd	nd	7.07	9.56	11.48	12.54	19.62	12.00	7.01
Acids												
acetic acid	14.21	1472	pungent, sour	0.50	12.09	22.43	35.13	36.78	38.81	74.48	81.90	98.18
propanoic acid	16.25	1555	pungent	nd	nd	nd	nd	13.41	22.99	21.02	28.14	30.00
butanoic acid	18.32	1643	sour	nd	nd	5.75	10.26	14.02	19.52	26.03	54.11	64.44
hexanoic acid	23.07	1862	sour, sweat	nd	nd	nd	nd	20.04	28.11	29.02	36.82	51.46
Ketones												
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	27.79	36.18	51.64	60.03	32.55	34.86	22.93	15.58
Phenol												
phenol	26.38	2027	phenol	2.89	12.45	19.03	41.00	50.01	54.90	42.13	20.01	10.00

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = Total ion current; nd = Not detected

Table 33 Volatile flavor compounds and their attributes in longkong during storage under active modified atmosphere packaging with intermittent warming treatment on the day 8th (8th+IW)

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D] Storage time (days)								
				0	3	6	9	12	15	18	21	24
Esters												
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	2.80	28.87	24.49	25.25	28.22	13.13	10.17	6.87	nd
Alcohols												
2-methyl propanol	4.96	1107	fruity, sweet	nd	15.46	22.85	31.00	42.22	35.14	24.63	13.00	nd
n-butanol	6.19	1157	fruity	1.25	5.67	10.81	15.21	18.91	12.00	12.84	3.08	nd
iso-amylalcohol	7.72	1218	fermented	0.00	6.24	35.49	48.32	55.51	29.00	38.15	59.24	89.11
n-hexanol	11.05	1347	green	1.25	3.11	4.32	6.03	5.01	13.42	25.50	15.21	6.55
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	2.09	4.56	15.99	16.34	17.55	22.25	7.21	nd
benzyl alcohol	23.77	1895	floral, sweet	nd	25.86	40.86	54.50	47.54	67.77	68.06	68.16	69.61
phenylethyl alcohol	24.45	2134	floral	nd	23.87	44.19	65.31	63.36	78.00	81.41	85.00	92.51
Terpenes and their derivatives												
cis-linalool oxide	14.39	1479	floral, sweet	nd	14.37	15.55	19.66	23.91	41.26	60.11	14.14	6.30
alpha-copene	14.58	1486	woody	nd	nd	8.47	9.63	14.54	56.09	45.00	26.54	2.23
beta-bourbonene	15.22	1512	herbaceous	nd	nd	5.98	11.67	16.76	44.17	14.19	12.32	5.85
laevo-linalool	16.35	1559	fruity, sweet	nd	7.17	9.03	14.21	18.21	52.67	26.06	16.13	nd
delta-germacrene	19.75	1706	herbaceous	0.82	22.89	41.05	57.38	53.21	55.77	30.01	10.02	nd
bicyclogermacrene	20.29	1731	woody	nd	nd	11.39	20.31	37.09	40.09	30.54	24.23	11.32
delta-cadinene	20.84	1756	herbaceous	nd	nd	18.31	21.08	28.96	35.48	32.02	25.87	19.95

Table 33 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Terpenes and their derivatives												
epoxylinalool	21.21	1707	flower	nd	nd	22.67	24.00	27.00	35.78	13.41	nd	nd
spathulenol	28.37	2184	herbaceous, fruity	nd	nd	7.07	9.56	11.43	18.99	10.67	nd	nd
Acids												
acetic acid	14.21	1472	pungent, sour	0.50	12.09	22.43	35.13	41.49	58.67	77.99	80.00	83.42
propanoic acid	16.25	1555	pungent	nd	nd	nd	nd	13.64	50.23	65.76	78.54	88.03
butanoic acid	18.32	1643	sour	nd	nd	5.75	10.26	18.96	28.56	53.65	64.87	86.24
hexanoic acid	23.07	1862	sour, sweat	nd	nd	nd	nd	12.22	32.00	41.54	63.21	74.67
Ketones												
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	27.79	36.18	51.64	61.92	48.08	32.09	23.12	12.98
Phenol												
phenol	26.38	2027	phenol	2.89	12.45	19.03	41.00	54.56	39.00	31.01	15.32	13.36

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = Total ion current; nd = Not detected

Table 34 Volatile flavor compounds and their attributes in longkong during storage under active modified atmosphere packaging with intermittent warming treatment on the day 11th (11th+IW)

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Esters												
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	2.80	13.24	15.17	17.11	34.81	12.23	10.00	5.87	nd
Alcohols												
2-methyl propanol	4.96	1107	fruity, sweet	nd	nd	6.95	8.14	24.87	32.24	26.63	11.00	nd
n-butanol	6.19	1157	fruity	1.25	10.11	17.31	27.41	35.65	32.00	24.44	13.02	5.66
iso-amylalcohol	7.72	1218	fermented	0.00	3.24	3.97	4.56	10.64	29.00	38.15	59.24	48.64
n-hexanol	11.05	1347	green	1.25	1.86	2.82	3.00	12.21	10.42	5.50	3.21	nd
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	1.95	2.17	8.11	13.33	17.55	23.25	17.13	nd
benzyl alcohol	23.77	1895	floral, sweet	nd	35.25	37.13	39.23	38.97	57.77	48.13	40.22	38.97
phenylethyl alcohol	24.45	2134	floral	nd	4.52	6.96	27.72	40.62	58.00	61.61	45.00	36.62
Terpenes and their derivatives												
cis-linalool oxide	14.39	1479	floral, sweet	nd	nd	5.95	8.01	13.63	21.17	58.11	13.14	12.60
alpha-copene	14.58	1486	woody	nd	10.03	12.09	26.81	35.12	46.19	35.00	25.04	8.88
beta-bourbonene	15.22	1512	herbaceous	nd	6.77	7.27	8.14	11.81	24.17	34.09	12.32	7.12
laevo-linalool	16.35	1559	fruity, sweet	nd	nd	13.04	13.65	19.78	32.47	20.01	11.13	9.87
delta-germacrene	19.75	1706	herbaceous	0.82	10.03	12.09	26.81	28.28	45.27	30.01	10.02	13.25
bicyclogermacrene	20.29	1731	woody	nd	nd	11.39	20.31	28.43	40.09	30.54	nd	nd
delta-cadinene	20.84	1756	herbaceous	nd	nd	18.31	21.08	26.66	35.48	32.02	25.87	6.28

Table 34 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Terpenes and their derivatives												
epoxylinalool	21.21	1707	flower	nd	6.77	7.27	8.14	10.78	21.28	16.71	nd	nd
spathulenol	28.37	2184	herbaceous, fruity	nd	nd	nd	9.11	11.63	16.69	9.67	2.01	1.15
Acids												
acetic acid	14.21	1472	pungent, sour	0.50	2.14	7.14	13.56	20.00	68.67	80.99	83.33	85.14
propanoic acid	16.25	1555	pungent	nd	nd	nd	9.11	13.73	52.83	68.16	78.54	84.00
butanoic acid	18.32	1643	sour	nd	5.06	12.11	20.81	24.44	30.26	43.15	67.87	76.59
hexanoic acid	23.07	1862	sour, sweat	nd	nd	nd	nd	20.13	35.00	43.14	64.21	75.54
Ketones												
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	50.33	58.09	61.01	63.58	48.08	30.00	21.12	12.79
Phenol												
phenol	26.38	2027	phenol	2.89	5.34	13.05	43.81	51.51	33.00	25.01	10.32	12.02

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = Total ion current; nd = Not detected

Table 35 Volatile flavor compounds and their attributes in longkong during storage under modified atmosphere packaging with intermittent warming treatment on the day 14th (14th+IW)

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Esters												
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	2.80	13.24	15.17	17.11	19.95	12.83	8.00	nd	nd
Alcohols												
2-methyl propanol	4.96	1107	fruity, sweet	nd	nd	6.95	8.14	12.76	15.26	11.13	10.00	nd
n-butanol	6.19	1157	fruity	1.25	10.11	17.31	27.41	31.83	22.00	20.84	10.02	7.02
iso-amylalcohol	7.72	1218	fermented	0.00	3.24	3.97	4.56	5.97	32.00	50.15	51.24	59.94
n-hexanol	11.05	1347	green	1.25	1.86	2.82	3.00	4.13	20.92	14.43	12.21	8.16
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	1.95	2.17	8.11	15.70	18.14	26.45	nd	nd
benzyl alcohol	23.77	1895	floral, sweet	nd	35.25	37.13	39.23	41.17	52.83	62.36	40.22	38.97
phenylethyl alcohol	24.45	2134	floral	nd	4.52	6.96	27.72	45.27	48.00	31.47	25.00	16.62
Terpenes and their derivatives												
cis-linalool oxide	14.39	1479	floral, sweet	nd	nd	5.95	8.01	14.98	34.76	15.66	3.14	-
alpha-copene	14.58	1486	woody	nd	10.03	12.09	26.81	27.76	31.95	22.19	19.04	4.48
beta-bourbonene	15.22	1512	herbaceous	nd	6.77	7.27	8.14	11.81	22.17	30.09	10.32	7.12
laevo-linalool	16.35	1559	fruity, sweet	nd	nd	13.04	13.65	14.33	46.79	21.01	10.13	5.87
delta-germacrene	19.75	1706	herbaceous	0.82	10.03	12.09	26.81	28.28	48.27	26.01	15.32	22.25
bicyclogermacrene	20.29	1731	woody	nd	nd	11.39	20.31	27.17	41.17	30.54	nd	nd
delta-cadinene	20.84	1756	herbaceous	nd	nd	18.31	21.08	26.66	32.48	nd	nd	nd

Table 35 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas (×10⁴) TIC[D] Storage time (days)								
				0	3	6	9	12	15	18	21	24
Terpenes and their derivatives												
epoxylinalool	21.21	1707	flower	nd	6.77	7.27	8.14	10.78	21.28	nd	nd	nd
spathulenol	28.37	2184	herbaceous, fruity	nd	nd	nd	9.11	11.63	16.69	nd	nd	nd
Acids												
acetic acid	14.21	1472	pungent, sour	0.50	2.14	7.14	13.56	17.14	36.51	47.2	85.33	88.14
propanoic acid	16.25	1555	pungent	nd	nd	nd	9.11	12.42	15.87	28.48	81.54	84.00
butanoic acid	18.32	1643	sour	nd	5.06	12.11	20.81	25.81	32.7	36.36	65.87	73.59
hexanoic acid	23.07	1862	sour, sweat	nd	nd	nd	nd	24.98	45.83	69.51	68.21	74.54
Ketones												
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	50.33	58.09	61.01	98.78	74.06	38.61	18.12	9.51
Phenol												
phenol	26.38	2027	phenol	2.89	5.34	13.05	43.81	62.72	54.12	41.26	10.32	12.02

Note: [A] Rt = Retention time (min);   [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = Total ion current; nd = Not detected

Table 36 Volatile flavor compounds and their attributes in longkong during storage under active modified atmosphere packaging with intermittent warming treatment on the day 17th (17th+IW)

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Esters												
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	2.80	13.24	15.17	17.11	19.95	12.83	8.00	nd	nd
Alcohols												
2-methyl propanol	4.96	1107	fruity, sweet	nd	nd	6.95	8.14	12.76	15.26	11.13	10.00	nd
n-butanol	6.19	1157	fruity	1.25	10.11	17.31	27.41	31.83	22.00	20.84	10.02	7.02
iso-amylalcohol	7.72	1218	fermented	0.00	3.24	3.97	4.56	5.97	32.00	50.15	51.24	59.94
n-hexanol	11.05	1347	green	1.25	1.86	2.82	3.00	4.13	20.92	14.43	12.21	8.16
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	1.95	2.17	8.11	15.70	18.14	26.45	nd	nd
benzyl alcohol	23.77	1895	floral, sweet	nd	35.25	37.13	39.23	41.17	52.83	62.36	40.22	38.97
phenylethyl alcohol	24.45	2134	floral	nd	4.52	6.96	27.72	45.27	48.00	31.47	25.00	16.62
Terpenes and their derivatives												
cis-linalool oxide	14.39	1479	floral, sweet	nd	nd	5.95	8.01	14.98	34.76	15.66	3.14	nd
alpha-copene	14.58	1486	woody	nd	10.03	12.09	26.81	27.76	31.95	22.19	19.04	4.48
beta-bourbonene	15.22	1512	herbaceous	nd	6.77	7.27	8.14	11.81	22.17	30.09	10.32	7.12
laevo-linalool	16.35	1559	fruity, sweet	nd	nd	13.04	13.65	14.33	46.79	21.01	10.13	5.87
delta-germacrene	19.75	1706	herbaceous	0.82	10.03	12.09	26.81	28.28	48.27	26.01	15.32	22.25
bicyclogermacrene	20.29	1731	woody	nd	nd	11.39	20.31	27.17	41.17	30.54	nd	nd
delta-cadinene	20.84	1756	herbaceous	nd	nd	18.31	21.08	26.66	32.48	nd	nd	nd

Table 36 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Terpenes and their derivatives												
epoxylinalool	21.21	1707	flower	nd	6.77	7.27	8.14	10.78	21.28	nd	nd	nd
spathulenol	28.37	2184	herbaceous, fruity	nd	nd	nd	9.11	11.63	16.69	nd	nd	nd
Acids												
acetic acid	14.21	1472	pungent, sour	0.5	2.14	7.14	13.56	17.14	36.51	47.2	84.13	87.64
propanoic acid	16.25	1555	pungent	nd	nd	nd	9.11	12.42	15.87	18.48	62.54	84.00
butanoic acid	18.32	1643	sour	nd	5.06	12.11	20.81	25.81	42.7	46.36	68.87	75.31
hexanoic acid	23.07	1862	sour, sweat	nd	nd	nd	nd	24.98	45.83	70.41	72.11	74.54
Ketones												
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	50.33	58.09	61.01	98.78	74.06	48.34	22.12	12.51
Phenol												
phenol	26.38	2027	phenol	2.89	5.34	13.05	43.81	52.72	43.12	30.26	13.32	10.02

Note: [A] Rt = Retention time (min);   [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = Total ion current; nd = Not detected

Table 37 Volatile flavor compounds and their attributes in longkong during storage under active modified atmosphere packaging with intermittent warming treatment on the day 20th (20th+IW)

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Esters												
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	2.80	13.24	15.17	17.11	19.95	15.83	13.57	nd	nd
Alcohols												
2-methyl propanol	4.96	1107	fruity, sweet	nd	nd	6.95	8.14	12.76	15.16	14.23	9.00	nd
n-butanol	6.19	1157	fruity	1.25	10.11	17.31	27.41	31.83	32	32.84	12.02	6.02
iso-amylalcohol	7.72	1218	fermented	0.00	3.24	3.97	4.56	5.97	29	33.25	41.40	49.90
n-hexanol	11.05	1347	green	1.25	1.86	2.82	3.00	3.63	17.92	29.83	10.21	6.16
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	1.95	2.17	8.11	15.7	18.14	26.45	nd	nd
benzyl alcohol	23.77	1895	floral, sweet	nd	35.25	37.13	39.23	41.17	87.83	38.36	30.22	28.97
phenylethyl alcohol	24.45	2134	floral	nd	4.52	6.96	27.72	35.27	48	51.47	25.00	14.62
Terpenes and their derivatives												
cis-linalool oxide	14.39	1479	floral, sweet	nd	nd	5.95	8.01	14.98	34.76	20.27	16.14	nd
laevo-linalool	16.35	1559	fruity, sweet	nd	nd	13.04	13.65	14.33	46.79	21.01	10.13	5.87
delta-germacrene	19.75	1706	herbaceous	0.82	10.03	12.09	26.81	27.17	71.17	17.19	15.32	12.25
bicyclogermacrene	20.29	1731	woody	nd	nd	11.39	20.31	27.17	31.17	20.54	8.61	nd
delta-cadinene	20.84	1756	herbaceous	nd	nd	18.31	21.08	22.66	30.48	nd	nd	nd

Table 37 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Terpenes and their derivatives												
epoxylinalool	21.21	1707	flower	nd	6.77	7.27	8.14	8.96	72.67	22.06	nd	nd
Acids												
acetic acid	14.21	1472	pungent, sour	0.50	2.14	7.14	13.56	17.14	36.51	47.2	85.33	88.14
propanoic acid	16.25	1555	pungent	nd	nd	nd	9.11	12.42	15.87	28.48	81.54	84.00
butanoic acid	18.32	1643	sour	nd	5.06	12.11	20.81	25.81	32.7	36.36	65.87	73.59
hexanoic acid	23.07	1862	sour, sweat	nd	nd	nd	nd	24.98	45.83	69.51	68.21	74.54
Ketones												
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	50.33	58.09	61.01	78.78	74.06	38.61	18.12	9.51
Phenol												
phenol	26.38	2027	phenol	2.89	5.34	13.05	43.81	62.72	54.12	41.26	10.32	12.02

Note: [A] Rt = Retention time (min); [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = Total ion current; nd = Not detected

Table 38 Volatile flavor compounds and their attributes in longkong during storage under modified atmosphere packaging with intermittent warming treatment on the day 23rd (23rd+IW)

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Esters												
ethyl-3-hydroxy butyrate	15.68	1532	fruity, grape-like	2.80	13.24	15.17	17.11	19.95	15.83	13.57	9.87	nd
Alcohols												
2-methyl propanol	4.96	1107	fruity, sweet	nd	nd	6.95	8.14	12.76	15.16	14.23	10.5	nd
n-butanol	6.19	1157	fruity	1.25	10.11	17.31	27.41	31.83	32.00	32.84	33.08	nd
iso-amylalcohol	7.72	1218	fermented	0.00	3.24	3.97	4.56	5.97	29	33.25	49.94	59.94
n-hexanol	11.05	1347	green	1.25	1.86	2.82	3	3.63	17.92	29.83	18.21	8.16
2-ethyl hexanol	14.89	1499	citrus, floral, sweet	1.28	1.95	2.17	8.11	15.7	18.14	26.45	9.21	nd
benzyl alcohol	23.77	1895	floral, sweet	nd	35.25	37.13	39.23	41.17	87.83	38.36	27.86	38.97
phenylethyl alcohol	24.45	2134	floral	nd	4.52	6.96	27.72	35.27	48	51.47	40.8	16.62
Terpenes and their derivatives												
cis-linalool oxide	14.39	1479	floral, sweet	nd	nd	5.95	8.01	14.98	54.76	70.27	54.44	nd
laevo-linalool	16.35	1559	fruity, sweet	nd	nd	13.04	13.65	14.33	46.79	35.95	20.54	12.87
delta-germacrene	19.75	1706	herbaceous	0.82	10.03	12.09	26.81	28.28	48.27	36.01	12.32	10.25
bicyclogermacrene	20.29	1731	woody	nd	nd	11.39	20.31	27.17	41.17	30.54	nd	nd
delta-cadinene	20.84	1756	herbaceous	nd	nd	18.31	21.08	26.66	32.48	nd	nd	nd

Table 38 continued

Compounds	Rt[A]	RI[B]	Attribute[C]	Peak areas ($\times 10^4$) TIC[D]								
				Storage time (days)								
				0	3	6	9	12	15	18	21	24
Terpenes and their derivatives												
epoxylinalool	21.21	1707	flower	nd	6.77	7.27	8.14	9.96	52.67	20.06	19.03	nd
Acids												
acetic acid	14.21	1472	pungent, sour	0.50	2.14	7.14	13.56	17.14	36.51	47.2	85.33	88.14
propanoic acid	16.25	1555	pungent	nd	nd	nd	9.11	12.42	15.87	28.48	81.54	84.00
butanoic acid	18.32	1643	sour	nd	5.06	12.11	20.81	25.81	32.7	36.36	65.87	73.59
hexanoic acid	23.07	1862	sour, sweat	nd	nd	nd	nd	24.98	45.83	69.51	68.21	74.54
Ketones												
3-hydroxy-2-butanone	9.78	1298	sweet	12.40	50.33	58.09	61.01	98.78	74.06	38.61	18.12	9.51
Phenol												
phenol	26.38	2027	phenol	2.89	5.34	13.05	43.81	62.72	54.12	41.26	10.32	12.02

Note: [A] Rt = Retention time (min);   [B] RI = Retention index (FFAP column)

[C] References: http://www.thegoodscentscompany.com/rawmatex.html, http://www.flavornet.org/flavornet.html

[D] TIC = Total ion current; nd = Not detected

Headspace gases composition

Changes in headspace oxygen (O_2) and carbon dioxide (CO_2) inside package were monitored throughout storage time. The interaction between a different day of IW treatment and storage time showed a significantly affected on headspace gases composition ($p<0.05$). The decreases in headspace O_2 and the increases in headspace CO_2 inside a package were observed as can be seen in Figure 48. Headspace O_2 significantly decreased from 5% to a range of 0.42-1.48% (Figure 48A). An increase in headspace CO_2 from 5% to a range of 39.18-88.93% was observed (Figure 48B). Almost constant of headspace of O_2 and CO_2 was associated with a cycle of IW treatment to ambient condition in every 2 days. Under this condition, high concentration of headspace CO_2 was eliminated. It had better not accumulate too much of CO_2 which induces anaerobic metabolism, giving a result of low ethanol and acid formation as mentioned in Figures 46 and 49.
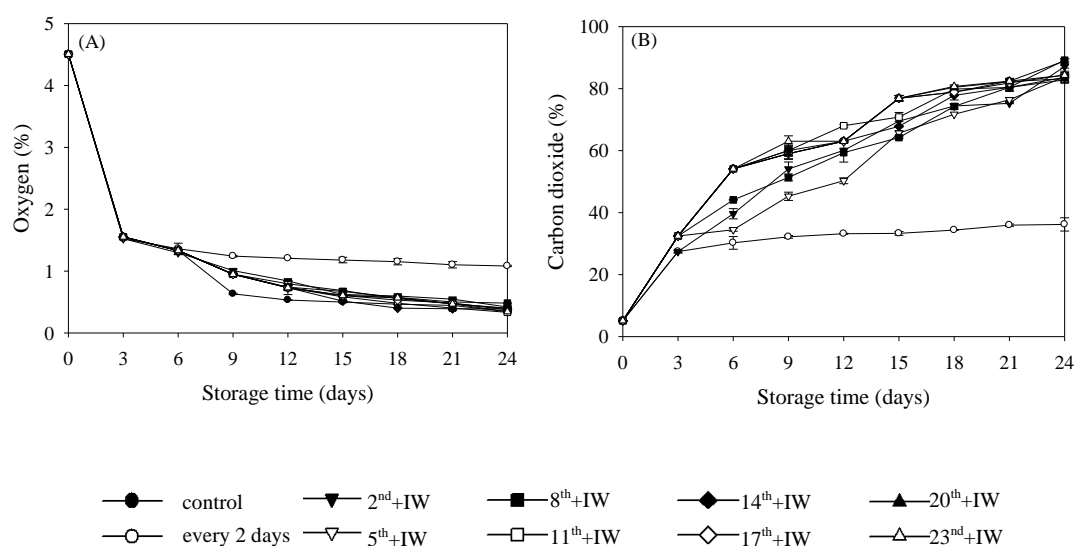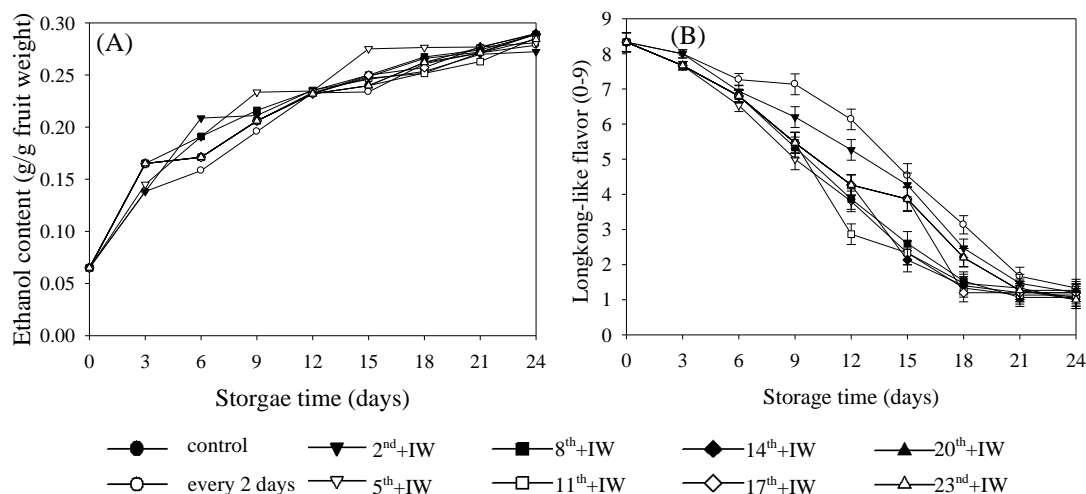

Figure 48 Headspace oxygen (A) and carbon dioxide (B) inside package of longkong during storage under active modified atmosphere packaging on a different day of intermittent warming treatment

Note: Data are mean $\pm$ standard deviation (in four replicates).

Ethanol concentration and longkong-like flavor

In response to high CO_2 and/or O_2 limitation, fruit attempts to adapt or survive by diverting its carbon to ethanol via ethanol fermentation (Knee, 2002). The ethanol fermentation gives an off-flavor characteristic in longkong. Ethanol was produced by the action of ADH enzyme under anaerobic metabolism (Ke and Kader, 1991). Normally, high ethanol concentration in longkong that kept under active MAP storage for a long-term occurred. High concentration of ethanol provided mild of longkong-like flavor. In this study, ethanol accumulation and sensation of longkong-like flavor during storage under active MAP+IW treatment for 24 days were monitored. The interaction between a different day of IW treatment and storage time shows a significantly affected on ethanol concentration and longkong-like flavor ($p < 0.05$). An increase in ethanol concentration with a decrease in longkong-like flavor is presented in Figure 49. Ethanol concentration continuously increased until the end of storage in all conditions. Ethanol concentration significantly increased from 0.06 g/g FW to a range of 0.21-0.28 g/g FW at the end of storage longkong under active MAP+IW treatment for 24 days (Figure 49A).

High concentration of ethanol was responsible for the sensation of off-flavor which was directly effect on the organoleptic characteristic. It was indicated by reduction in longkong-like flavor ($p < 0.05$). At the end of storage, longkong-like flavor was rated from 8.33 to a range of 2.33-4.53 (Figure 49B). In addition, to retard off-flavor and maintain longkong-like flavor, IW treatment was applied. The highest longkong-like flavor correlated with the lowest ethanol concentration which was found in longkong treated with a cycle of IW treatment to an ambient condition in every 2 days. It might be due to frequent turning longkong to an ambient condition. Under ambient condition, aerobic metabolism occurs and ethanol fermentation pathway is inhibited (Knee, 2002). Less ethanol accumulation gives a unique flavor profile of longkong.
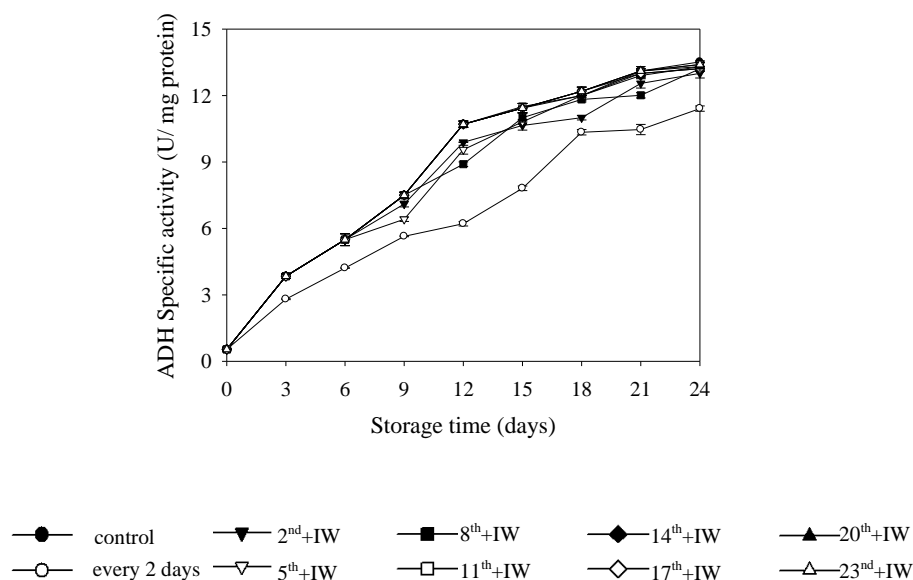
Figure 49 Ethanol content (A) and longkong-like flavor (B) of longkong during

storage under active modified atmosphere packaging on a different day of

intermittent warming treatment

Note: Data are mean $\pm$ standard deviation (in four replicates for ethanol content

and in fifteen replicates for longkong-like flavor score).

Alcohol dehydrogenase (ADH) enzyme activity

The activity of ADH enzyme in longkong during storage under active

MAP+IW treatment for 24 days was monitored. The interaction between a different

day of IW treatment and storage time showed a significantly affected on activity of

ADH enzyme ($p < 0.05$). The increase in activity of ADH enzyme is presented in

Figure 50. The activity of ADH enzyme significantly increased from 0.54 U/mg

protein to a range of 11.41-13.40 U/mg protein at the end of storage longkong under

active MAP+IW treatment for 24 days. An increase in the activity of ADH enzyme

showed a positive correlation with high ethanol accumulation in longkong as

discussed and demonstrated in Figure 49A. In long-term storage under active MAP,

high concentration of CO_2 induces anaerobic pathway in fruit cell. An anaerobic

condition favored an active the activity of ADH enzyme. The ADH enzyme generates

ethanol from acetaldehyde (Knee, 2002). In addition, Sachs *et al*. (1980) reported that

O_2 limitation and high CO_2 concentration induces the expression of genes, including

ADH in the ethanolic fermentation pathway (Cossins, 1978; Ke *et al*., 1994). Low

activity of ADH enzyme was noticed in longkong treated with a cycle of IW treatment to an ambient condition in every 2 days. It was probably due to frequent times to turn longkong to ambient condition inhibits ethanol fermentation pathway via the inhibition of ADH enzyme activity (Knee, 2002).

Figure 50 ADH specific activity of longkong during storage under modified atmosphere packaging with different day of intermittent warming treatment

Note: Data are mean $\pm$ standard deviation (in four replicates).

Microbiological determination

Total viable count in longkong at the initial and the end of storage under active MAP+IW treatment for 24 days was reported in Table 39. The results show that total viable count increased with storage time in all conditions.

Table 39 Total viable counts in longkong at the initial and the end of storage under active MAP on a different day of intermittent warming (IW) treatment

IW	Storage time (days)	Total viable counts (CFU/g)
	0	1.50×10^3
The end of storage		
Control	24	7.53×10^5
every 2 days	24	8.00×10^5
2nd+IW	24	7.87×10^5
5th+IW	24	7.67×10^5
8th+IW	24	7.75×10^5
11th+IW	24	7.86×10^5
14th+IW	24	7.78×10^5
17th+IW	24	7.68×10^5
20th+IW	24	7.65×10^5
23nd+IW	24	7.96×10^5

Note: Data are mean $\pm$ standard deviation (in four replicates).

8.5 Conclusion

Active modified atmosphere can be used as an effective tool for prolonging longkong shelf-life. However, longkong becomes stress in long-term storage under high CO_2 accumulation. The IW treatment was can be used for longkong quality improvement and for a reduction in CO_2 accumulation. A cycle of IW treatment to ambient condition in every 2 days was successfulness tool to retard brownish color and soften flesh. The undesirable off-flavor in longkong such as ethanolic-flavor occurred during storage. It reduced longkong-like flavor. The IW treatment as a cycle of IW treatment to an ambient condition in every 2 days was an effective way for aerobic condition refreshment, resulting in low ethanol accumulation and good flavor acceptability.

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APPENDIX

Appendix A

Fruit flavor acceptance

Name………………………………..   Date…………………………

Phone Number…………………………   e-mail………………………...

Instruction:

1. Please consume some pieces of longkong, determine and rate the score of fruit flavor acceptability (9=like extremely and 1=dislike extremely).

2. Please take a rest between the samples for 5 min and rinse your mouth with water.

Code………………………….

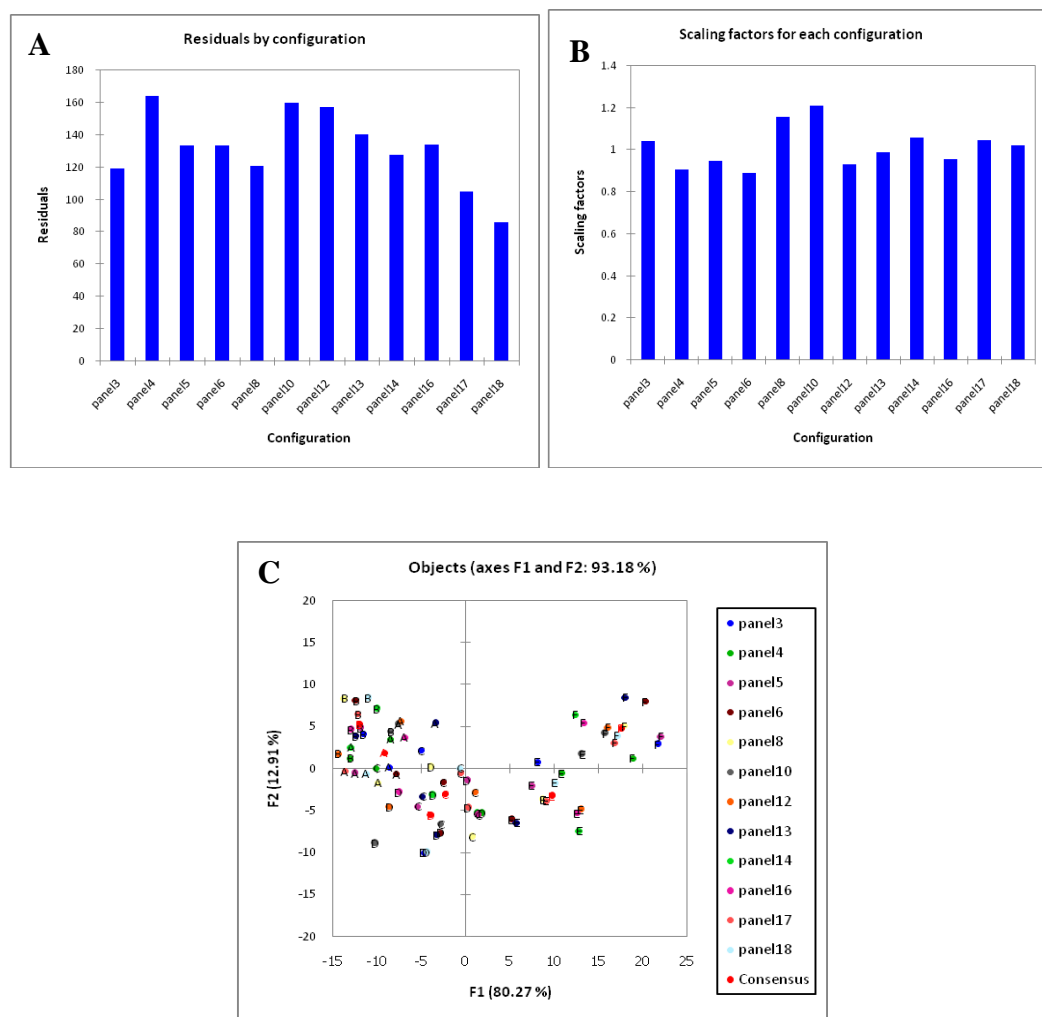…… (9) Like extremely

…… (8) Like very much

…… (7) Like moderately

…… (6) Like slightly

…… (5) Neither like nor dislike

…… (4) Dislike slightly

…… (3) Dislike moderately

…… (2) Dislike very much

…… (1) Dislike extremely

Thank you very much

Appendix B

Perceived ethanolic-flavor intensity rating

Name……………………………….. Date…………………………

Phone Number……………………… e-mail………………………...

Instruction:

1. Please sniff and taste some pieces of longkong (start on your left first).

2. Please determine and rate the intensity of ethanolic-flavor.

3. Please write down the sample code on the top of your mark.

4. Please take rest between the samples for 5 min, sniff the air through a filter paper and rinse your mouth with water.

5. The scale represents by 20 cm-category line scale, the scores ranged from 0 to 100 (0=imperceptible ethanolic-flavor, 50=moderate ethanolic-flavor and 100=strong ethanolic-flavor).

Code……………………………….

| 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |

Imperceptible
ethanolic-
flavor

Moderate
ethanolic-
flavor

Strong
ethanolic-
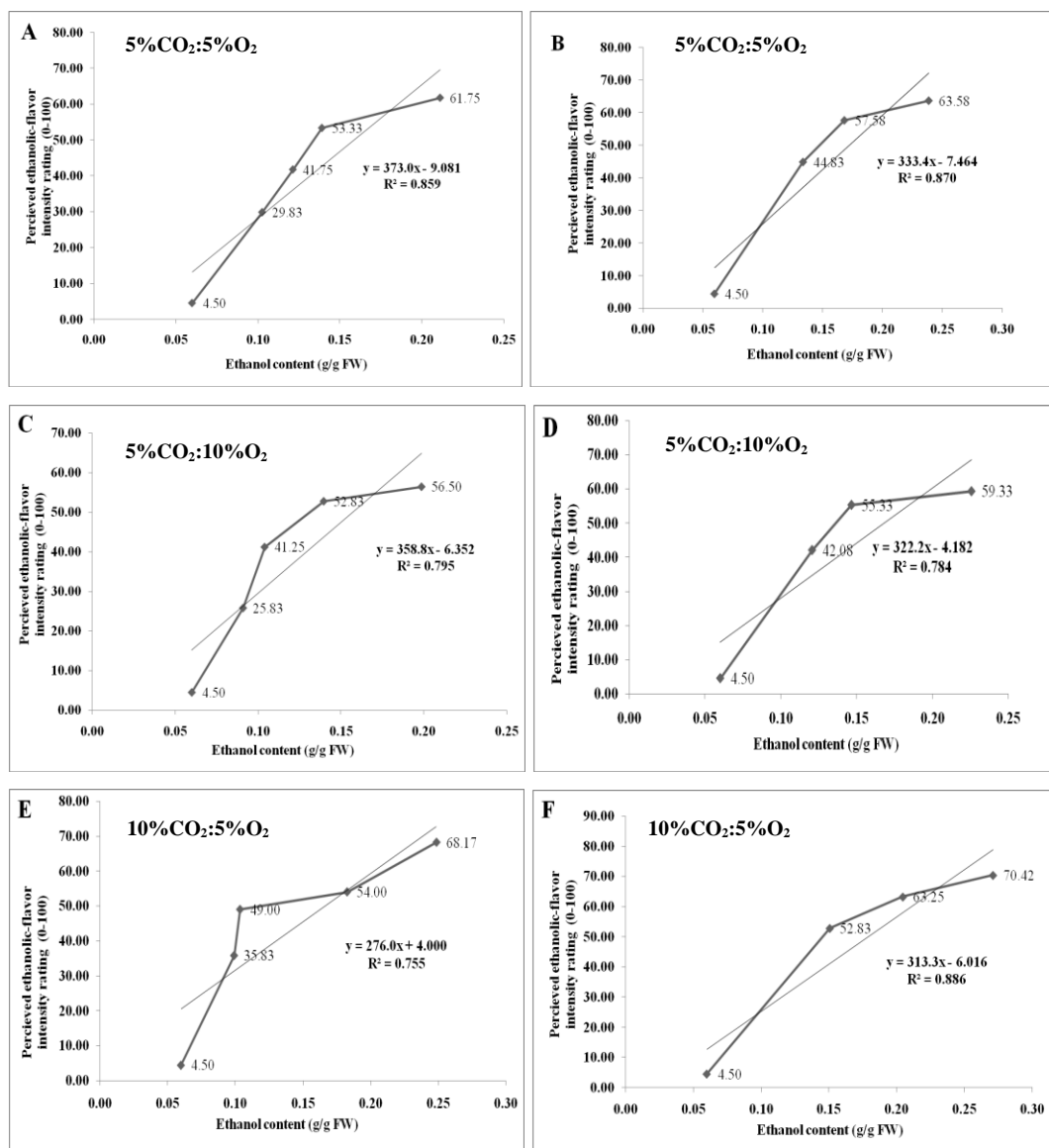flavor

Thank you very much

Appendix C

Figure 51 Residuals by configuration (A), Scaling factor for configuration (B) and
Objects coordinates (C) from Generalized Procrustes analysis (GPA)

Note: The residuals by configuration and objects coordinates show the consensus
among panels for intensity rating and the average positions of the 6
concentrations of standard ethanol at 0, 1, 1.25, 2.5, 5 and 10 %. The
scaling factor for configuration shows the consensus among panels for
scaling.

12 train-panels consisted of panel3, panel4, panel5, panel6, panel8,
panel10, panel12, panel13, panel14, panel16, panel17 and panel18.

Appendix D

Figure 52 Positive correlations between perceived ethanolic-flavor intensity rating
and ethanol content in longkong during storage under modified
atmosphere packaging at different atmospheric conditions and temperatures

Note: Storage at 18ºC (A, C, E) and room temperature (~30ºC) (B, D, F)

Appendix E

Lonkong-like flavor

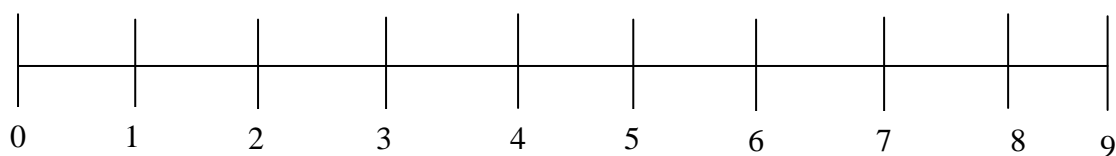Name…………………………………..   Date…………………………
Phone Number…………………………   e-mail………………………...
Instruction:

1. Please consume some pieces of longkong, determine and rate the score of longkong-like flavor.

2. Please take a rest between the samples for 5 min and rinse your mouth with water.

3. The scale was represented by numeric scale nine point, oriented by score point (the scores ranged from 0 to 9) with the two poles (0=no longkong-like flavor; 9=intense longkong-like flavor).

Code………………………

```
|   |   |   |   |   |   |   |   |   |   |
0   1   2   3   4   5   6   7   8   9
```

Thank you very much