Factors Affecting Browning and Crystallisation of Palm Sugar Syrup and Palm Sugar Cake

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Thesis Title	Factors Affecting Browning and Crystallisation of Palm Sugar Syrup and Palm Sugar Cake
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ABSTRACT

Palm sugar syrup and palm sugar cake are the local products in Songkhla province. These products have not yet been produced industrially. The properties of these products are extremely varied across the samples and within individual local producer. Some factors affecting the properties of these products are harvesting time of palm sap, processing methods, ingredient added and storage condition. Therefore, this research was to study the properties and factors affecting browning and crystallisation of palm sugar syrup and palm sugar cake. Ten palm sap samples were characterised. The pH of all palm sap varied from 4.19 to 5.23 and total acidity ranged from 0.13% to 0.19%. Ethanol was also found in all samples that indicating the fermentation. Commercial ten palm sugar syrup samples were characterised. HMF content was found to vary between 15.35 mg/kg to 96.76 mg/kg. HMF content of three out of ten samples was higher than the maximum limited as recommend by Codex Alimentarious (40 mg/kg). The TSS ranged from 62.97^{o}Brix to 67.50^{o}Brix. TSS of two out of ten samples did not meet the requirements of Thai industrial standards institute ministry of industry. Ten palm sugar cake samples were also characterised. The hardness was ranged from 30.83 N to 69.00 N. Crystallinity of these samples was found in a range of 73.40%-78.56%. HMF content was found to vary between 21.81 mg/kg to 341.80 mg/kg. HMF content of eight out of ten samples was higher than the maximum limited as recommend by Codex Alimentarious.

The changes in physical and chemical properties during the production of palm sugar syrup by palm sap at different harvesting times (6 h, 12 h, 18 h and 24 h) were monitored. The highest nonenzymatic browning reactions and inversion reaction

were found in palm sugar syrup sample which was taken from harvested palm sap at 24 h. Moreover, HMF of a sample that produced by palm sap after harvesting time for 24 h (113.07 mg/kg) was higher than the standard requirement. The effect of storage temperatures (4^{o}C and 30^{o}C) and storage time (0-12 months) on the properties changed of palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h were investigated. Maillard reaction took place in all samples that stored under 4^{o}C lower than those that stored under 30^{o}C as evidenced by lower a* value, IBP, BI and HMF content and higher L* value, fructose, glucose and FAG content ($P<0.05$). Low temperature (4^{o}C) can be used to retard dark colour and HMF formation in palm sugar syrup during storage.

The changes in physical, chemical properties and antioxidant activity during the production of palm sugar syrup by an open pan (110^{o}C) and a vacuum evaporator (70^{o}C and 80^{o}C) were studied. The results showed that the a*, b*, IBP, BI, HMF content, TSS, and reducing sugar as well as glucose and fructose increased with heating time in each heating process. The increase in browning development (IBP, BI and HMF content) with heating time was concomitant with the increase in DPPH radical scavenging activity, FRAP and reducing power in each heating process. Among all heating processes, palm sugar syrup that produced by an open pan rendered the highest browning development and antioxidant activity. The effect of storage temperatures (4^{o}C and 30^{o}C) and storage time (0-12 months) on the properties changed of palm sugar syrup that produced by an open pan and a vacuum evaporator were also investigated. During storage, Maillard reaction took place in a sample that stored under 4^{o}C lower than those that stored under 30^{o}C. This was shown by lower a* value, IBP, BI and HMF content, and higher L* value, fructose, glucose and FAG content during storage for 12 months ($P<0.05$). Only the sample produced by an open pan and stored under 30^{o}C contained higher HMF content (50.58 mg/kg) than the permitted maximum limit.

The effect of sucrose (30%, 40%, and 50%) and glucose syrup (10% and 20%) addition on properties of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator were investigated. It was found

that an increase in hardness and crystallinity was found in all samples with increasing sucrose content ($P<0.05$). There was a decrease in hardness and crystallinity with increasing glucose syrup content ($P<0.05$). The highest dark colour and lowest hardness was observed in a sample that produced from 100% palm sugar syrup as indicated by the lowest L* and hardness and the highest a*, IBP, BI and HMF content. Furthermore, the highest overall acceptability score was found in samples that produced from 50% palm sugar syrup, 40% sucrose and 10% glucose syrup. Thus, this formulation was selected to study the effect of storage temperature (4^oC and 30^o) and storage time (12 months for 11% of RH and 4 weeks for 75% of RH) on properties changes of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. During storage, Maillard reaction took place in samples stored under 4^oC lower than those stored under 30^oC in both RHs. This was shown by lower a* value, BI and higher L* value at the end of storage ($P<0.05$). Storage temperature did not affected on hardness and crystallinity of all samples that stored in both RHs. Continuous decrease in hardness and crystallinity was found in all samples stored under 75% of RH during storage ($P<0.05$). Thus, storage condition under 30^oC and 75% of RH is improper to store palm sugar cake due to these conditions greatly promote the decrease in hardness and increase in dark colour of palm sugar cake.

The effect of storage temperature (20^oC and 30^oC) on MSI characteristic, EMC and Tg of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator was investigated. It was found that MSI for all palm sugar cake samples that stored under both temperatures was Type-III isotherms. Storage temperature did not affect on EMC when a sample stored under 11-75% of RH ($P\geq0.05$). The EMC of all samples that stored under 20^oC was higher than those stored under 30^oC and 85% of RH ($P<0.05$). Storage temperature did not affect on Tg of all samples. There was no significant difference in Tg of all samples that stored under 11-51% RH ($P\geq0.05$). However, the Tg of all samples decreased with increasing RH in a range of 75-85%. In addition, a decrease in crystallinity and increase in IBP and BI during storage was detected in a sample that stored under high RH (75-85%).

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CHAPTER 1

INTRODUCTION AND REVEIW OF LITERATURE

1.1 Introduction

Appearance, flavour, texture, and nutritional values are four major quality attributes used by consumers when selecting food choices. Appearance, on which a significant impact is made by colour, is one of the first attributes used by consumers for evaluation. Colour can be influenced by many compounds, particularly colour formed through processing. One of the most important colour reactions that affect plant syrup is the colour from nonenzymatic browning reactions caused by the heating process. In general, palm sugar syrup is produced by heating the sap derived from the tropical palm tree called Palmyra palm (*Borassus flabellifer* Linn*)*. Normally, some factors affected the quality of palm sugar syrup such as quality of raw material (palm sap) and processing methods. One important factor affected the property of palm sap is collection time. Generally, fresh sap is sweet, oyster white colour and translucent, with nearly neutral pH (Lasekan *et al.*, 2007). The sap is sterile (free of microorganisms) while flowing in Palmyra inflorescences. However, microorganisms are found in the sap coming from environment during collecting process. Firstly, harvesting time affected the quality of palm sap especially, pH of sap and reducing sugar content as reported by Phaichamnan *et al.* (2010). Long harvesting time also induced in the increase of microorganisms. The microorganisms use sugars in the sap as an energy source and produce organic acids. Thus, the chemical properties of palm sap are changed and may affect the quality of palm sugar syrup and palm sugar cake afterward. Hence, the effect of harvesting time of palm sap on the properties of palm sugar syrup is investigated. The second factor affected the quality of palm sugar syrup is processing method. During heating process, chemical and physical changes occur which impart the unique colour and flavour to palm sugar syrup.

In traditional production of palm sugar syrup, a large volume of filtered palm sap is heated on the wood fired stove above 100^{o}C until it becomes concentrated to obtain typical aromas (Ho *et al.*, 2006). However, it requires a long time to evaporate water until the concentration of total soluble solid reach 65^{o}Brix or above. The longer sap is boiled, the darker it becomes. Major reactions occuring during the heating process of palm sap include inversion reaction and nonenzymatic browning reactions (Maillard reaction and Caramelization). The syrup was dark and affected the quality during storage. Alternative way, evaporation at low temperature, under vacuum, reduces thermal degradation of food properties due to the decrease in processing temperatures and times. Thus, the effect of processing methods such as using an open pan and a vacuum evaporator as well as storage temperature and time affecting on the properties of palm sugar syrup were also investigated.

Palm sugar cake is perhaps the most common product produced from either palm sap or palm sugar syrup. The stability of palm sugar cake depends on water content, sugar type, and storage temperature, time and relative humidity (RH). The glass transition temperature (Tg) in sugar products are of interest since it can be used as a tool to understand the properties and shelf life stability of sugar products (Tanon *et al.*, 2009; Syamaladevi *et al.*, 2009; Mosquera *et al.*, 2010). This information is crucial because the stability and properties of foods are directly dependent on the water concentration. Typically, sugar products such as palm sugar cake become sticky and lose flavour when storage under temperature exceeds Tg. Moreover, the effect of Tg on chemical reactivity has been emphasized in a number of studies. One particular issue has been a possible relationship between the Tg and rate of nonenzymatic browning reactions. To predict the suitable storage condition of palm sugar cake, moisture sorption isotherm (MSI) will be monitored. Knowledge of MSI is also important to evaluate storage stability and quality change during packaging and storage of intermediate moisture foods. Therefore, in this research, Tg and MSI is introduced for prediction storage stability of palm sugar cake. There is no information regarding to Tg and MSI of palm sugar cake available in published domain. Therefore, in this research was performed to determine Tg and MSI of

palm sugar cake. To date, scientific supports on palm sugar products in Thailand have rarely been yet investigated, therefore the aim of this work are studied factor affecting browning and crystallisation of palm sugar syrup and palm sugar cake. The information obtained from this study could be used as a guideline for optimizing or designing thermal processes to reduce the loss in property of these products. In addition, more detailed knowledge of palm sugar syrup and palm sugar cake during processing and storage will be of benefit for producers and consumers.

1.2 Review of Literature

1.2.1 Palmyra palm

Palm is one of the most important crops in many countries. The most common types of palm tree available in Thailand are palmyra palm and coconut palm. Palmyra palm, botanically known as *Borassus flabellifer* Linn., belongs to genus of *Borassus*, which is an important member of the monocotyledons. Palmyra palm can be found in tropical countries such as India, Thailand, Myanmar, Sri Lanka and Cambodia. In Thailand, palmyra palms are crowded in southern part of Thailand from Phetchaburi to Songkhla province, beside they can be found in other part such as Phitsanulok, Buriram, Singburi, Chainat, Suphanburi, Nakhonphathom and Nakhon Sri Thammarat provinces. Palmyra palms are the most populated in Songkhla province about 3 millions plants (Department of agricultural extension Thailand, 2001 and Trèbul, 1984). The important product of Palmyra palm is the sap or juice.

Sap is the fluid transported in xylem cells (tracheids or vessel elements) or phloem sieve tube elements of a plant. Xylem and phloem sap consists primarily of water with sugar, hormones, mineral elements and other nutrients (Davis and Johnson, 1987). In addition to being a high value product, palm sap can be converted to many products in commercial such as fresh palm sap, pasteurized sap, syrup and sugar cake. Some products especially fresh sap, syrup and sugar cake are discussed as follow.

1.2.2 Palm sap and its quality

The most important product of palmyra palm is the sap or juice. The tapping process involves the bruising of the interior of the developing inflorescences by means of a wooden mallet or tong, thereby stimulating sap flow. Sap is collected by cutting the inflorescences grown with a very sharp sickle or knife at the apex of the palm tree. Sap collector cut the outer end of the inflorescence for collecting sap. Each inflorescence was 25 cm to 30 cm in length and 2.0-2.5 cm in diameter. Sap was collected twice a day from each inflorescence which is collected in morning and evening. Three to six inflorescences were tied together and inserted into a suitable container for sap collection, usually using an earthenware pot (in Sri Lanka) or a bamboo tube (in Thailand). During collecting process, Kiam woods or Payorm woods were added in containers to prevent fermentation from microorganisms. Farmer stopped tapping the sap in rainy season because it has a little yield and also the contamination of water to the sap which was collected by an open container. After each harvesting season, inflorescences were removed by using sickle or knife to allow new inflorescences to grow (Davis and Johnson, 1987).

Fresh sap is sweet, oyster white colour and translucent, with nearly neutral pH (Gupta *et al.*, 1980). The sap is sterile (free of microorganisms) while flowing in Palmyra inflorescences. However, microorganisms are found in the sap coming from an environment during collecting process. Microbes are contaminated into the sap by unsanitary tapping procedures and unsanitary collection. Further contamination in sap occurs when the utensils are not completely cleaned and sanitized between sap runs, especially during summer season particularly, which favours the rapid growth of microbial. The growth of microorganisms is generally affected by three factors: temperature, time and nutrient availability. Increased temperatures favour rapid division of microorganisms, thereby increasing their population over time. As they divide and increase in number, they use sugars in the sap as an energy source, since palm sap is rich in sugars (10-15%). The fermenting organisms are dominated by yeasts, particularly

Saccharomyces cerevisiae and lactic acid bacteria. These microorganisms converted sugars to acids and alcohols (Sanni, 1993; Iwuoha and Eke, 1996). Kiam wood and Payorm wood is commonly added to the collection receptacle because it can delay spoilage in palm sap by reducing microbial populations and thereby keeping quality of the product (Department of agricultural extension Thailand, 2001).

Some works have been reported the chemical quality of fresh palm sap as shown in Table 1. The pH of fresh palm sap (2 h after collection) was neutral (7.55) while the pH of palm sap after collection 12 h was 4.69 as reported by Jitbunjerdkul (1989). The decrease in pH of palm sap during collection was due to the increase in microbial loads. This result was in agreement with the increasing in total acidity. When pH decreased, sucrose inversion took place. It was indicated from the increasing of reducing sugar content (from 0% to 0.78%). Similar results were found in the works of Jamfa (2002), Tiapaiboon (2004) and Loetkitsomboon (2004) (Table 1). Thus, long collection time resulted in the low quality of palm sap as evidenced by low pH and high amount of total acidity and reducing sugar contents.

As mentioned previously, microorganism is the major parameter affecting on the quality of palm sap. The quality of palm sap including turbidity, colour, pH, acidity and sugar type and sugar concentration could be changed due to microorganisms. The quality of palm sap also affected the quality of palm sugar syrup and palm sugar cake. Acid condition of palm sap caused by microorganisms can promote the inversion of sucrose. Consequently, high concentration of reducing sugar was presented in palm sap. The initial sap which contained high reducing sugar could promote Maillard reaction during heating process and storage (Willits and Hills, 1976; Shallenberger and Birch, 1989).

Table 1. Chemical qualities of palm sap

Qualities	**Values[1]**	**Values[2]**	**Values[3]**	**Values[4]**	**Values[5]**	**Values[6]**
pH	5.09	7.55	4.69	5.76	5.63	5.60
Total soluble solid (^{o}Brix)	13.80	13.50	13.93	11.20	12.67	16.00
Total sugar (%,w/w)	12.34	13.48	11.54	10.91	11.72	15.82
Reducing sugar (%,w/w)	-	-	0.78	0.67	0.44	1.79
Total acidity (%,w/v)	0.036	0.068	0.098	0.032	0.051	0.022
Total solid (%,w/w)	-	-	-	-	1.27	-
Protein (%,w/w)	0.37	-	-	-	-	-
Ash (%,w/w)	1.04	-	-	-	-	-
Moisture (%,w/w)	84.47	-	-	-	-	-
Vitamin C (mg/ml)	0.084	-	-	-	-	-

Note: [1] Jamfa (2002) analysed the chemical qualities of palm sap harvested 12 h which was added Payorm wood from Watpood district, Phitsanulok province.

[2] Jitbunjerdkul (1989) analysed the chemical qualities of palm sap harvested 2 h from Satringphra district, Songkhla province.

[3] Jitbunjerdkul (1989) analysed the chemical qualities of palm sap harvested 12 h which was added Kiam woods in Satringphra district, Songkhla province.

[4] Tiapaiboon (2004) analysed the chemical qualities of palm sap harvested 15 h which was added Kiam wood in Singhanakhon district, Songkhla province.

[5] Loetkitsomboon (2004) analysed the chemical qualities of palm sap harvested 15 h which was added Kiam wood in Singhanakhon district, Songkhla province.

[6] Chantachum (2004) analysed the chemical qualities of palm sap harvested 15 h which was added Kiam wood in Singhanakhon district, Songkhla province.

1.2.3 Plant syrup

Syrups are products with high concentrations of sugar content approximately 65-90% of all soluble solids (Kallio *et al.,* 1989; Collins and Dincer 1973). Plant sap represents the raw material to produce syrup including palm sugar syrup (*Borassus flabellifer* Linn.), date syrup (*Phoenix dactylifera*), coconut syrup (*Cocos nucifera*), birch syrup (*Betula pubescens*) and maple syrup (*Acer saccharum*). The

processing methods for syrup production are by water removal from the sap by different methods such as an open pan or kettle, concentration under reduce pressure and reverse osmosis (Kallio *et al.,* 1989; Perkin and Van den Berg, 2009).

Plant sap starts with various total soluble solid (TSS). The TSS of palm sap was approximately 10-16^{o}Brix. When palm sap is produced to palm sugar syrup, it takes approximately 6-7 parts of sap to produce 1 litre of syrup (TSS approximately 65 ^{o}Brix). Syrup must contain TSS at least 65 ^{o}Brix. This criteria was set by the regulation of Thai industrial standards institute ministry of industry (number 155, in the year 2003). It is in agreement with the regulation of maple syrup procedure that defined maple syrup means the liquid food derived by concentration and heat treatment of maple sap. The TSS content of finished maple syrup shall not be less than 66% by weight (^{o}Brix). While birch sap and maple sap contain TSS approximately 1^{o}Brix, they take approximately 65 parts of sap to produce 1 litre of syrup (Kallio *et al.,* 1989; Paradkar *et al.,* 2002; Perkin and Van den Berg, 2009). Therefore, palm sap represents better source for the production of syrup when compared to other saps.

1.2.3.1 Plant syrup production

Concentration of liquid foods is a vital operation in many food processes. Plant sap could be concentrated to provide increased shelf-life and/or increased value. Concentration process will reduce weight and volume and resulting in economic advantages. Normally, syrup with concentration approximately 70^{o}Brix is stable to store under room temperature (Potter and Hotchkiss, 1995).

Evaporation involves removal of water by boiling, with a steam or wood fire using a stove, a pan or a metal kettle. In commercial, an open pan and a kettle are still widely used by manufactures for producing maple syrup and palm sugar syrup (Potter and Hotchkiss, 1995; Ho *et al.,* 2007; Perkin and Van den Berg, 2009).

Generally, palm sugar syrup that presents in Thailand is usually produced by evaporation palm sap with an open pan. In general, the sap is boiled for many hours to produce palm sugar syrup. For the traditional production of palm sugar syrup, a large volume of palm sap is heated on the wood fired stove above 100^{o}C until its becomes

concentrate (Figure 1) (Ho *et al.*, 2007; Phaichamnan *et al.*, 2010). This leads to typical colour and flavour. The TSS of finished palm sugar syrup shall not be less than 65^{o}Brix according to Thai industrial standards institute ministry of industry.

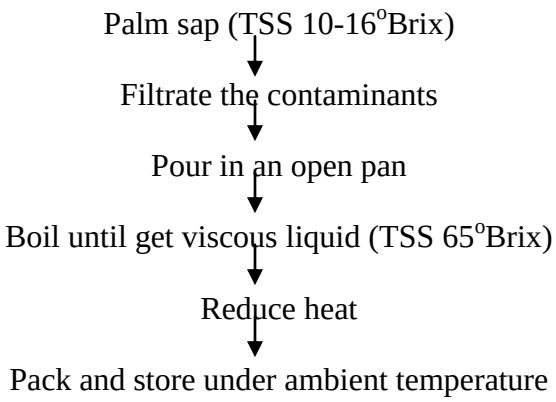


Figure 1. Production of palm sugar syrup

Source: Adapted from Department of agricultural extension Thailand (2001)

However, a traditional production uses high temperature (approximately 110-120^{o}C) for a long time. It has been reported that thermal deterioration can take place during thermal processing and affect on the quality of product, especially colour, flavour and nutritional values. The most common chemical reactions that influence on the quality of syrup are inversion reaction, Maillard reaction and Caramelisation (Akochi-K *et al.*, 1997; Apriyantono *et al.*, 2002; Ho *et al.*, 2007; Perkin and Van den Berg, 2009). In order to reduce thermal degradation during evaporation, it is necessary to minimize heating temperature and time. An alternative way, evaporation under low temperature and vacuum condition could reduce thermal degradation of food properties (Potter and Hotchkiss, 1995; Heldman and Hartel, 1998).

1.2.3.2 Quality of plant syrup after processing and during storage

Jitbunjerdkul (1989) investigated the chemical qualities of palm sugar syrup and found that the pH value of palm sugar syrup (5.30) was higher than its palm sap (4.69) due to the loss of volatile acids during heating process with an open pan. The increase in a ratio of reducing sugar/ total sugar of palm sugar syrup (from 0.067 to 0.088) could be attributed to the inversion of sucrose during high temperature boiling and acidic condition. Upon hydrolysis, glucose and fructose are formed (Pancoast and Junk, 1980).

Jamfa (2002) studied the physical and chemical qualities such as colour (L and a value), pH, total acidity, TSS and protein content of palm sugar syrup after processing by an open pan and a vacuum evaporator. It was found that the L value of palm sugar syrup produced by an open pan (L* = 8.48) was lower than that produced by a vacuum evaporator (L* = 13.63). The a values of palm sugar syrup produced by an open pan and a vacuum evaporator were 1.71 and -1.76, respectively. Since, L value is a measure of colour in the light-dark axis, this low value indicated that syrup was turning dark. Palm sugar syrup produced by an open pan showed darker colour than that produced by a vacuum evaporator (consider to L and a value) due to it took place high rate of Maillard reaction and Caramelisation with the long processing temperature and time. However, pH, total acidity, TSS and protein content of palm sugar syrup that obtained from two processing methods were not different ($P \geq 0.05$).

As mentioned previously, syrup with TSS more than 65% is stable to store at any temperatures for at least one year (Potter and Hotchkiss, 1995; Perkin and Van den Berg, 2009). However, nonenzymatic browning reaction especially Maillard reaction and inversion reaction can take place during storage. This reaction results in undesirable colour and flavour changes. It is followed by the formation of brown pigment (Bozkurt *et al.*, 1999).

Microorganisms are also affected the quality loss of palm sugar syrup during storage. Generally, Foods of high sugar content such as syrup, honey and juice concentrates are selective environments for the growth of microorganisms. Tilbury

(1980) has tabulated syrup and honey along with their sugar contents and water activity values which, generally, are above 60% and below 0.85 respectively. If contaminated with yeasts and inadequately processed and stored, these products may undergo fermentative spoilage to acquire an estery, fruity odour and sour taste. Almost invariably, these high sugar products are spoiled by a few species of osmotolerant (osmophilic, xerotolerant) yeasts of which *Saccharomyces rouxii*, *Saccharomyces bailii*, *Saccharomyces Bisporus*, *Torulopsis candida* and *Torulopsis versatilis* are most frequently encountered.

Chantachum (2004) monitored the quality of palm sugar syrup during storage (60 days) and found that L and b values decreased during storage for 60 days. L value of palm sugar syrup was reduces from initially 14.31 to 0.31 at 60 days of storage. Decreasing of L value indicated that colour of palm sugar syrup change to brown. The pH of palm sugar syrup was stable during storage for 60 days. TSS and total sugar content decreased with storage time, while reducing sugar increased. The initial values of TSS and total sugar were 66.60^{o}Brix and 61.37%, respectively. After 60 days, these values decreased to 48.41^{o}Brix and 46.29% (total sugar), while reducing sugar increased from 6.12% to 9.31%. During storage palm sugar syrup, the darkening is attributed mainly to Maillard reaction. The increase in reducing sugar during storage was due to inversion of sucrose.

1.2.4 Sugar cake

Sugar cakes are made from various materials such as sucrose and plant sap. Plant sap such as palm sap, coconut sap and maple sap are common used to produce sugar cakes. Sugar cake allowed easier to transport and storage as well as providing a source of sugar which was used as a sweetener (Apriyantono *et al.*, 2002; Pattnayak and Misra, 2004; Rao and Das, 2009; Aider *et al.*, 2006; Aider *et al.*, 2007).

1.2.4.1 Sugar cake production

Sugar cake can produce either plant sap or plant syrup. Generally, the processing method for the production of palm sugar cake is water removal from sap or syrup by an open pan until it becomes concentrate more than 80^{o}Brix. After that, it was allowed to cool and poured in a mould. In maple cake production, maple cake is produced by heating maple syrup until a boiling temperature of 120^{o}C is reached. As soon as the cooked syrup reaches this temperature it is removed from the heat and stirred. Stirring is continued until the solution begins to crystallize and stiffen. At this time, it can be poured into a mould, either of a small size or a large block. Coconut cake is obtained by evaporating coconut sap through boiling in an open vessels at 118-120^{o}C then allowed to cool and solidify. Major components of coconut cake are sucrose (70-79%) followed by glucose (3-9%) and fructose (3-9%). The variations in sugar content can be occurred due to the differences in processing method, raw material quality and variety of coconut.

In Southern Thailand, palm sugar cake has been produced in commercially by heating palm sugar syrup and other ingredients such as sucrose and glucose syrup until its became concentrate and thick consistency. Sucrose is added as a seeding to induce a process of sugar crystallisation. The quality of palm sap or palm sugar syrup influences on the quality of palm sugar cake. Generally, most sugars can induce crystallise, especially nonreducing sugar but reducing sugar is difficult to obtain a crystallise form. Sugar cake can be produced by sap or syrup due to its has high concentration of sucrose. If sap or syrup is fermented, it will be obtained a lot of reducing sugar contents due to sucrose inversion. Therefore, this syrup cannot use to produce sugar cake due to sugar cake cannot solidified. In addition, during the production of palm sugar cake, nonenzymatic browning reactions can be formed (Perkin and Van den Berg, 2009).

1.2.4.2 Quality of sugar cake

In general, palm sugar cake deteriorates fast and become watery within 1 or 2 weeks due to its hygroscopic nature. Quality changes in sugar cake such as colour, flavour and texture depends on many factors including quality of the initial sap,

processing methods, type of seeding and storage temperature, storage time and relative humidity. More details will be discussed as follows.

Sap quality is the main factor influenced the quality of sugar cake. Uttraporn (2006) studied the influence of pH (4.0, 5.5, 7.0 and 8.5) of coconut sap affecting on the quality of coconut cake. Coconut sap (pH 4.8 and TSS 17^{o}Brix) was first adjusted to a desired pH with citric acid and sodium hydroxide, evaporated using an electrical pan until TSS reached 85^{o}Brix. After that, viscous coconut syrup was beaten by kitchen aid, then poured in a mould and allowed to cool at ambient temperature. It was found that the highest L* value was found in coconut cake with using coconut sap (pH 4) as a raw material. On the other hand, the lowest L* value was found in coconut cake with using coconut sap (pH 8.5) as a raw material. This phenomenon is mainly related to Maillard reaction that accelerates easily in basic medium. The L* values were in accordance with browning intensity (BI). The highest BI was found in sample with pH 8.5 adjustment. In addition, the coconut cake with pH 4.0 adjustment was not solidified, having the lowest hardness while coconut cake with pH 8.5 adjustment was solidified, having the highest hardness. The lowest sucrose and highest fructose and glucose content were found in coconut cake with pH 4 adjustment. During heating process, sucrose inversion occurred easily with temperature increased especially with acid condition. Upon hydrolysis glucose and fructose are formed. These invert sugars are difficult to form a crystalline, resulting in a soft of coconut cake.

Processing methods also influenced on the quality of sugar cake. Uttraporn (2006) investigated the influence of evaporation method (using an electrical pan; fast heating and a double-jacket kettle; slow heating) on the physical (L*, BI and hardness) and chemical quality (sucrose, glucose and fructose content) of coconut cake. It was found that higher L* and lower BI was observed in coconut cake produced by fast heating compared to slow heating. This is due to the rate of the Maillard reaction and Caramelisation increased exponentially as the heating time increased (Martins *et al*., 2001). Moreover, higher hardness was found in coconut cake produced by fast heating compared to slow heating.

Ingredients such as sucrose and glucose syrup also affected the quality of sugar cake. During sugar cake production, glucose syrup and/or sucrose were added to induce a crystallization of sugar and its stability. Nuanchamnong and Soumjinda (2005) studied the effect of sucrose added on the quality of coconut cake. Coconut sap was first adjusted to pH 7.0 with sodium hydroxide and then sucrose at different concentration including 0%, 5%, 10% and 15% (w/w) was added. The evaporation process was started using an electrical pan until the TSS reached 85^{o}Brix. After that, viscous coconut syrup was beaten by kitchen aid and then poured in a mould. Coconut cake was allowed to cool at ambient temperature. It was observed that a decrease in moisture content was observed with increasing sucrose. The highest sucrose crystals dispersing in a continuous phase as observed were found in coconut cake added 15% sucrose.

Finally, relative humidity (RH), storage temperature and storage time also influenced on the quality of sugar cake. Uttraporn (2006) monitored the effect of relative humidity (RH) (50%, 65% and 80%), storage temperature (30^{o}C, 40^{o}C and 50^{o}C) and storage time (0-6 months) on the qualities of coconut cake. The results showed that an increase in moisture content, water activity and decrease in hardness with increasing RH was found. The sample stored in 80% RH (at 30^{o}C) was liquefied within 1 month. The sample stored at the relative humidity of 50% had the highest hardness. In addition, a decrease in L* value and increase in BI was found with increasing RH, storage temperature and time.

Chantachum (2004) monitored the qualities of palm sugar cake packed in polypropylene bag and stored at ambient temperature during storage for 60 days. It was found that L and b values decreased during storage. With increasing storage time, palm sugar cake became darker which corresponded to a decrease in L value. Total and reducing sugars decreased in all samples due to Maillard reaction occurred corresponding to the decrease of L value during storage. Palm sugar cake is highly hygroscopic product, therefore moisture content increased with storage time.

1.2.5 Inversion reaction

Sucrose is α-D-glucopyranosyl-β-D-fructofuranoside. It is a disaccharide with one molecule of D-glucose in the pyranose or 6-membered ring and is condensed with one molecule of β-D-fructose in the furanose or 5-membered ring form (Pancoast and Junk, 1980). Sucrose inversion occurs when the glycosidic linkage of disaccharide is hydrolyzed, releasing the monosaccharide units. Upon hydrolysis, α-D-glucose (α-D-glucopyranose) and β-D-fructose (β-D-fructofuranose) are formed. The reaction is dependent on pH, temperature and hydrolyzing reagent such as enzyme. Sucrose hydrolyzed easily in an acid medium. The sucrose inverting capacity of acid varies with their degree of ionization or dissociation constants. Moreover, the inversion of sucrose increases with temperature (Shallenberger and Wienen, 1988).

1.2.6 Nonenzymatic browning reaction

Many foods undergo browning due to nonenzymatic browning reactions that occur during processing or storage (Babsky *et al.*, 1986; Ibarz *et al.*, 1999). These reactions are the most complex reaction in food industry and are composed of Maillard reaction and Caramelization (Eskin, 1990).

1.2.6.1 Maillard reaction

The Maillard reaction has been named after the French chemist Louis Maillard who first described it. The first coherent scheme of Maillard reaction was put forward by Hodge (Figure 2). It can induce browning of foods, has an effect on nutritive value, has toxicological implications (such as the formation of acrylamide), produce antioxidative components. Moreover, it has also a large effect on flavour (Martins *et al.*, 2001). The mechanisms of Maillard reaction are generally divided into three stages including early, advanced and final stages (Ajandouz *et al.*, 2001).

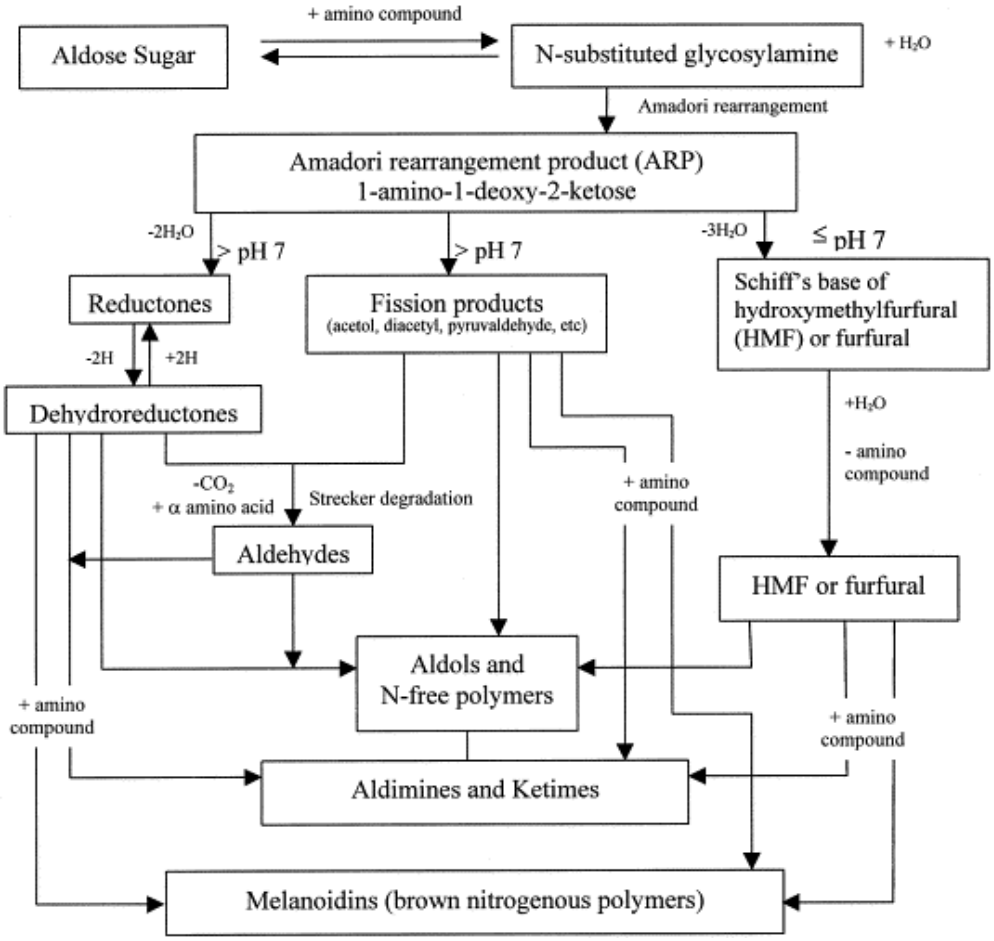


Figure 2. Maillard reaction scheme adapted from Hodge

Source: Martins *et al.* (2001)

The initial stage starts with a condensation between a free amino group (of an amino acid or in protein mainly the ε-amino group of lysine, but also the α-amino groups of terminal amino acids) and an α-hydroxyl carbonyl moiety of a reducing sugar to form a reversible Schiff"s base, which is subsequently rearranged to form stable products. When reducing sugar (e.g. glucose) is reacted with amino compounds, the reversible Schiff's base refers to aldosylamine or N-substitued glycosylamine (e.g. glucosylamine) that unstable. The undergoes a reversible rearrangement to aldosylamine is known as Amadori rearrangement product namely 1-amino-1deoxy-2-ketose. Fructose

reacts in a similar way to give the corresponding rearranged product is known as Heyns rearrangement product namely 2-amino-2-deoxy- 2-aldose. The Amadori or Heyns rearrangement are reversible and the reaction products, aldosamine or ketoseamine, are still colorless (Berk, 1976; Surmaya-Maertinez *et al*., 2005). No browning colour occurs at this stage (Coca *et al.,* 2004).

Next step in the advanced stage, many reactions such as dehydration and enolisation, the Amadori rearrangement product (ARP) or Heyns rearrangement product degrades into a variety of carbonyl compounds. The Amadori rearrangement products undergo further transformation to fluorescent, colour substances and cross-linked polymers (Ames, 1990; Van Boekel, 2002; Surmaya-Maertinez *et al*., 2005). The degradation of the Amadori rearrangement product is dependent on the pH of the system. At pH 7 or below, it undergoes mainly 1,2-enolisation with the formation of furfural (when pentoses are involved) or hydroxymethylfurfural (HMF) (when hexoses are involved). At pH more than 7, the degradation of the Amadori rearrangement product is thought to involve mainly 2,3-enolisation, where reductones, such as 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one and a variety of fission products, including acetol, pyruvaldehyde and diacetyl are formed. All these compounds are highly reactive and take part in further reaction. Carbonyl groups can condense with free amino groups, which results in the incorporation of nitrogen into the reaction products. Dicarbonyl compounds will react with amino acids with the formation of aldehydes and α- aminoketones. This reaction is known as the Strecker degradation (Martins *et al.,* 2001). In this stage, any browning colour occurs and absorb near UV light spectrum.

In final stage, the formation of melanoidin (MW = 12,000-100,000 Daltons) polymeric compounds is the result of polymerisation reactions of highly reactive intermediates that are formed during the advanced stage of Maillard reaction. This compound is recognised as being acidic compounds with a charged nature. A wide range of reactions takes place, including cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations and further condensations proceeds (Coca *et al.,* 2004). These reactions increased with increasing reaction time and temperature.

Maillard reaction development is generally monitored by the increase in the absorbance either at 280 (early MRPs), which is considered to indicate formation of furfural compounds (Hodge and Osman, 1976; Resnik and Chirife, 1979) and is used to detect products of early stage of browning (Flink, 1983). Furthermore, the degree of browning or browning intensity (BI), usually measured via absorbance at 420 nm, is often used to follow the extent of the Maillard reaction (brown pigments or melanoidins) (Ajandouz *et al.,* 2001; Billuad *et al.,* 2004).

1.2.6.2 Formation of colour in the Maillard reaction

The development of colour is extremely important and is associated with the extent of Maillard reaction (Martins and Van Boekel, 2003a). The colour produced range from pale yellow to dark brown, depending on the type of food and/or the extent of the reactions. Colour occurs due to the formation of high molecular weight polymeric compounds known as melanoidins (Coca *et al.,* 2004).

1.2.6.3 Formation of flavour in the Maillard reaction

Most studies on Maillard flavours deal with volatile aroma components, while only few reported exist on taste. Maillard-derived aromas are extremely complex and many components are formed. The most important steps of thermal flavour formation via the Mailard reaction are discussed as follows.

The first step of reaction is the condensation of amino acids and reducing sugar and the rearrangement to Amadori or Heyns rearrangement. The intermediate endiol structures give rise to a facile sugar decomposition by which different aliphatic or cyclic mono- or di- carbonyl compounds are formed. Dicarbonyl compounds react with amion acid to yield the Strecker aldehydes and amino ketone which can be converted via dimerisation to yield pyrazine. Sugars are the main precursors of α-dicarbonyls. The amino/sugar ratio is decisive for the proportion of pyrazines, furans, furanones and pyranones in the volatile fractions. The most abundant flavour compounds formed via the Maillard reaction are aliphatic aldehydes, ketones, diketones and fatty acids. Moreover, heterocyclic compounds containing oxygen, nitrogen, sulfur, are much more numerous and significant to the flavour of thermally processed foods via Maillard reaction. The

furanones and pyranones are oxygen-containing heterocyclic compounds associated with both flavour formation via Caramelisation and Maillard reaction. Oxygenated furans and pyrans, such as furfural, 5-methyl furfural, 2-acetyl furan, generally impact caramel-like flavour, sweet, fruity characteristics to foods (Reineccius, 2006).

During heating process, typical flavour of plant syrup could be formed via Maillard reaction and Caramelisation. Akochi *et al.* (1994) identified alkylpyrazine in maple syrup. It was found that no pyrazine was found in maple sap while pyrazines were detected in maple syrup. The content of total identified pyrazines in maple syrup was 57.29 ng/g. The most abundant pyrazine in the maple syrup samples was 2,6-dimethylpyrazine, representing 34–43% (20.16 ng/g) of the total pyrazines in the samples. 2,6-Dimethylpyrazine showed nutty, roasty and sweet aroma. 2,5-Dimethyl- and 2-methylpyrazines were also presented in considerable amounts. Trimethylpyrazine was presented in maple syrup (7.79 ng/g). This suggests that trimethylpyrazine could play a role in imparting the characteristic maple flavour and showed roasted bean aroma. The results indicated that pyrazine formed due to concentration process via Maillard reaction and Caramelisation during heating. This result was in accordance with Akochi *et al.* (1997) who characterized pyrazine formation in maple syrup during boiling of maple sap at 105^{o}C for 220 min. They also found that no pyrazine was detected during heating from 0 to 60 min at 105^{o}C. However, 2,5-dimethyl- and trimethylpyrazine were formed after 60 min of heating time. Whereas methyl-, 2,6-dimethyl-, ethyl-, 2,3-dimethyl-, and 2-ethyl-3-methylpyrazine were detected after 120 min of heating time. Total level of pyrazines increased from 3.42 ng/g after 60 min of heating time to 72.32 ng/g in the end of heating time (220 min). This indicated that pyrazine formed due to heating process via Maillard reaction and Caramelisation.

Ho *et al.* (2007) monitored the volatile flavour compound of palm sap (*Arenga pinnata*) during heating process for production of palm sugar cake. The samples were collected at every 30 min interval during heating process at 150^{o}C for 4 h. The analyses were performed by GC-MS after HS-SPME. The results showed that N-heterocyclic (pyrazine derivatives) chemical class possessed the highest relative

percentage area (RPA) 83.69%, followed by O-heterocyclic group (furan derivatives) with RPA of 14.5%. Main volatile compounds were 5-methyl-6,7-dihydro-5H-cyclopenta pyrazine and 4-hydroxy-2,5-dimethyl-3(2H) furanone which were responsible for roasty and sweet caramel-like notes, respectively. The pyrazine compounds were detected after 30 min of heating time and increased exponentially with heating time. On the other hand furan derivatives compounds were formed at 180 min of heating time.

1.2.6.4 Factor affecting on the Maillard reaction

Maillard reaction is strongly affected by several factors such as types of sugar and amino acid (Jing and Kitts, 2002), temperature, time, pH (Ajandonz *et al.*, 2001) and water activity (Buera *et al.*, 1987).

Sugars

Reducing sugars are essential ingredients in Maillard reaction, as they provide the carbonyl groups for interaction with the free amino groups of amino acids, peptides and proteins. The initial kinetics of glycation are dependent on the proportion of the reducing sugar existing in the acyclic or active form under the reaction condition (Yaylayan *et al.*, 1993) and on the electrophilicty of the sugar carbonyl group (Bunn and Higgins, 1981). Starch and non-reducing sugars such as sucrose may be hydrolyzed to form reducing sugars that can participate in the Maillard reaction (Camire *et al.*, 1990). The reactivity of reducing sugars was reported to decrease in the following order: aldopentoses> aldohexoses> aldoketoses> disacchrides (Spark, 1969). Pentoses yield stronger colour intensity than hexoses (Lingnert, 1990). Among the four sugars, fructose, maltose, lactose and glucose, fructose has the highest proportion of opened chain form, but aldoses would react faster than ketoses because they are more electrophilic (Naranjo *et al.*, 1997).

Amino acid and protein

Amino acid participating in the generation of melanoidins is more influence on the melanoidin formation than carbonyl compounds (Eskin, 1990). The reactivity of the amino acids to form Maillard reaction products is different. The reactivity decreases in the order of lysine>glycine>alanine (Morales and Jimenez-Perez,

2001). Additionally, the increase in reaction rate was observed with increasing amino acid concentration (Toribio and Lozano, 1986). Kwak and Lim (2004) found that the reactivity of lysine to form Maillard reaction products was 2-3 times higher than other amino acid. High reactivity of lysine is attributed to the two α- and ε- amino groups. Cysteine was found to have the lowest contributory effect to browning due to the sulfur amino acid (Kwak and Lim, 2004).

Temperature and heating time

Temperature and heating time is also affected on the rate of chemical reactions especially, Maillard reaction (Martins *et al.*, 2001; Brands and Van Boekel, 2002). Maillard reaction proceeds effectively at temperature above 50^{o}C. Yaylayan *et al.* (1993) reported that the percentage of fructose in its acylic form at neutral pH is about 0.7% at room temperature and 13.1% at 80°C. Temperature affects the activities of the reactants. The active form of sugar is considered to be opened chain, which is formed markedly with increasing temperature (Van Boekel, 1998, 2001).

pH

The carbonyl-amino reaction can either develop in acidic or alkaline media, although it is favoured under alkaline condition. The reactivity of sugar and amino acid is also highly influenced by pH. The opened chain form of the sugar and the unprotonated form of the amino group, considered to be the reactive form, are favoured at higher pH. At lower the pH, the more protonated amino group is present in the equilibrium and therefore, less reactive with the sugar (Martins *et al*., 2001)**.**

Water activity

Water activity (Aw) is an important factor for Maillrad reaction determination. The rate of browning decreases continuously with increasing water activity (Buera *et al.*, 1987; Bell *et al.*, 1988). A maximal browning rate was generally observed at water activity between 0.5 and 0.8. However, the rate of Maillard reaction decreases when water activity reaches 0.9-1.0 due to the substrate is diluted (Eichner and Karel, 1972; Bell, 2007).

1.2.6.5 Caramelisation

One of the most methods of causing colour and flavour in food is the heating of sugar and sugar rich foods. The reactions which occur are responsible for the caramel-like flavour and the development of a brown colour. The requirement for Caramelisation reaction is temperature approximately 120^{o}C or above and pHs between 3-9. (Kroh, 1994).

Caramelisation is a complex reaction. The first step involves the stepwise conversion of glucose, fructose and mannose (Figure 34A). These transformations can be mediated by organic acid catalysts. The interconversion of these sugars occurs primarily through the 1, 2-enolic form. Further heating results in the dehydration of sugars that leading to the formation of 5-hydroxymethylfurfural (HMF). This process is initiated by the removal of a hydroxyl group from the 1, 2-enediol form located in the α-position to the carbonyl group. The initial product, a dicarbonyl, undergoes further degradation. The postulated intermediate in this reaction is though to 3-deoxyosulose (Eskin, 1990). If the initial sugar is pentose, then the final dehydration product is 2-furaldehyde (furfural). The polymerization of furfural derivatives leads to the formation of coloured pigment. If the heating of sugar occurs under alkaline conditions, then 1, 2- and 2, 3-enediols are formed, which may undergo cleavage and fragmentation reactions leading to formation of various flavour compounds such as saccharinic acid, lactic acid, 2, 4-dihydroxybutric acid, ethyl alcohol and aromatic compounds. The formation of flavour varies from mild, caramel-like and sweet to burning bitter (Kroh, 1994). The enolization reaction is of particular important because it initiates the subsequent chain of events. These reactions give rise to the aliphatic sugar degradation products which can react further to produce oxygen heterocyclic and carbocyclic compounds via aldol condensation (Kroh, 1994). In fairly late stage of the Caramelisation reaction, brown coloured polymeric substances are formed via radical polymerization (Kroh, 1994).

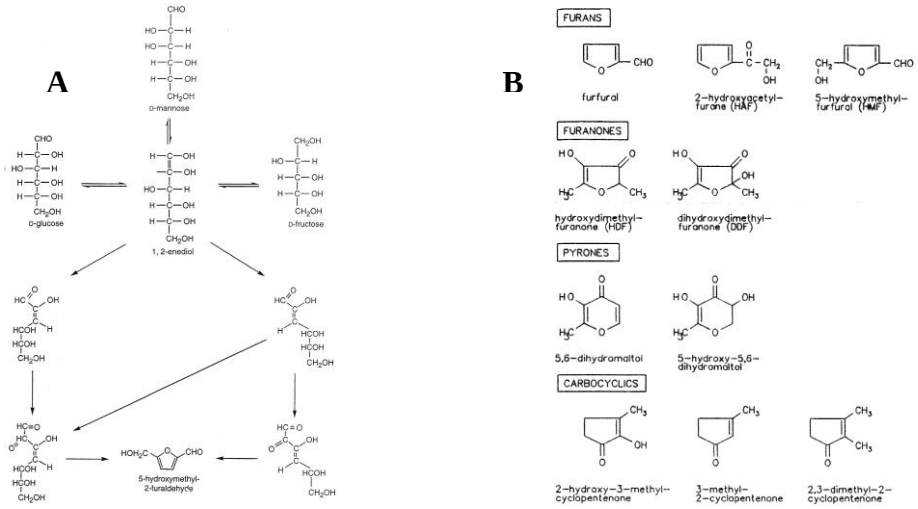


Figure 3. Mechanism of sugar dehydration and formation of HMF, a precursor of caramel (A) and typical caramel aromatics formation during caramalisation (B)

Source: Eskin (1990) and Kroh (1994)

From these principle sugar degradation reactions, key intermediates of the thermal Caramelisation are α-dicarbonyl compounds such as 3-deoxyosulose. These not only lead to the formation of caramel colour but give rise to the important volatile products which are typical of caramel flavour. Some typical components of caramel flavour are shown in Figure 3B (Kroh, 1994).

1.2.6.6 Antioxidant activity of Maillard reaction products and Caramelisation products

Antioxidant in food is defined as any substances which is capable of delaying, retarding or preventing the development of rancidity or other flavour deterioration due to oxidation (Gordon, 2001). These substances can occur as natural constituents of foods, but they also can be intentionally added to products or formed during processing. Their role is not to enhance or improve the quality of foods, but they

do maintain food quality and extend shelf-life. Based on their function, food antioxidants are classified as primary and secondary antioxidant (Rajalakshmi and Narasimhan, 1996). Primary antioxidants terminate the free radical chain reaction by donating hydrogen or electron to free radicals and converting them to more stable products. Many of naturally antioxidants occurring phenolic compounds likes flavonoids, eugenol and tannin also have chain breaking properties (Rajalakshmi and Narasimhan, 1996).

The Maillard reactions products (MRPs) have a direct impact on the overall acceptability of food by consumer. Antioxidant activity is one of the functional properties of MRPs. MRPs derived from glucose-lysine, xylose-lysine, fructose-lysine and casein-sugar model system also possess DPPH radical scavenging activity, reducing power and FRAP (Yen and Hsieh, 1995; Yoshimura *et al.,* 1997; Ajandouz *et al.,* 2001; Kim and Lee, 2009). Turkmen *et al.* (2006) monitored the change in DPPH radical scavenging activity of honey during heating at 70^{o}C and reported that MRPs in honey exhibited DPPH radical scavenging activity and increased with heating time.

The Caramelisation products (CPs) can be formed during heating sugar in the absence and present of amino acid when temperature reached above 100^{o}C. CPs also showed antioxidants activity as reported by many researchers. CPs from glucose, fructose, xylose and ribose prepared by heating at 100^{o}C at pH 7 and pH 10 have a DPPH radical scavenging activity (Benjakul *et al.,* 2005). DPPH radical scavenging activity of CPs prepared under neutral conditions increased linearly as the heating time increased. For CPs prepared under alkaline conditions, an exponential increase in DPPH radical scavenging activity was observed with increasing heating time. Reducing power of CPs from 1.47 M sucrose dissolved in buffer pH 4 and heated at 200^{o}C increased with prolonged heating time (Lee and Lee, 1997).

1.2.7 Sugar crystallisation

Crystallisation is the term that describes several different phenomena related to the formation of a crystalline lattice structure. The four steps in crystallisation

typically include: (1) generation of supersaturated state, is a prerequisite for crystallisation, (2) nucleation- the formation of crystalline lattice structure from solution or melt, (3) growth-subsequent growth of nuclei until equilibrium is attained and (4) recrystallisation- a reorganization of the crystalline structure to a lower energy state, generally without any further change in the amount of crystalline phase volume (Hartel, 2001). Monosaccharides and disaccharides in a solid form can be found either in stable crystals (ordered structure) or in amorphous (noncrystalline) material. Crystallisation may occur from a melt or from a solution. Crystallisation from a melt may occur at temperature below equilibrium melting temperature (T_m) and from a solution due to supersaturation (Roos, 1995).

1.2.7.1 Crystallisation in solution

The crystallisation in solution was illustrated to explain formation of nuclei and crystals in an unseeded solution. This theory is shown in Figure 4, where line AB is the normal solubility curve. If a sample of solution at point a is cooled, it first crosses the solubility curve. The sample will not crystallise until it has supercooled to point b where crystallisation begin and the concentration drop to point c if no further cooling is done. The curve CD, called the supersolubility curve, represent the limit at which nucleus formation starts spontaneously and hence where crystallisation can start. Crystals in the metastable region will grow. The present tendency is to regard the supersolubility curve as a zone where the nucleation rate increases sharply (Hartel, 2001; Joupplia, 2006).

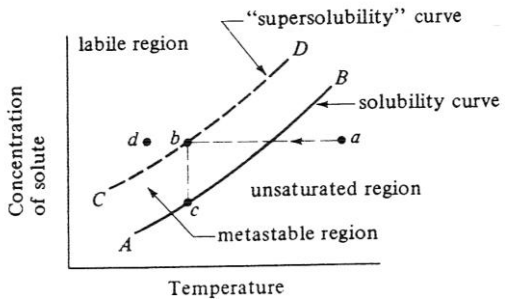


Figure 4. Qualitative explanation of crystallisation

Source: Geankoplis (1993)

1.2.7.2 Factors affecting on the sugar crystallisation

Sugar crystallisation is strongly affected by several factors such as type of sugar, superstauration and temperature (Hartel, 2001).

Type of sugars

Most sugars can be induced to crystallise, particular the non-reducing oligosaccharides. However, it is difficult to obtain certain reducing sugars in a crystalline form because the presence of anomers and ring isomers in solution make the reducing sugars intrinsically "impure". The presence of rings isomer hinders the crystallisation of fructose and unstable conformation. This leads to significant amounts of conformational isomer in equilibrium solution and seems also to present barrier to crystallizsation. Sugars are generally very soluble in water and frequently form supersaturated syrups when their solutions are concentrated. The syrups of reducing sugars are particularly resistant to crystallisation (Shallenberger and Birch, 1989).

Supersaturation

The term supersaturation refers to a solution that contains more of the dissolved material than could be dissolved by the solvent under normal circumstances. In primary nucleation, as the supersaturation is increased due to concentration increases, the nucleation rate initially increases sharply once the critical supersatutation has been exceeded. This critical supersaturation may be defined functionally as the boundary between the metastable and labile zones. Below this critical supersaturation, the solution is metastable and nucleation does not occur. If the supersaturation increased sufficiently by increasing the concentration, eventually the viscous barrier to nucleation, becomes dominant and rate of nucleation begins to decrease (Hartel and Shastry, 1991).

Temperature

The effect of temperature is intimately linked to the driving force for crystallisation. In solution system, especially where solubility is strong function of temperature, supersaturation changes as temperature is changed. Under low temperature, supersaturation increased due to decreased temperature usually reduced the solubility of

solids in liquids. Thus, low temperature accelerates the increment of nucleation rate (Hartel and Shastry, 1991).

1.2.8 Moisture sorption isotherm (MSI) and phase transition in sugar-based product

The MSI depicts the relationship between equilibrium moisture content and water activity at a constant temperature (Labuza, 1968). Foods with different moisture contents will have different water activity depending upon the interactions between water and food solid. Therefore, each food product will have its own unique MSI (Bell and Labuza, 2000). In water adsorption, material adsorbs water when stored at various relative humidities (RH) higher than an initial equilibrium relative humidity (ERH) of material (ERH = Aw × 100) at constant temperature, resulting in the material gaining weight. In water desorption, a material desorbs water when stored at relative humidities lower than the initial ERH of material at the same temperature, resulting in a loss of weight of the material. Water sorption of dehydrated material is usually determined gravimetrically by weighing the samples stored at various RHs (established using various saturated salt solutions) at constant temperature and observing the changes in water content as a function of storage time (Roos, 1995; Labuza, 1968). Information derived from MSIs are useful (1) for concentration and dehydration processes, because the ease or difficulty of water removal is related to relative humidity (RH), (2) for formulating food mixtures so as to avoid moisture transfer among the ingredients, (3) for determination the moisture barrier properties needed in a packaging material, (4) for determination what moisture content will curtail growth of microorganisms of interest, and (5) for prediction the chemical and physical stability of food as a function of water content (Fennema, 1996).

1.2.8.1 MSI of crystalline and amorphous sugar

Each type of an isotherm is influenced by a structure or a structure of a solid. In a simplest term, a solid can exist as a crystal (i.e. an ordered molecular lattice) or

as an amorphous substance (i.e. non-crystalline, random molecular arrangement) (Bell and Labuza, 2000). The general types of MSI for this range are depicted in Figure 6. The following classification scheme was introduced by Brunauer (1945).

Type I isotherm is typical of anticaking agents. This type of ingredient adsorbs water on specific sites with a high binding energy and into a network of non swelling capillaries, resulting in the holding of large amounts of water at low water activity. Figure 5 shows the shape vertical rise in moisture at low water activity. When all the binding sites, as well as any narrow capillaries, are filled, any further increase in moisture content causes a large water activity increase. This large increase in water activity occurs because the material does not dissolve or swell, so the added water interacts only with the water already present through weak hydrogen bonding, forming a surface solution. Most foods, such as cereal, followed the sigmoidal-shaped curve representative of a type II isotherm. The resultant curve is caused by the combination of colligative effects, capillary effects, and surface-water interactions. The type III curve represents the MSI of a pure crystalline substance such as sucrose and shows very little moisture gain until the water activity goes above the point where water begins to dissolve the crystal surface (water activity 0.7-0.8 for sucrose). The shape of this curve is due to water interacting only via hydrogen bonds with the hydroxyl groups on the surface of the crystal. At low water activity, the interaction of water with the sugar molecules is not strong enough to break the interactive forces between individual sugar molecules in the crystal. However, as the water activity is increased, the overall water-sugar interactions increase enough to cause disruption of the sugar-sugar interactions, and thus water begins to penetrate into the crystal, dissolving sugar molecules and exposing new surfaces. At this water activity, the moisture content rises dramatically because a solution is being created. The water activity at the beginning point of solution generally corresponds to the water activity of a saturation solution and is where the vapour pressure lowering begins to occur (Bell and Labuza, 2000).

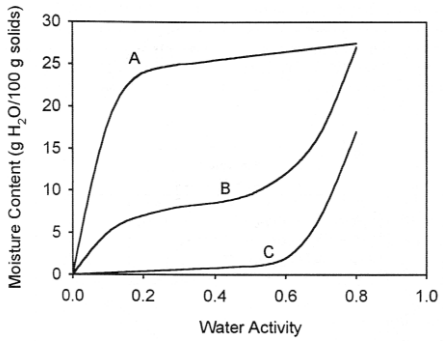


Figure 5. Classification of MSI: A = type I (anticaking agent), B = type II (most foods), C = type III (crystalline substance)

Source: Bell and Labuza (2000)

Water adsorption in foods affects their physical state and stability. Therefore, prediction of water adsorption is needed to establish water activity and water content relationships for food materials. Such prediction is often based on the determination of sufficient experimental data and fitting adsorption models to the data. Several empirical and theoretical adsorption models are available. These models have proved to be useful in predicting water adsorption, since experimental data are usually obtained only at a few relative vapour pressures. The most frequently used models for food materials are the Guggenheim– Anderson–de Boer (GAB) and Brunauer–Emmett–Teller (BET) models. The biggest difference between these models is the range of water activity over. The range of water activity in BET model is 0 to 0.5 and a wider water activity range for the GAB model. The GAB isotherm is a particular useful a sorption model, since it can be applied over a wide water activity range (Roos, 1995).

1.2.8.2 Glass transition temperature

The glass transition temperature (Tg), is the temperature at which the amorphous phase of the polymer is converted between rubbery and glassy states. The molecular diffusion in glassy state of material is extremely slow. The product may be shelf stable when stored below Tg since deterioration due to microbial growth and chemical reaction is greatly reduced (Sablani *et al.,* 2007). The rules of glass-transition

concept are: (1) the food is most stable at Tg and below its Tg, and (2) higher the T-Tg, higher the deterioration or reaction rates (Rahman, 2006). In food systems, the glass transition temperature is mainly affected by the water content and the average molecular weight. The glass to rubber or liquid transition is accompanied by rather abrupt changes in the physical properties of the material with temperature, e.g. increase in entropy, heat capacity, and volume and a decrease in both rigidity and viscosity. These changes in physical properties can be used to determine the Tg of the material (Roudaut *et al.*, 2004).

Amorphous monosaccharides and disaccharides, like almost all biomaterials, can be plasticized by water, and an increase in water content causes a decrease in Tg. Water plasticization can occur if a material is stored at relative humidities that lead to an increase in the water content of the material (i.e., increased water sorption). Storage of amorphous food materials at temperatures above their glass transition temperatures results in thermal plasticization of material; the viscosity decreases dramatically, and the molecular mobility increases (Roos, 1995). Thus, the physical state of the material changes rapidly (i.e., the material undergoes glass transition). Glassy material becomes rubbery, sometimes even a viscous, syrup-like liquid with obvious viscous flow. The Tg of a sugar mixture depends on the proportion of different sugars in the mixture. Lower molecular weight sugar has been shown to plasticize higher than high molecular weight sugar (Roos and Karel, 1991). Mono- and disaccharides can be used as plasticizers for higher molecular weight carbohydrates; for example, the Tg of a maltodextrin–sucrose mixture decrease with increasing amounts of sucrose (Roos and Karel, 1991). Correspondingly, the Tg of amorphous sugars increase with increasing amounts of high-molecular-weight carbohydrates (Roos, 1995). The Tg of the amorphous mixture of sucrose and corn syrup saccharides increased with an increasing proportion of corn syrup saccharides (Gabarra and Hartel, 1998).

1.2.8.3 Crystallisation of amorphous sugar

When amorphous sugars or products containing amorphous sugars are stored at temperatures above their Tg, crystallisation of the amorphous sugars may occur due to increased molecular mobility**.** The rate of crystallisation of amorphous sugars has

been shown to increase with an increasing temperature difference T–Tg due to decreasing viscosity and increasing diffusion and translational mobility (Roos, 1995); thus, the storage temperature and water content of amorphous sugar are the key parameters in predicting the tendency of amorphous sugar to crystallise during storage. Crystallisation of amorphous sugar may occur when the relative humidity during storage is higher than the critical storage RH at the storage temperature. Sugar crystallisation rates have been shown to increase with increasing RH at a constant temperature or with increasing storage temperature at constant water (Roos and Karel, 1990; Kedward *et al.,* 2000). X-ray diffraction techniques are used to confirm the amorphous state of material at the beginning of crystallisation studies and to observe the extent of crystallisation from increasing intensities.

Wang and Langrish (2007) measured crystallisation rates of amorphous sucrose powders from spray drying. The effects of temperature and RH on the crystallisation process within amorphous solids have been explored. Lactose and sucrose were spray dried, and the products were exposed to different RH (32%, 51% and 75%) at both room temperature and 40^{o}C. The rate of crystallisation for both sucrose and lactose was monitored through sorption measurement and XRD techniques. The main peaks associated with lactose were 19.1^{o}, 19.6^{o}, 20^{o} and 21^{o}. The main peaks associated with sucrose were 11.6^{o}, 19^{o}, and 24.7^{o}. The rate of the crystallisation was more than doubled for every increase of temperature by 10^{o}C, up to three times for the case of lactose. The effect of increasing RH was observed to increase the rate of crystallisation. The 95% crystallisation times for sucrose are both about two hours at RH of 75% (2.2 h) and 51% (1.9 h). This time, however, is more than doubled at 32% RH (5.1 h). The time taken for 95% of crystallisation to occur at 40^{o}C was less than 2 h, and that at room temperature (20^{o}C) was 9 h.

Crystallisation of amorphous sugar may be delayed by the presence of polysaccharides or other macromolecules and other sugars or various anomeric forms of sugars. Polysaccharides may impede the diffusion of sugar molecules and cause steric

hindrance, which results in decreased nucleation and crystal growth in amorphous sugars (Hartel and Shastry, 1991).

Nowakowski and Hartel (2002) studied the impact of formulation on the stability of sugar glasses in model systems. Model systems were made with mixtures of sucrose and commercial corn syrup products. Sucrose was combined with several corn syrups, 43 DE (control), and 65 DE very high maltose (maltose 95%), at concentrations of 15, 37, and 57% corn syrups. Sugar glasses in powder form made with different ratios of corn syrup to sucrose and with different types of corn syrups were equilibrated in different RH, varied between 11-80%. It was found that Tg values were higher for the 57% corn syrup formulation when compared to that of the 15% corn syrup formulation. This corn syrup has a higher Tg than sucrose, so higher addition levels led to higher Tg. It was similar to the result of sucrose with 65 DE corn syrup solid. This could be due to the effect of small molecular weight compounds acting as plasticizers (lowering Tg) in this corn syrup, despite the increase in average molecular weight (which would tend to increase Tg). Hardness of the amorphous sugar products decreased as moisture content increased. The high maltose corn syrup (65 DE) led to slightly harder products at equivalent moisture content than corn syrup (43 DE). Higher corn syrup concentration increased hardness. Therefore, incorporation of corn syrup into amorphous sugar formulations can be used to prevent sugar crystallisation and increases Tg (Gabarra and Hartel, 1998).

1.2.8.4 Nonenzymatic browning in glassy system

Nonenzymatic browning reactions are one of the most important chemical reactions in foods. In sugar products during storage, Maillard reaction can take place because this reaction can effective at low and high temperature. However, Caramelisation can not occur because this reaction progress at temperature 120^{o}C or above. Therefore, only Maillard reaction caused the quality of sugar products at ambient temperature. Nonenzymatic browning reactions have effects on the flavour, colour and texture of food materials. It also affects the nutritional quality and toxicological characteristics (Eskin, 1990; Coultata, 1993).

The nonenzymatic browning reactions rate was increased with RH and temperature storage. Sample stored at high RH adsorbed water and tended to increase moisture content more than stored under low RH.

Miao and Roos (2006) monitored the rate of nonenzymatic browning reactions in freeze-dried lactose, trehalose, and lactose/trehalose-based food model systems containing L-lysine and D-xylose (2% w/w) as reactants on four different RHs (33.2%, 44.1%, 54.5%, 65.6%) environments at room temperature. It was found that lactose/reactant, lactose/trehalose/reactant and trehalose/reactant systems showed different nonenzymatic browning reactions kinetic during storage at various RHs. At RH 33.2% and 44.1%, BI of freeze-dried model systems increased linearly with zero order kinetics (Labuza and Baisier, 1992).

When materials were stored at 54.5% RH, BI in freeze-dried lactose/reactant, trehalose/reactant systems was observed increase with times and divided in three stages suggesting stepwise reaction kinetics. Firstly, browning developed at a low rate, secondly, BI increased rapidly and linearly, and this was followed by the third plateau stage. This interesting stepwise nonenzymatic browning reaction behaviour seemed to be related to the water sorption properties of the matrix materials, particularly to the loss of water released during crystallisation of the component sugars. When lactose/reactant and trehalose/reactant systems were exposed to 54.5% RH, increasing sorption of water was followed by a decrease in the sorbed water content resulting from the crystallization of the component sugars until a maximum extent of crystallisation of the component sugars was achieved. The second stage of rapid nonenzymatic browning reaction development in the trehalose/reactant system occurred earlier than in the lactose/reactant system. When freeze-dried materials were stored at 65.6% RH, BI of the systems was observed at 11 h, 24 h and then at 24 h intervals up to 240 h. BI of the freeze-dried trehalose/reactant system increased linearly after 11 h, which was followed by a leveled-off plateau. When freeze-dried was exposed to high RH (65.5%), mobility of the molecules increased. Crystallisation of component sugars seemed to affect nonenzymatic browning reaction kinetics through the amount of released absorbed water.

Uttraporn (2006) determined the effect of RHs (50%, 65% and 80%), storage temperature (30^{o}C, 40^{o}C and 50^{o}C) and storage time (0-6 months) on the nonenzymatic browning reactions of coconut cake. The results showed that water activity and moisture content of samples increased with increasing RH. The coconut cake stored at the RH of 80% was liquefied within 1 month due to mass transfer of water from high moisture content of environment to product. BI increased with increasing of RH, storage temperature and storage time corresponded to the decrease in L* value. Decreasing of L* value indicated that colour of coconut cake change to brown.

1.3 Objectives of study

1. To characterise palm sap, palm sugar syrup and palm sugar cake.
2. To study the effect of harvesting time of palm sap, heating time and storage condition on property changes in palm sugar syrup.
3. To study the effect of processing method, heating time and storage condition on property changes in palm sugar syrup.
4. To study the effect of sucrose and glucose syrup addition and storage condition on the property changes in palm sugar cake.
5. To study the effect of storage temperature on moisture sorption isotherm (MSI) characteristic and glass transition temperature of palm sugar cake.

CHAPTER 2

CHARACTERISATION OF PALM SAP, PALM SUGAR SYRUP AND PALM SUGAR CAKE (*BORASSUS FLABELLIFER* LINN.)

2.1 Abstract

Palm sap is dehydrated to get products of palm sugar syrup and palm sugar cake. So far, these products are produced using an own experience of each producer with a conventional method. No nation or international standard to control a property of these products yet. Therefore, the purpose of this study was to characterise the property of palm sap, palm sugar syrup and palm sugar cake. Ten palm sap samples harvested in Songkhla province were analysed. The results showed differed in physical and chemical property among samples ($P<0.05$). The results showed range of L*, a* and b* values between 61.49 to 87.53, 1.46 to 3.52 and 12.41 to 19.31, respectively. The transmittance value was ranged from 39.56% to 79.95%. The pH value was varied from 4.19 to 5.23, while total acidity was ranged from 0.13% to 0.19%. The total soluble solids ranged from 10.67^{o}Brix to 17.40^{o}Brix. Total and reducing sugars were varied in a range of 11.65% to 21.66% and 0.95% to 4.06%, respectively. The sucrose, glucose and fructose contents were found vary in a range from 10.45% to 20.91%, 0.56% to 2.21% and 0.54% to 2.17%, respectively. Protein content varied from 0.31 mg/g to 0.39 mg/ml. Ethanol was also found in all samples that indicating the fermentation. All results indicated a large variation property of palm sap although they harvested in the same production area in Songkhla province. The different property of palm sap was mainly due to the fermentation of sugars by the activity of microorganisms during palm sap collecting time.

Ten palm sugar syrup samples from primary producers in Songkhla province were also analysed for their physical and chemical properties. The results showed differences among the samples ($P<0.05$). The results showed ranges in L*, a* and b* values of between 8.32 to 27.98, 23.23 to 34.70 and 14.30 to 54.60, respectively.

The transmittance value ranged from 1.00% to 4.52%. The IBP ranged from 0.47 to 0.91 and the BI ranged from 0.62 to 1.32, respectively. The pH value varied between 4.52 and 5.36, while the total acidity varied from 0.21% to 0.48%. The total soluble solids ranged from 62.97^{o}Brix to 67.50^{o}Brix. The moisture content ranged from 27.31% to 29.63% and water activity varied from 0.79 to 0.81, respectively. The sucrose content ranged from 60.37% to 83.26%; the glucose and fructose content varied from 4.01% to 23.20% and 4.44% to 22.20%, respectively. The protein content varied from 7.24 mg/ml to 8.36 mg/ml. The HMF content was found to vary between 21.71 mg/kg and 137.50 mg/kg. Ethanol and acetic acid content were observed in all samples because of the activity of microorganisms. Furthermore, pyrazine and furan derivatives were also detected in all samples due to Maillard reaction and Caramelisation during the heating process.

Ten palm sugar cake samples from producers in Songkhla province were also analysed for their physical and chemical properties. The results showed differences among the samples ($P<0.05$). The results showed ranges in L*, a* and b* values of between 28.28 to 55.61, 6.43 to 14.23 and 11.77 to 27.37, respectively. The IBP and BI ranged from 0.52-1.15 and 0.38-0.84, respectively. The hardness ranged from 30.83 N to 69.00 N and the stickiness ranged from 0.11 N to 0.33 N, respectively. The solubility varied from 96.28% to 98.57%. The pH value ranged from 4.09 and 4.82, while total acidity ranged from 0.21% to 0.30%. Water activity ranged from 0.72 to 0.77, respectively. Reducing sugar and total sugar contents were found in a range of 5.09% to 9.16% and 80.76% to 100.73%, respectively. The sucrose content was mainly found to range from 79.24% to 87.72%. Meanwhile, only a small amount of glucose, fructose and maltose content were found to range from 1.04% to 5.74%, 0.16% to 3.05% and 0.00% to 4.41%, respectively. The HMF content was found to vary between 23.26 mg/kg to 358.06 mg/kg. The crystallinity of these samples was found to range from 73.40%-78.56%. The Tg of samples were in a range of 5.38^{o}C-26.76^{o}C. The variations in the characteristics of the palm sugar cake samples were probably due to differences in the properties of palm sap or palm sugar syrup, the ingredients added and the heating conditions.

2.2 Introduction

Palmyra palm (*Borassus flabellifer* Linn.) can be found in tropical countries such as Thailand, Malaysia, Indonesia, India, Myanmar, Sri Lanka and Cambodia. In Thailand, palmyra palms are crowded in southern part of Thailand from Phetchaburi to Songkhla provinces. Most populations of Palmyra palms are in Songkhla province, approximately 3 millions plants (Department of agricultural extension Thailand, 2001; Taybui, 1984). The most important product of palmyra palm is the sap, syrup and cake. The tapping process of palm sap involves the bruising of the interior of the developing inflorescences by means of a wooden mallet or tong, thereby stimulating sap flow. Sap is collected by cutting the outer end at the head of the inflorescences. Sap is collected twice a day from each inflorescence, normally in the morning and the evening. Three to six inflorescences are tied together and inserted into a suitable container for sap collection, usually using an earthenware pot (in Sri Lanka) or a bamboo tube (in Thailand) (Davis and Johnson, 1987). Fresh sap is sweet, oyster white colour and translucent, with nearly neutral pH (Gupta *et al.,* 1980). The sap is sterile (free of microorganisms) while flowing in palmyra inflorescences. However, microorganisms are found in the sap which is coming from an environment during collecting process. Microbes are introduced into the sap by unsanitary tapping procedures and unsanitary collection. Consequently, the growth of microorganisms in the sap will be manipulated. Further contamination of sap occurs when the utensils are not completely cleaned and sanitized between sap runs, especially during summer season such as in the southern Thailand, which favours the rapid growth of microbial loads. Bacteria account for most of the contamination. Increased temperatures favour the rapid growth of microorganisms, thereby increasing their populations over time. These microorganisms use sugars in the sap as an energy source and result in fermentation of palm sap. The fermenting organisms are dominated by yeasts, particularly *Saccharomyces cerevisiae* and lactic acid bacteria (Chanthachum and Beuchat, 1997). Since palm sap is rich in sugars (10-17%) and, unless it is collected under hygienic conditions, rapidly fermentation and conversion reactions to

acids and alcohols occur (Iwuoha and Eke, 1996). To prevent fermentation, Kiam wood (*Cotylelobium lanceotatum* Carih.) is commonly added to the collection receptacle because it can delay spoilage in palm sap by reducing microbial populations as well as keeping the quality of a product (Department of agricultural extension Thailand, 2001; Chanthachum and Beuchat, 1997).

Palm sap can be used as a raw material to produce palm sugar syrup. In the traditional production of palm sugar syrup, a large volume of filtered palm sap is heated on a wood fired stove at above 100^{o}C until it becomes concentrated to obtain a typical aroma (Phaichamnan *et al.*, 2010). Palm sap starts with a total soluble solid (TSS) approximately in a range of 10-19^{o}Brix. It takes approximately 6-7 parts of palm sap to make one part of syrup. The TSS of palm sap reaches a higher value during heating for production of palm sugar syrup. The minimum requirement for the TSS of palm sugar syrup is 65^{o}Brix. This is the minimum set by the Thai Industrial Standards Institute of the Ministry of Industry. Currently, palm sap is concentrated by two procedures; either by one step or two steps of heating processes. In the one step of heating process, palm sap is heated continuously until the final TSS reaches 65^{o}Brix or above. On the other hand, in the two steps of heating process, palm sap is first heated until it concentrates to obtain palm sugar syrup (the TSS is approximately 30^{o}Brix). Then new palm sap is added to the previous palm sugar concentrate and heated again until the TSS reaches 65^{o}Brix or above. During the heating process, physical and chemical changes occur which impart the unique colour and flavour features. The longer sap is boiled, the darker it becomes. Major reactions occur mainly during the process of heating the palm sap including the inversion reaction and nonenzymatic browning reactions (the Maillard reaction and Caramelisation). These reactions affected the property of palm sugar syrup.

Palm sugar cake is made from various materials, and mainly plant sap and sucrose. This form of sugar product is easy to transport and store and also provides a good source of sugar which is used as a sweetener ingredient. The purpose of the heating process during sugar cake production is to remove water from the sap or syrup until it becomes concentrated (the total soluble solid is more than 80^{o}Brix). As soon as the

temperature of this heated palm syrup reaches approximately 120^{o}C, it is removed from the heat and stirred. The stirring process is continued until the solution begins to crystallise and stiffen. At this stage, it can be poured into a mould. However, with low quality syrup it is hard to obtain a crystalline sugar cake. Therefore, sucrose is always used as a seeding agent in order to induce a crystallisation of the sugar. In addition, glucose syrup is usually added to increase the stability of this product (Nowakowski and Hartel, 2002). Each producer uses a different processing formula to produce a palm sugar cake product, thus resulting in the diversity of the properties of the palm sugar cake. Until now, scientific data has rarely been reported on the properties of palm sap, palm sugar syrup and palm sugar cake in Thailand even though they are commercial products. Therefore, the aim of this work was to characterise the properties of palm sap, palm sugar syrup and palm sugar cake.

2.3 Materials and Methods

Chemicals

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, methanol and phosphoric acid were obtained from Merck (Darmstadt, Germany). Bovine serum albumin (BSA) and Coomassie brilliant blue were obtained from Fluka (Buchs, Switzerland). Potassium sodium tartrate, lead acetate and potassium oxalate were purchased from Riedel-deHaen (Seelze, Germany).

Palm sap collection

Palm sap (ten samples) was randomly collected from farmers in Songkhla province, southern Thailand. Palm sap was harvested after 12-15 h of collecting time

with added Kiam wood (*Cotylelobrium lanceotatum* Carih.) 3 g/L during tapping process using bamboo tube. After that each bottle of palm sap was kept in an icebox (4^{o}C) during transportation (30 min) to the department of Food Technology, Prince of Songkla University, Hat-Yai Campus. The physical, chemical and microbiological property of each sample was determined within a day. Before analysing, the sample was filtrated by sheet cloth.

Palm sugar syrup collection

Ten commercial palm sugar syrup samples were randomly purchased from local manufacturers located in Songkhla province, southern Thailand. All samples were traditionally produced by thermally heating the palm sap on a wood fire stove. Palm sap was concentrated by two procedures, either the one step or two steps of heating process. In the one step of heating process, palm sap was heated between 3 and 5 h (at a temperature of approximately 110^{o}C) continuously until the final TSS reached 65^{o}Brix or above. In the two steps of heating process the palm sap was first heated (at a temperature approximately of 110^{o}C) until it concentrates (the TSS was approximately 30^{o}Brix) to obtain palm sugar concentrate. Then new palm sap was added to the previous palm sugar concentrate and heated again between 5 and 8 h (at a temperature of approximately 110^{o}C) until the final TSS reached 65^{o}Brix or above. The samples were kept in bottles and analysed immediately for physical, chemical and microbiological properties after collection.

Palm sugar cake collection

Ten commercial palm sugar cakes were also randomly purchased from producers in Songkhla province, southern Thailand. The physical, chemical and microbiological properties of each sample were determined within a day of collection.

Measurement of colour

The colour measurements of the samples were carried out using a Hunter Lab Colourflex colourimeter. A colourimeter was adjusted for reflectance, illuminant D 65, and angle of 10^{o}. Instrumental colour data was provided in accord with the CIE system in terms of L* (lightness), a* (redness and greenness) and b* (yellowness and blueness).

Measurement of clarity

The clarity of samples was estimated by measuring the transmittance at 650 nm using a spectrophotometer as describe by Taipaiboon (2004) and expressed in term of percentage.

Measurement of intermediate browning product and browning intensity

Intermediate browning product (IBP) and browning intensity (BI) palm sugar syrup and palm sugar cake were determined by monitoring the absorbance at 280 and 420 nm, respectively. The absorbance was measured by using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan), as described by Takano (2005) and Kawai *et al.* (2005). Appropriate dilutions of palm sugar syrup were 8-fold for IBP and 4-fold for BI in order to obtain a reliable absorbance reading. Appropriate dilutions of palm sugar cake were 20-fold for IBP and 10-fold for BI in order to obtain a reliable absorbance reading

Measurement of hardness and stickiness

The hardness and stickiness of palm sugar cake was measured by using a TA-XT2i Texture Analyzer (Stable Micro System, UK), calibrated by using a 5 kg

stainless steel weight. Hardness was measured as the force (Newton) required for a 6 mm diameter cylinder probe (P/6) to penetrate (0.1 mm/s) the surface to a depth of 30% of the height sample. For the measurement of stickiness, a cylinder probe (P/6) penetrated the surface to a depth of 30% of the height sample, and was held there for 3 sec. Stickiness was measured as the force (Newton) required to remove the probe (0.1 mm/sec) from the sample. Twenty samples were used for each testing (Nowakowski and Hartel, 2002).

Measurement of solubility

Solubility of palm sugar cake was determined according to Cano-Chauca *et al.* (2005). The sample (approximately 2 g) and distilled water (50 ml) were transferred into a beaker. The mixture was agitated with a magnetic stirrer, using a stirring bar with a size of 2 ×7 mm at 30^{o}C for 5 min. After that the solution was filtrated through pre-weighed filter paper (Whatman, No. 1) using a vacuum aspirator and immediately dried in vacuum oven at 60^{o}C for 5 h. Then, the percentage of solubility value was calculated by weight difference.

Measurement of glass transition temperature

The glass transition temperature (Tg) was determined by the differential scanning calorimetry model DSC7 (Perkin Elmer, U.S.A.). Palm sugar cake (5 mg) was powdered using a mortar and weighted in an aluminum pan, hermetically sealed and then taken for DSC analysis. First, a sample was cooled from 25^{o}C to -40^{o}C at the rate of 10^{o}C/min. After that a sample was heated at the rate of 10^{o}C/min from -40^{o}C to 200^{o}C followed by cooling to -40^{o}C at the rate of 120^{o}C/min. A repeat run was then performed. The reference was an empty pan. The mid-point of Tg was considered as the characteristic temperature of the transition. Only palm sugar cake was measured for Tg.

Measurement of crystallinity

The crystallinity of palm sugar cake was identified by the X-ray diffraction method. The powder sample was placed in a sample holder for the powder X-ray diffraction and the surface was smoothed with a glass slide. The measure was carried out with the powder X-ray diffraction meter using Cu radiation, under operational conditions of 40 kV of potency and 30 mA. Each sample was scanned with the 2θ being between 5^{o} and 40^{o}. Data acquisition was accomplished using a step size of 0.05° and a step time of 1 sec (Bhandari and Hartel, 2002). The crystallinity was calculated by intregrating total peak area.

Determination of pH

The pH value was measured at ambient temperature with a pH meter (Satorious, USA) which calibrated at pH 4.0 and 7.0.

Determination of total acidity

The sample was diluted with distilled water and titrated with 0.01 N NaOH using a few drops of 1% phenolphthalein solution as an indicator. The sample was transferred as a measured quantity (1-3 ml or g) into approximately 10 ml of distilled water. The result was calculated as a percentage of lactic acid (Rangana, 1986; Uttraporn, 2006).

Determination of total soluble solid

The total soluble solid (TSS) content of palm sap and palm sugar syrup was determined as degree Brix using a hand refractometer.

Determination of moisture content

The moisture content of palm sugar syrup and palm sugar cake was measured using a vacuum oven. About 2-5 g of the sample was placed in a pre-dried aluminum dish and dried in a vacuum oven at 60^{o}C (pressure<70mmHg) for 6 h. The dried sample was placed in a desiccator and cooled for 0.5 h to room temperature. The weight was recorded, and the percentage moisture based on the initial wet weight was calculated. Only palm sugar syrup and palm sugar cake were measured for moisture content.

Determination of water activity

Water activity of palm sugar syrup and palm sugar cake was measured at room temperature using a water activity meter (Novasina, Thermostanter). The sample was inserted into a sample cup and another water activity measurement was made immediately to restrict moisture transfer from the air to the samples. Only palm sugar syrup and palm sugar cake were measured for water activity.

Determination of total sugar and reducing sugar

The total sugar and reducing sugar content were quantified by the Lane and Eynon Volumetric method using titration with Fehling's reagents. The results were expressed as grams of glucose per 100 g of sample (Rangana, 1986).

Determination of type and concentration of sugars

The type and concentration of sugar was determined using HPLC (Shimadzu, CR6A Chromatopac) with a Hypersil NH_2 column and refractive index detector. The mobile phase was the solution of acetonitrile and water (80:20), pumped at

a flow rate of 1.5 ml/min and an injection volume of 20 µl. The samples were prepared by making appropriate dilutions with distilled water. All sample solutions were passed through a 0.45 µm nylon syringe filter to remove particulates prior to HPLC analysis. D-glucose, D-fructose, maltose and sucrose were used as the external standards. The calibration curve of each sugar was plotted between peak areas and concentrations (Stuckel and Low, 1996).

Determination of protein content

Coomassie brilliant blue dye (100 mg) was dissolved in 50 ml of methanol followed by the addition of 100 ml of phosphoric acid. This was then made up to 1 liter with deionized water. The dye mixture was filtered twice through Whatman No. 1 filter paper. The sample (2-5 g) was dissolved in deionized water and made up to 10 ml with deionized water. After that, 0.04 ml of the sample was mixed with 2 ml of dye solution. The absorbance was measured at 595 nm. Bovine serum albumin was used as an external standard (Boyes *et al.,* 1997).

Determination of 5-Hydroxymethylfurfural content

The palm sugar syrup and palm sugar cake (5-10 g) was dissolved in deionized water and made up to 50 ml with deionized water. After that it was centrifuged at 5,000 rpm for 15 min. The supernatant was used to measure 5-hydroxymethylfurfural (HMF) content. To determine the HMF content, 2 ml of supernatant was introduced into the tube. Two ml of 12% trichloroacetic acid and 2 ml of 0.025 M thiobabituric acid were subsequently added and mixed thoroughly. The tube with the sample was then placed in a water bath at 40^{o}C. After incubating for 50 min, the tube was cooled immediately, using water, and the absorbance was measured at 443 nm. A calibration curve of HMF was utilised to quantify the HMF concentration (Rattanathanalerk *et al.,* 2005). Only palm sugar syrup and palm sugar cake were measured for HMF content.

Determination of volatile flavour compounds

Volatile flavour compounds were analysed using the HS-SPME-GC-MS technique. A 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber was used (Supelco, Bellefonte, PA, USA). Each sample (2 ml for palm sap and palm sugar syrup or 2 g for palm sugar cake) was added in a 12 ml headspace vial and then an internal standard 2-methyl-3-heptanone was spiked into the sample. After that, the vial was sealed tightly with a crimp cap and a PTFE/silicone septum and equilibrated at 50^{o}C for 30 min in a water bath. A manual SPME holder containing fiber was inserted into the vial and exposed to the sample headspace at 50^{o}C for 15 min. The fiber was then transferred directly into the injector port of the GC-MS system. Thermal desorption of analytes from the fiber in the GC injector port was carried out with an SPME inlet liner (0.75 mm i.d., Supelco) in the splitless mode at a desorption temperature of 240^{o}C. The SPME fiber was conditioned at 250^{o}C for 10 min before starting the first measurement. It was then left in the injection port for re-conditioning during the whole GC run before taking the next sample. GC-MS analysis was conducted using the Agilent 6890 Plus GC/HP 5973 MSD, equipped with an HP-FFAP column (25 m×0.32 mm i.d.×25 μm film thickness). The injector temperature was 240^{o}C. The GC oven temperature was programmed from 40 to 230^{o}C at the rate of 8^{o}C/min and held at 230^{o}C for 10 min. The carrier gas was ultra high purity helium at a constant flow of 1.5 ml/min. The mass spectrometer condition was as follows: MSD capillary direct-interface temperature was 280^{o}C; the Ionization energy was 70 eV; and the mass range was between 20-450 a.m.u. Positive identification of a component was performed by comparison of the mass spectrum. Tentatively identified compounds were specifically identified in the basis of the mass spectra from the Wiley 275.L mass spectra database (Hewlett-Packard Co.). The integration of peaks was done on HP chemstation software (Hewlett-Packard Co.). The minimum peak area for detection was 10,000 counts.

In this study, the given concentration values were noted as equivalents to the internal standard. The relative concentrations of the investigated compounds (IC)

were calculated by relating the areas of the internal standard (IS) to the areas of the compounds of interest.

$$\text{Relative concentration} = \frac{\text{(Peak area of IC)}}{\text{(Peak area of IS)}} \times \text{Concentration of IS}$$

Determination of microbial load

Total microbial count and yeast and mold count were analysed in palm sap, palm sugar syrup and palm sugar cake. Only palm sap was analysed for lactic acid bacteria. Osmophilic yeast was analysed in palm sugar syrup and palm sugar cake. Each sample was aseptically taken and serially diluted in 0.1 g/100 g peptone water for microbial counts. Pour plating on Plate Count Agar (Merck KGaA, Darmstadt, Germany) was performed for the total microbial count, overlaid with the same medium, and the plates incubated at 35-37^{o}C for 1-2 days. Spread plating on Potato Dextrose Agar acidified with 10 g/100 g tartaric acid (Merck KGaA, Darmstadt, Germany) was performed for yeast and mold count and the plates incubated at 20-25^{o}C for 5 days. Lactic acid bacteria count was also analysed using the pour plate technique on MRS agar and the plates were incubated at 37^{o}C for 2 days. The osmophilic yeast count was also analysed using the spread plate technique on osmophilic potato dextrose agar and the plates were incubated at 37^{o}C for 3 days (Kiss, 1984).

Statistical analysis

All analysis and measurements were performed in triplicates. The experimental design was a completely randomized design (CRD). Data was subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test (Steel and Torrie, 1980). Analysis was performed using a SPSS package (SPSS 6.0 for windows, SPSS Inc, Chicago, IL). Principle Component analysis

(PCA) was applied to observe relationship among all properties indicators from ten palm sugar syrup samples by XLSTAT software (www.XLSTAT.com).

2.4 Results and Discussion

Physical properties

Colour

Table 2 shows physical properties of ten palm sap samples including L*, a*, b* and transmittance value. Ten palm sap samples were shown in Appendix Figure 1. All physical properties were significantly different among samples ($P<0.05$). The results showed that lightness (L* value) ranged from 61.49 to 87.53, redness (a* value) ranged from 1.46 to 3.25 and yellowness ranges (b* value) 12.41 to 19.31. Generally, fresh sap is oyster white colour and translucent. However, from this result, palm sap showed red shade colour as indicating from the positive a* values.

The red shade colour of palm sap could be attributed to the pigment of Kiam wood dissolved to palm sap during collecting time (Jamfa, 2002). In addition, enzymatic browning reaction can take place during collecting of palm sap (Taipaiboon, 2004; Loetkitsomboon, 2004). Polyphenol oxidase is responsible for this reaction. This enzyme catalyzes the hydroxylation of monophenols (from metabolite of plant and Kiam wood) to *o*-diphenols and oxidation reaction of *o*-diphenols to *o*-quinones. Quinones are very reactive compounds which strongly interact with other molecules, leading to a large pigment of high molecular weight with very red to brown colouring (Eskin, 1990). It was found that the highest a* value and lowest L* value was found in sample No. 7 while the lowest a* values and the highest a* value was obtained in sample No. 1.

The colour of palm sugar syrup as affected by thermal processing was also investigated using hunter parameters in the CIE system (L*, a* and b*). Nonenzymatic

browning reactions were considered as the major causes of colour change in palm sugar syrup. Table 3 shows the L*(lightness), a*(redness) and b*(yellowness) of ten palm sugar syrup samples. All colour parameters were found to be different ($P<0.05$). The results showed a range of L*, a* and b* values between 8.32-27.98, 23.23-34.70 and 14.30-54.60, respectively. The heating process affected the increase in a* and decrease in L* value of palm sugar syrup and palm sugar cake when compared to palm sap due to nonenzymatic browning reactions took place. Moreover, the steps of the heating process influenced the colour of the palm sugar syrup. The lowest L* value was observed in Sample No. 10 and that was in agreement with the highest IBP, BI and reducing sugar content. Moreover, sample No. 10 shows the lowest pH value. Low pH or acid condition can accelerate the hydrolysis of sucrose to glucose and fructose and that acts as a substrate of Maillard reaction (Whalen and Morselli, 1985). The effects of the heating process, such as heating temperature and time taken for the palm sugar syrup production, could be a main factor affecting the variation of colour values. Samples produced by the one step heating process presented higher L* values than those produced by the two steps heating process. This is probably due to the one step heating process using a shorter processing time than the two steps of heating process. Generally, the rate of chemical reactions increases with increasing temperature and time (Martins *et al.,* 2001). Concentration process that expose palm sap to temperatures of $100^{o}C$ or higher for prolonged periods can produce high reducing sugar content and cause dark colour and brown pigments. This corresponded to a decrease in L* value (Rattanathanalerk *et al.,* 2005).

The colour of palm sugar cake was also affected by heating process. Table 4 presents the physical properties of ten commercial palm sugar cake samples including L*, a*, b* values, hardness, stickiness, intermediate browning product (IBP) and browning intensity (BI). All physical properties showed significant differences among the samples ($P<0.05$). The results show that lightness (L* value) ranged from 27.28 to 55.61, redness (a* value) ranged from 6.49 to 14.23 and yellowness (b* value) ranged from 11.77 to 27.37. From the results, almost samples appeared in red-brown to brown colour

shades. This observation was reflected by a low L* value and a high a* value (Pua *et al.,* 2008; Rao *et al.,* 2009). The lowest L* value was found in sample No. 4 while the highest L* value was obtained in sample No. 10. In addition, sample No. 10 contained the lowest a* value while sample No. 4 and No. 6 had high a* values. Nonenzymatic browning reactions such as the Maillard reaction and Caramelisation are responsible for the colour of palm sugar cake. Many factors affect the colour of palm sugar cake that are caused by nonenzymatic browning reactions, including processing temperature, processing time and the ingredients added. Traditionally, palm sugar cake is produced by evaporating palm sugar syrup in a large open pan heated on a wood fire stove for a long time until it is concentrated (approximately 80^{o}Brix or above and a processing temperature reaching 120^{o}C). After that, it is cooled to room temperature to obtain a crystalline solid. High heating temperature and long time during palm sugar cake production cause the dark colour of the product.

Clarity

The clarity of palm sap was measured in term of transmittance value (%). High transmittance value shows the juice more clarified than low transmittance value. The transmittance values of ten palm sap samples were found to vary from 39.56% to 79.95% (Table 2). In general, the presence of cell fragments has been found to be responsible for the clarity in fresh juice. Additionally, haze formation caused in clarity of juice. The clarity of palm sap depends greatly on its protein concentration and the polyphenol compounds, which is dissolved from Kiam wood and as presented in natural of palm sap itself (Phaichamnan *et al.,* 2010). Balange and Benjakul (2009) reported that the total phenolic content in Kiam wood (intact form) as extracted by water contained tannin 29.33 mg/g of dry Kiam wood. The complex between protein and polyphenol can be induced and therefore, a large colloid size or haze can be developed (Kermasha *et al.,* 1995; Siebert *et al.,* 1996). The development of haze may result from interactions between sugars or metal ions, and proteins. In general the oxidative polymerization of

polyphenols, with protein-polyphenol interactions being considered as the most frequent cause of haze formation in juice. The protein-phenol haze forms via hydrogen and/or hydrophobic interaction. The hydrogen bonds occur between the hydroxyl groups of polyphenols and the carbonyl oxygen in the peptide backbone, whereas the hydrophobic interactions are generated via attraction between the aromatic structure of polyphenols and the nonpolar moiety in proteins (Katrine *et al.,* 2006). Moreover, a positive correlation between pH and transmittance value was found. The growth of microorgnisms was responsible for the low pH of palm sap. Microorganisims are also responsible for clarity of palm sap. Uzochukwu *et al.* (1994a, 1994b and 1999) suggested that the microorganisms important for the fermentation of palm sap were mainly *Saccharomyces* yeasts and lactic acid bacteria. The lactic acid bacteria have been shown to be responsible for the consistency and soluble white colouration of palm sap through their production of gum likely dextrans in the early stage of fermentation in the beverage, which change the consistency and the colour from transparent to whitish. In addition, a heavy suspension of yeast and bacteria also gave a milky-white appearance (Lasekan *et al.,* 2007). This phenomenon was also contributed to the decrease clarity of palm sap.

The transmittance value of palm sugar syrup ranged from 1.00% to 4.52% and showed significant differences ($P<0.05$) (Table 3). The clarity of palm sugar syrup also depended greatly on its protein content and polyphenolic compounds as well as the undissolved particles that were concentrated during heating process (Phaichamnan *et al.,* 2010). Moreover, the concentrated colloid particles from brown pigment during heating also responsible for the clarity in palm sugar syrup (Takano, 2005).

Intermediate browning product and browning intensity

Nonenzymatic browning reactions are one of the major causes of colour change in palm sugar syrup and palm sugar cake. Therefore the accumulation of intermediate browning product (IBP) and brown pigment (BI) formation or melanoidin due to the heating process was monitored in this study. The IBP of all palm sugar syrup samples was found to vary from 0.47 to 0.91, while the BI of samples ranged from 0.62 to 1.32 (Table 3). The effect of the heating process such as heating temperature and time taken for palm sugar syrup production could be a main factor affecting the variations in IBP and BI. The highest IBP and BI was found in sample No. 10 that corresponded to the lowest L* value, while the lowest INB and BI was obtained in sample No. 5. This result indicated that sample No. 10 took place the highest nonenzymatic browning reactions, including Maillard reaction and Caramelisation, when compared to other samples. Samples produced by the one step of heating process presented higher IBP and BI than those produced by the two steps of heating process. This is probably because the one step of heating process used a shorter processing time than the two steps of heating process.

The IBP and BI of palm sugar cake samples are shown in Table 4. The heating process had a marked effect on the formation of brown pigment in palm sugar cake. The IBP was found to vary between 0.52 and 1.15, while the BI of samples ranged from 0.38 to 0.84, respectively. Sample No. 10 showed the lowest IBP and BI in accord with L* and a* values. However, sample No. 4 and No. 6 appeared high for IBP (1.15 for sample No. 4 and 1.01 for sample No. 6) and BI (0.84 for sample No. 4 and 0.87 for sample No. 6) that also corresponded to the L* and a* values. It may be noted that the highest nonenzymatic browning reactions occurred in samples No. 4 and No. 6.

Table 2. Physical properties of ten palm sap samples

Sample/Property	L*	a*	b*	Transmittance (%)
1	87.53 ± 0.01^{a}	1.46 ± 0.01^{g}	13.10 ± 0.05^{h}	79.95 ± 0.76^{a}
2	81.04 ± 0.23^{d}	2.04 ± 0.02^{e}	13.83 ± 0.04^{g}	67.64 ± 0.40^{f}
3	75.06 ± 0.69^{e}	2.87 ± 0.08^{b}	15.15 ± 0.13^{e}	59.44 ± 0.41^{g}
4	83.41 ± 0.38^{c}	2.32 ± 0.08^{d}	17.51 ± 0.15^{b}	73.09 ± 0.64^{c}
5	85.74 ± 0.20^{b}	1.48 ± 0.04^{g}	14.43 ± 0.10^{f}	75.95 ± 0.25^{b}
6	82.35 ± 0.50^{c}	1.79 ± 0.05^{f}	12.41 ± 0.13^{i}	71.26 ± 0.05^{d}
7	61.49 ± 1.18^{h}	3.25 ± 0.05^{a}	14.95 ± 0.15^{e}	39.56 ± 0.08^{j}
8	68.94 ± 0.67^{g}	2.75 ± 0.03^{c}	19.31 ± 0.17^{a}	47.55 ± 0.23^{i}
9	70.76 ± 1.35^{f}	2.10 ± 0.01^{e}	17.19 ± 0.11^{c}	49.01 ± 0.03^{h}
10	80.65 ± 0.90^{d}	2.28 ± 0.08^{d}	16.87 ± 0.14^{d}	69.57 ± 0.41^{e}

Note: Each value is the mean of triplicate determinations ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

Table 3. Physical properties of ten palm sugar syrup samples

Sample/ Property	Processing method[a]	L*	a*	b*	Transmittance (%)	IBP[b]	BI[c]
1	1	27.98 ± 0.01^{h}	32.26 ± 0.08^{g}	47.77 ± 0.13^{h}	4.37 ± 0.41^{a}	0.91 ± 0.01^{f}	0.88 ± 0.01^{c}
2	1	21.46 ± 0.33^{g}	30.20 ± 0.06^{f}	36.78 ± 0.53^{g}	1.85 ± 0.04^{a}	0.77 ± 0.01^{d}	0.90 ± 0.00^{d}
3	1	18.81 ± 0.04^{e}	28.95 ± 0.05^{e}	32.24 ± 0.09^{e}	1.83 ± 0.05^{a}	0.83 ± 0.01^{e}	0.95 ± 0.01^{f}
4	1	18.76 ± 0.04^{e}	28.80 ± 0.11^{de}	32.34 ± 0.35^{e}	1.76 ± 0.02^{a}	0.83 ± 0.01^{e}	0.92 ± 0.01^{e}
5	1	32.71 ± 0.26^{i}	23.23 ± 0.30^{a}	54.06 ± 0.42^{i}	1.00 ± 0.02^{a}	0.48 ± 0.01^{b}	0.62 ± 0.00^{a}
6	2	17.63 ± 0.34^{d}	28.91 ± 0.50^{de}	30.27 ± 0.56^{d}	1.42 ± 0.08^{a}	0.71 ± 0.01^{c}	1.15 ± 0.00^{g}
7	2	13.41 ± 0.28^{b}	28.54 ± 0.15^{cd}	23.05 ± 0.52^{b}	1.16 ± 0.03^{a}	0.82 ± 0.04^{e}	1.28 ± 0.00^{h}
8	2	15.34 ± 0.06^{c}	28.37 ± 0.08^{c}	26.32 ± 0.15^{c}	1.31 ± 0.06^{a}	0.47 ± 0.01^{a}	0.75 ± 0.01^{b}
9	2	20.29 ± 0.34^{f}	34.70 ± 0.22^{h}	34.87 ± 0.66^{f}	3.03 ± 0.03^{a}	0.83 ± 0.01^{e}	0.95 ± 0.01^{f}
10	2	8.32 ± 0.03^{a}	24.56 ± 0.02^{b}	14.30 ± 0.10^{a}	4.52 ± 0.01^{a}	0.91 ± 0.00^{f}	1.32 ± 0.00^{i}

Note: Each value is the mean of triplicate determinations ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

[a] 1 refers to one step of heating process, 2 refers to two steps of heating process.

[b] The IBP was measured by diluting the sample with distilled water in the ratio of sample:water = 1:8.

[c] The BI was measured by diluting the sample with distilled water in the ratio of sample:water = 1:4.

Table 4. Physical properties of ten palm sugar cake samples

Sample/ Property	L*	a*	b*	IBP[a]	BI[b]
1	33.02 ± 1.01^{f}	11.44 ± 0.33^{b}	17.73 ± 0.49^{f}	0.52 ± 0.00^{h}	0.61 ± 0.01^{c}
2	41.64 ± 0.90^{b}	10.91 ± 0.11^{c}	19.95 ± 0.82^{e}	0.64 ± 0.01^{g}	0.66 ± 0.03^{b}
3	34.30 ± 0.17^{e}	9.50 ± 0.31^{ef}	18.30 ± 0.88^{f}	0.68± 0.01^{f}	0.57± 0.03^{d}
4	27.28± 0.25^{h}	14.17 ± 0.61^{a}	22.35 ± 0.27^{c}	1.15 ± 0.02^{a}	0.84 ± 0.01^{a}
5	41.29 ± 1.63^{b}	8.86 ± 0.83^{f}	21.18 ± 1.03^{d}	0.74 ± 0.01^{d}	0.38 ± 0.02^{f}
6	27.33 ± 0.30^{h}	14.23 ± 0.19^{d}	25.45 ± 0.68^{b}	1.01 ± 0.03^{b}	0.87 ± 0.01^{a}
7	29.05 ± 1.01^{g}	9.31 ± 0.34^{f}	11.77 ± 0.86^{g}	0.76± 0.01^{cd}	0.63 ± 0.01^{c}
8	38.56 ± 1.07^{c}	11.74 ± 0.15^{b}	22.10 ± 0.80^{cd}	0.72 ± 0.01^{e}	0.58 ± 0.01^{d}
9	36.46 ± 0.97^{d}	9.07 ± 0.72^{f}	17.39 ± 0.87^{f}	0.78 ± 0.02^{c}	0.67 ± 0.02^{b}
10	55.61 ± 0.82^{a}	6.49 ± 0.14^{g}	27.37 ± 0.29^{a}	0.32± 0.00^{i}	0.44 ± 0.02^{e}

Note: Each value is the mean ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

[a] The IBP was measured by diluting the sample with distilled water in the ratio of sample:water = 1:10.

[b] The BI was measured by diluting the sample with distilled water in the ratio of sample:water = 1:20.

Hardness and stickiness

Hardness is the property of a material that enables it to resist deformation, usually by penetration or compression. It is generally characterised by strong intermolecular bonds via hydrogen bond. During the production of palm sugar cake, syrup was concentrated until the crystalline form was obtained. Thus, the degree of crystallisation of the sugar affected the hardness of the palm sugar cake. Some factors affected the degree of crystallisation of palm sugar cake such as the reducing sugar; acidity, processing conditions (processing temperature and time), the formulation of palm sugar cake, and storage conditions.

The texture of the palm sugar cake was measured in term of hardness and stickiness as shown in Table 5. The hardness of the ten palm sugar cake samples ranged from 30.83 N to 69.00 N. Sample No. 9 showed the lowest hardness, indicating the

weakest intermolecular bonding. This is probably because this sample contained a low sucrose content and high reducing sugar content as well as fructose and glucose. Generally, palm sugar cake contains sucrose as the main sugar. Sucrose is easily hydrolyzed to fructose and glucose in acidic conditions during the heating process. The presence of reducing sugar as well as fructose and glucose could retard sugar crystallisation and cause the low hardness of the product (Aider *et al.,* 2006).

Some foods exhibit a marked tendency to adhere to a contact surface; this is generally known as stickiness. Stickiness in food greatly depends on water, temperature and the ingredients of the food. The interaction of water with solids is the prime cause of stickiness in low moisture food. Water is a suitable catalyst for stickiness (Adhikari *et al.,* 2001). Stickiness was also measured in the palm sugar cake as shown in Table 5. The stickiness ranged from 0.11 N to 0.33 N. It was found that sample No. 10 showed the highest stickiness because this sample contained high moisture content.

Solubility

Solubility is a process relating to hydration. The interaction between water and solute molecules is called hydration. In the presence of polar molecules such as sugars and water, these interactions are dominated by hydrogen bonding (Joupplia, 2006). Sugar is generally very soluble in water at room temperature (Coultate, 1993). Palm sugar cake showed solubility in the range of 96.28%-98.57% (Table 5). High solubility was found in the samples showing low hardness and low crystallinity. Normally, crystalline solids can hold less water than amorphous solids. This is due to the amorphous noncrystalline material that can form hydrogen bond with water internally, not just on the surface, which is the only way water can interact with perfect crystals (Bell and Labuza, 2000).

Crystallinity

Normally, palm sugar cake is produced by heating palm sap or syrup until its crystalline structure is formed. The properties of the palm sap or palm sugar syrup, such as reducing sugar content, pH and total acidity, influence the crystallinity of palm sugar cake. Low pH and high acidity of palm sap or syrup can promote an increase in the content of reducing sugar during the production of palm sugar cake. Most sugars can be induced to become crystalline, particular non reducing sugars. However, it is difficult to obtain certain reducing sugars in crystalline form because of the presence of anomers and ring isomers in the solution make the reducing sugars intrinsically "impure". Thus the crystallisation of non reducing sugar could be retarded by the presence of reducing sugars (Shallenberger and Birch, 1989; Hartel, 2001). XRD patterns of ten palm sugar cake samples were shown in Appendix Figure 3. A sharp in XRD pattern was found in all samples. The crystallinity of ten palm sugar cake samples is shown in Table 5 and range from 73.40% to 78.56%. Sample No. 4 shows the highest crystallinity. This was probably because these samples contained low reducing sugar content.

Glass transition temperature

The glass transition temperature (Tg) is the temperature at which an amorphous phase is converted between rubbery and glassy states. In food systems, Tg is mainly affected by the water content and the average molecular weight (Sablani *et al.,* 2007). Normally, monosaccharide and disaccharides, like almost all biomaterials, can be plasticised by water, and an increase in water content causes a decrease in Tg. However, the moisture content of all palm sugar cake samples did not show a wide range (4.54%-6.23%). Therefore, the Tg of palm sugar cake may mainly depend on the type and concentration of sugar. Generally, the Tg of a sugar mixture depends on the proportion of different sugars in the mixture. Lower molecular weight sugar has been shown to plasticise higher than high molecular weight sugar (Roos and Karel, 1991).

Monosaccharide such as fructose and glucose act as plasticisers for higher molecular weight carbohydrates (sucrose). The Tg of ten palm sugar cake samples is shown in Table 5. The Tg ranged from 5.39^{o}C to 25.66^{o}C. Sample No. 7 showed the lowest Tg, possibly because these samples contained the highest reducing sugar content.

Table 5. Physical properties of ten palm sugar cake samples

Sample/ Property	Hardness (Newton)	Stickiness (Newton)	Solubility (%)	Tg (^{o}C)	Crystallinity (%)
1	36.82 ± 9.42^{de}	0.30 ± 0.08^{ab}	98.07 ± 0.46^{abc}	11.70 ± 0.05^{g}	76.42 ± 0.09^{d}
2	49.82 ± 9.63^{c}	0.15 ± 0.06^{d}	97.70 ± 0.22^{bcd}	25.66 ± 0.09^{a}	78.16 ± 0.05^{b}
3	41.93 ± 10.57^{d}	0.11 ± 0.04^{d}	97.22 ± 0.45^{de}	20.09 ± 0.01^{c}	76.32 ± 0.07^{e}
4	69.00 ± 5.24^{a}	0.12 ± 0.07^{d}	97.09 ± 0.16^{de}	24.16 ± 0.09^{b}	78.56 ± 0.06^{a}
5	57.64 ± 9.54^{b}	0.20 ± 0.05^{c}	96.28 ± 0.60^{f}	19.52± 0.13^{d}	76.70± 0.04^{c}
6	33.43 ± 9.43^{ef}	0.13 ± 0.06^{d}	98.34 ± 0.45^{ab}	7.47 ± 0.10^{h}	73.40 ± 0.05^{h}
7	36.63 ± 7.58^{de}	0.11± 0.08^{d}	97.39 ± 0.59^{cde}	5.39± 0.06^{i}	74.60± 0.04^{g}
8	36.82 ± 9.42^{de}	0.13 ± 0.07^{d}	97.29 ± 0.57^{cde}	11.87 ± 0.06^{f}	76.41 ± 0.04^{d}
9	30.83 ± 6.40^{f}	0.28 ± 0.07^{b}	98.57 ± 0.28^{a}	24.08 ± 0.06^{b}	73.40 ± 0.02^{h}
10	38.73± 6.81^{de}	0.33 ± 0.07^{a}	96.77 ± 0.29^{ef}	12.13 ± 0.03^{e}	75.93 ± 0.05^{f}

Note: Each value is the mean ± standard deviation.

The different superscripts in the same column denote the significant different (P<0.05).

Chemical properties

pH and total acidity

The pH and total acidity of all palm sap samples were significantly different among the samples (P<0.05). The pH value of all palm sap samples varied from 4.19 to 5.23 while total acidity found in a range from 0.13% to 0.19% (Table 6). Since, lactic acid is the main organic acid presented in palm sap (Taipaiboon, 2004; Loetkitsomboon, 2004). Microorganisms, mainly lactic acid bacteria have produced

organic acids (lactic acid), which then increase in total acidity and decrease in pH value. Normally, natural palm sap showed neutral pH approximately 7 as reported by Jitbunjerdkul (1989) and Lasekan *et al*. (2007). Hence, a high percentage of total acidity and low pH indicates the initial fermentation step of palm sap, for example, during collecting time.

The ten palm sugar syrup samples showed pH in a range of 4.52-5.36, while the total acidity ranged from 0.21% to 0.48% (Table 7). The pH value and total acidity showed significant differences among the samples ($P<0.05$). These variations of pH and total acidity can be used for indicating food safety. They might be due to the effects of sugar fermenting process, which is based on activity caused by microorganisms. Microorganism contamination is responsible for the low pH and high total acidity of palm sugar syrup. Generally, palm sugar syrup that contains high sugar content is a very selective environment for the growth of microorganisms. During the evaporation process, microorganisms present in the palm sap are destroyed since the syrup reaches a temperature 100^{o}C or above at the terminal stage. However, during post-production, syrup could be contaminated by microorganisms from the air, equipment and packaging (Dumont *et al.,* 1993). Microorganisms that survived after processing, such as osmophilic yeasts, can grow and produce organic acids yielding low pH and high total acidity. High temperature during the summer season in the southern Thailand favors the rapid growth of osmophilic yeasts (such as *Saccharomyces rouxii*). These microorganisms are normally found in syrup which is capable of the growth at a low water activity value (<0.85) or high solute concentrations (Tilbury, 1980). High acidity and low pH will accelerate the Maillard reaction and the brown colour of the palm sugar syrup will be promoted during a heating step. Therefore, dark colour formation will be increased.

Table 8 shows the chemical property of ten palm sugar cake samples. The pH value and total acidity of the ten palm sugar cake samples varied from 4.09 to 4.82 and 0.21% to 0.30%, respectively. Nonenzymatic browning reactions affected the pH and total acidity of the palm sugar cake. The reduction in pH values occurring in the Maillard

reaction was due to the formation of organic acids such as formic acid and acetic acid (Ames, 1998; Brands and Van Boekel, 2002; Lertittikul *et al.*, 2007).

Total soluble solid

The total soluble solid (TSS) of the ten palm sap samples varied from 10.8^{o}Brix to 17.4^{o}Brix (Table 6). The variation of TSS of palm sap depends on different source of palm sap and fermentation of sugar caused by microorganisms (Iwuoha and Eke, 1996).

The TSS of palm sugar syrup samples ranged from 62.97^{o}Brix to 67.50^{o}Brix ($P<0.05$) (Table 7). According to the definition of the Thai Ministry of Industrial Standards Institute, palm sugar syrup is syrup that is made by the evaporation of palm sap and contains TSS that is at least 65^{o}Brix in order to prevent the growth of microorganisms during storage under room temperature. From this definition, two samples (No. 1 and No. 10) in this study did not meet this standard.

Moisture content and water activity

The moisture content (MC) of the ten palm sugar syrup ranged from 27.31% to 29.63%. The MC of the ten palm sugar cake samples ranged from 4.54% to 6.81%. The MC of palm sugar syrup depends on heating temperature, processing time and the TSS. The water activity (Aw) of all palm sugar syrup samples ranged from 0.79 to 0.81. The Aw of all palm sugar cake samples ranged from 0.72 to 0.77. Aw is an intrinsic product characteristic that most influences the microbial ecology of a sugar-rich product. The MC and Aw are highly important for control of the shelf life of syrup during storage (de Rodriguez, 2004). Generally, osmophilic yeast can grow at low water activity (0.65-0.80) and may spoil products containing high concentrations of sugar. It may cause a decrease of the pH value and alcohol present in syrup during storage. Moreover, a

positive corrlelation of MC and Aw with stickiness was found. This probably due to water caused an increase in the stickiness of palm sugar cake.

Protein content

Protein content of all palm sap samples was found in a range of 0.31-0.39 mg/ml. The variation of protein content in palm sap may also due to the different sources of palm sap. In addition, microorganisms may use protein as a carbon source or as a nitrogen source for their metabolism and genetic material (Adams and Moss, 1995). Protein acts as a substrate of Maillard reaction that occurring during the production of palm sugar syrup. High protein content in palm sap influenced on the properties of palm sugar syrup afterward. Protein content of all palm sugar syrup samples varied from 7.24 mg/ml to 8.36 mg/ml while protein content of all palm sugar cake samples was 4.26 mg/g to 5.46 mg/g. High heating temperatures and times could accelerate the Maillard reaction that is responsible for a lower protein content (Wang *et al.*, 2006).

Table 6. Chemical properties of ten palm sap samples

Sample/ Property	pH	Total acidity (%)	TSS (^{o}Brix)	Protein (mg/ml)
1	5.10 ± 0.05^{b}	0.13 ± 0.01^{e}	10.67 ± 0.42^{i}	0.33 ± 0.01^{ce}
2	4.49 ± 0.01^{f}	0.16 ± 0.00^{c}	15.93 ± 0.12^{c}	0.34 ± 0.01^{c}
3	4.60 ± 0.01^{e}	0.15 ± 0.01^{d}	16.57 ± 0.06^{b}	0.34 ± 0.00^{c}
4	5.23 ± 0.06^{a}	0.13 ± 0.00^{e}	12.07 ± 0.12^{f}	0.32 ± 0.00^{ef}
5	4.78 ± 0.04^{d}	0.13 ± 0.01^{e}	12.40 ± 0.00^{e}	0.31 ± 0.00^{f}
6	4.90 ± 0.01^{c}	0.15 ± 0.01^{d}	11.07 ± 0.12^{g}	0.39 ± 0.00^{a}
7	4.19 ± 0.01^{fg}	0.18 ± 0.01^{b}	16.00 ± 0.00^{c}	0.31 ± 0.01^{f}
8	4.53 ± 0.01^{h}	0.19 ± 0.01^{a}	16.33 ± 0.12^{b}	0.31 ± 0.02^{f}
9	4.46 ± 0.03^{g}	0.18 ± 0.01^{b}	17.33 ± 0.12^{a}	0.34 ± 0.01^{c}
10	4.89 ± 0.01^{c}	0.13 ± 0.01^{e}	12.00 ± 0.00^{f}	0.36 ± 0.01^{b}

Note: Each value is the mean of triplicate determinations ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

Table 7. Chemical properties of ten palm sugar syrup samples

Sample/ Property	pH	Total acidity (%)	TSS (^{o}Brix)	MC (%)	Aw	HMF (mg/kg, dry basis)	Protein (mg/ml)
1	5.04 ± 0.01^{c}	0.27 ± 0.01^{e}	63.07 ± 0.12^{g}	27.49 ± 0.68^{d}	0.80 ± 0.01^{ab}	33.70 ± 0.45^{f}	7.24 ± 0.07^{d}
2	4.81 ± 0.01^{e}	0.36 ± 0.00^{d}	68.17 ± 0.15^{a}	27.59 ± 0.88^{cd}	0.80 ± 0.00^{b}	30.52 ± 0.16^{g}	8.19 ± 0.11^{a}
3	5.01 ± 0.01^{d}	0.39 ± 0.05^{cd}	67.10 ± 0.10^{c}	27.31 ± 0.26^{d}	0.80 ± 0.00^{b}	30.15 ± 0.21^{g}	7.32 ± 0.23^{cd}
4	5.24 ± 0.01^{b}	0.36 ± 0.00^{d}	65.00 ± 0.20^{f}	28.04 ± 0.11^{bcd}	0.80 ± 0.00^{b}	30.57 ± 0.14^{g}	7.55 ± 0.11^{c}
5	5.03 ± 0.01^{cd}	0.24 ± 0.05^{e}	67.53 ± 0.06^{b}	29.33 ± 0.11^{a}	0.81 ± 0.00^{a}	21.71 ± 0.19^{h}	7.36 ± 0.20^{cd}
6	5.36 ± 0.01^{a}	0.21 ± 0.05^{e}	67.07 ± 0.12^{c}	27.67 ± 0.09^{cd}	0.79 ± 0.00^{c}	45.80 ± 0.21^{e}	7.89 ± 0.24^{b}
7	4.75 ± 0.01^{f}	0.42 ± 0.05^{ab}	66.03 ± 0.06^{e}	28.32 ± 0.23^{bc}	0.80 ± 0.00^{b}	61.77 ± 0.19^{c}	7.51 ± 0.05^{cd}
8	5.03 ± 0.01^{cd}	0.36 ± 0.00^{d}	66.53 ± 0.06^{d}	28.55 ± 0.24^{b}	0.80 ± 0.00^{b}	50.67 ± 0.21^{d}	7.90 ± 0.15^{b}
9	4.73 ± 0.01^{g}	0.45 ± 0.00^{ab}	66.53 ± 0.06^{d}	27.43 ± 0.51^{d}	0.80 ± 0.00^{b}	65.94 ± 0.19^{b}	8.15 ± 0.48^{ab}
10	4.52 ± 0.01^{h}	0.48 ± 0.05^{a}	62.97 ± 0.06^{g}	29.63 ± 0.15^{a}	0.81 ± 0.00^{a}	137.50 ± 0.41^{a}	7.88 ± 0.12^{b}

Note: Each value is the mean of triplicate determinations ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

Table 8. Chemical properties of ten palm sugar cake samples

Sample/ Property	pH	Total acidity (%)	MC (%)	Aw	HMF (mg/kg, dry basis)	Protein (mg/g)
1	4.19 ± 0.02^{e}	0.30 ± 0.03^{a}	5.74 ± 0.12^{a}	0.73 ± 0.00^{a}	26.67 ± 0.12^{i}	4.26 ± 0.34^{d}
2	4.65 ± 0.01^{b}	0.21 ± 0.03^{c}	5.66 ± 0.22^{cd}	0.72 ± 0.00^{f}	43.27 ± 0.21^{h}	4.28 ± 0.03^{d}
3	4.46 ± 0.04^{d}	0.22 ± 0.03^{c}	5.81 ± 0.41^{d}	0.73 ± 0.00^{e}	68.67 ± 0.21^{f}	4.87 ± 0.11^{c}
4	4.82 ± 0.00^{a}	0.21 ± 0.05^{c}	4.54 ± 0.11^{f}	0.72 ± 0.00^{ef}	358.06 ± 0.35^{a}	4.11 ± 0.12^{d}
5	4.11 ± 0.03^{f}	0.24 ± 0.03^{bc}	5.18 ± 0.42^{d}	0.74 ± 0.00^{c}	181.05 ± 0.20^{c}	5.32 ± 0.10^{ab}
6	4.09 ± 0.03^{f}	0.30 ± 0.03^{a}	4.64 ± 0.20^{f}	0.72 ± 0.01^{f}	322.92 ± 0.27^{b}	4.06 ± 0.19^{d}
7	4.19 ± 0.02^{e}	0.29 ± 0.02^{a}	5.55 ± 0.20^{d}	0.75 ± 0.01^{c}	179.23 ± 0.16^{d}	5.11 ± 0.03^{bc}
8	4.09 ± 0.03^{f}	0.25 ± 0.02^{abc}	5.53 ± 0.13^{d}	0.75 ± 0.00^{bc}	101.16 ± 0.20^{e}	5.46 ± 0.19^{a}
9	4.10 ± 0.02^{f}	0.30 ± 0.01^{a}	5.41 ± 0.15^{cd}	0.73 ± 0.00^{de}	50.99 ± 0.16^{g}	5.01 ± 0.05^{bc}
10	4.58 ± 0.07^{c}	0.25 ± 0.03^{abc}	6.23 ± 0.11^{b}	0.74 ± 0.01^{b}	23.26 ± 0.24^{j}	5.32 ± 0.29^{ab}

Note: Each value is the mean ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

Total sugar, reducing sugar, type and concentration of sugar

Total sugars, reducing sugars as well as fructose and glucose contents of all palm sap samples were analysed in this study and showed significant different across samples ($P<0.05$). Total sugars were varied from 11.65% to 21.66%. Reducing sugars of all palm sap samples were found to vary between 0.95% and 4.06%. Glucose content was ranged from 0.56% to 2.21% and fructose content was ranged from 0.54% to 2.17%, respectively (Table 9). The sucrose content was found to vary between 10.45% and 20.91% (Table 9). The presence of fructose and glucose might be due to they presented naturally and the inversion reaction caused by invertase activity and acid condition. The occurrence of invertase in palm sap was due to its present naturally and also synthesised by microorganisms (Taipaiboon, 2004). The microorganisms can convert sucrose to glucose and fructose by invertase and finally to organic acids and alcohols in palm sap (Willits and Hills, 1976). It is generally known that the primary sources of invertase are from yeasts such as *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis* and fungi

such as *Aspergillus oryzae* and *Aspergillus niger* (Pancoast and Junk, 1980; Takano, 2005). Moreover, an increase in total acidity and decrease in pH are also responsible for the inversion reaction. The inversion reaction occurs when the glycosidic linkage of disaccharide is hydrolysed, releasing the monosaccharide units. Upon hydrolysis glucose and fructose are formed (Wiene and Shallenberger, 1988). Reducing sugars act as a substrate of Maillard reaction occurring during the production of palm sugar syrup and palm sugar cake. High reducing sugars content presented in palm sap also influence on the browning colour of palm sugar syrup and palm sugar cake afterward, due to Maillard reaction.

Total sugar content and reducing sugar content of all palm sugar syrup samples were depicted in Table 10. Reducing sugar content ranged from 7.46% to 39.72% and total sugar content ranged from 84.12% to 92.63%. In addition, the results from HPLC show that all palm sugar syrup samples consisted of mainly sugars such as sucrose, glucose and fructose. The most abundant sugar found in the palm sugar syrup sample was sucrose, in a range from 60.37 to 83.26%. Glucose and fructose content ranged from 4.01% to 23.32% and 4.44% to 22.20%, respectively (Table 10). Sample No. 10 contained the highest reducing sugar content. This is probably due to the highest sucrose inversion that took place. Therefore in sample No. 10 the highest browning reaction may occur that corresponds with the lowest L* value and highest IBP and BI as mentioned previously.

Total sugar content and reducing sugar content of all palm sugar cake samples were depicted in Table 11. Total sugar and reducing sugar content were found to range from 80.76% to 100.73% and 5.09% to 9.16%. The results of analysis by HPLC showed that all the palm sugar cake samples consisted of mainly sugar such as sucrose, fructose, glucose and maltose. The most abundant sugar found in the palm sugar cake samples was sucrose, in a range of 79.24-87.72%. Other sugars such as fructose, glucose and maltose ranged from 0.16% to 3.05%, 1.04% to 5.74% and 0.00% to 4.41%, respectively (Table 11). Generally, the ingredients of palm sugar cake production are palm sugar syrup, sucrose and trace content of glucose syrup. During heating, the sucrose

in palm sugar syrup and the added sucrose are hydrolysed to glucose and fructose. This reaction is accelerated by thermal processes and acidic conditions (Wiene and Shallenberger, 1988). Glucose syrup was added in the palm sugar cake as evidenced by the presence of maltose. Glucose syrup is the most viable substitute for sugar. It is an aqueous solution of several compounds principally glucose and maltose. From the results, glucose syrup was added in almost all palm sugar cake samples, as indicated by the presence of maltose. The highest maltose content was found in sample No. 10, thus a high amount of glucose syrup was probably added to this sample. Nowakowski and Hartel (2002) reported that high amounts of corn syrup resulted in an increment of stickiness that, given that it had the highest stickiness, was found in sample No. 10. However, maltose was not detected in sample No. 7. This is probably because glucose syrup was not added to this sample.

HMF content

HMF has been found to be a well known indicator of heating processing and/or the storage capacity of sugar-based products (Mendoza *et al.,* 2004). HMF formation depends greatly on the processing method, degree of heating, acid condition and storage condition. Table 7 indicates the HMF content in ten palm sugar syrup samples. This was found to vary from 21.71 mg/kg to 137.50 mg/kg and was significantly different among samples ($P<0.05$). From this result, the HMF content of seven samples was lower than the allowed maximum limit of 40 mg/kg as recommended by the Codex Alimentarius (Turhan *et al.,* 2008). However, all samples produced by the two steps of heating process did not meet these standards. Moreover, samples produced by the two step heating process tended to contain higher HMF content than those produced by the one step heating process.

The HMF content of the ten palm sugar cake samples was found to vary from 23.26 mg/kg to 358.06 mg/kg and was significantly different among the samples ($P<0.05$) (Table 8). The HMF content of eight out of ten samples was higher than the

allowed maximum limit of 40 mg/kg as recommended by the Codex Alimentarius (Turhan *et al.,* 2008). Sample No. 4 contained the highest HMF content and that was in agreement with the lowest L* value and highest IBP. During the production of palm sugar syrup and palm sugar cake by a heating process, HMF can be formed. In the acid medium of this product, the dehydration of carbohydrates, especially hexose, led to the formation of HMF. Moreover, a Maillard reaction can also take place, giving rise to Amadori compounds during the first steps of the reaction, and HMF as a consequence of further reaction (Mendoza *et al.,* 2004). Additionally, it is well known that HMF is a precursor of coloured compounds in the Caramelisation reaction (Kroh, 1997). Therefore, the considerable variations of HMF found in the samples may be an indication of overheating.

Table 9. Type and concentration of sugar of ten palm sap samples

Sample/ Property	Reducing sugar (%)	Total sugar (%)	Fructose (%)	Glucose (%)	Sucrose (%)
1	1.11 ± 0.01^{f}	12.10 ± 0.08^{h}	0.56 ± 0.01^{h}	0.56 ± 0.02^{g}	10.52 ± 0.25^{h}
2	2.20 ± 0.04^{c}	17.03 ± 0.68^{e}	1.13 ± 0.01^{e}	1.15 ± 0.03^{c}	14.56 ± 0.75^{e}
3	1.99 ± 0.11^{ce}	21.66 ± 0.07^{a}	0.96 ± 0.03^{f}	0.90 ± 0.03^{e}	20.91 ± 0.64^{a}
4	0.95 ± 0.02^{f}	12.71 ± 0.21^{g}	0.59 ± 0.01^{h}	0.58 ± 0.02^{g}	12.04 ± 0.42^{g}
5	1.25 ± 0.01^{f}	13.72 ± 0.29^{f}	0.71 ± 0.02^{g}	0.67 ± 0.01^{f}	13.09 ± 0.24^{f}
6	1.15 ± 0.21^{f}	11.65 ± 0.11^{h}	0.54 ± 0.02^{h}	0.59 ± 0.02^{g}	10.45 ± 0.17^{h}
7	2.99 ± 0.38^{b}	11.78 ± 0.22^{c}	1.54 ± 0.03^{b}	1.46 ± 0.09^{b}	15.78 ± 0.51^{c}
8	4.06 ± 0.60^{a}	18.01 ± 0.64^{c}	2.17 ± 0.04^{a}	2.21 ± 0.02^{a}	14.38 ± 0.16^{e}
9	2.93 ± 0.12^{b}	19.55 ± 0.94^{b}	1.49 ± 0.03^{c}	1.47 ± 0.01^{b}	16.98 ± 0.14^{b}
10	1.51 ± 0.17^{ef}	12.60 ± 0.09^{g}	0.56 ± 0.01^{h}	0.58 ± 0.05^{g}	11.90 ± 0.11^{g}

Note: Each value is the mean of triplicate determinations ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

The concentration of all sugars was calculated in terms of dry basis.

Table 10. Type and concentration of sugar of ten palm sugar syrup samples

Sample/ Property	Reducing sugar (%)	Total sugar (%)	Fructose (%)	Glucose (%)	Sucrose (%)
1	7.46 ± 0.07^{j}	88.38 ± 0.45^{d}	4.92 ± 0.08^{f}	4.01 ± 0.05^{i}	83.26 ± 0.37^{a}
2	10.68 ± 0.04^{i}	90.08 ± 0.81^{abcd}	4.44 ± 0.19^{g}	4.36 ± 0.31^{h}	77.85 ± 1.08^{cd}
3	11.72 ± 0.33^{g}	90.85 ± 0.90^{ab}	4.64 ± 0.55^{fg}	4.85 ± 0.12^{fg}	80.55 ± 1.04^{b}
4	12.08 ± 0.08^{f}	90.73 ± 1.00^{abcd}	4.34 ± 0.07^{g}	4.66 ± 0.05^{gh}	79.54 ± 0.90^{c}
5	11.12 ± 0.12^{h}	92.63 ± 0.45^{abc}	5.60 ± 0.02^{e}	5.27 ± 0.13^{f}	77.62 ± 0.55^{de}
6	14.69 ± 0.46^{e}	91.57 ± 1.16^{a}	7.78 ± 0.09^{d}	7.20 ± 0.11^{e}	72.62 ± 1.00^{e}
7	22.06 ± 0.90^{c}	91.83 ± 0.95^{abc}	13.09 ± 0.42^{c}	12.39 ± 0.15^{c}	71.41 ± 0.26^{f}
8	19.22 ± 0.71^{d}	84.12 ± 1.19^{e}	7.69 ± 0.08^{d}	7.29 ± 0.20^{d}	64.42 ± 0.60^{h}
9	24.76 ± 0.31^{b}	89.40 ± 0.43^{bcd}	13.56 ± 0.13^{b}	13.45 ± 0.65^{b}	67.89 ± 0.75^{g}
10	37.92 ± 0.36^{a}	91.82 ± 0.43^{cd}	22.20 ± 0.38^{a}	23.20 ± 0.20^{a}	60.37 ± 0.98^{i}

Note: Each value is the mean of triplicate determinations ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

The concentration of all sugars was calculated in term of dry basis.

Table 11. Type and concentration of sugar of ten palm sugar cake samples

Sample/ Property	Reducing sugar (%)	Total sugar (%)	Fructose (%)	Glucose (%)	Maltose (%)	Sucrose (%)
1	7.27 ± 0.16^{d}	91.41 ± 0.684^{d}	1.33 ± 0.00^{h}	2.11 ± 0.04^{g}	3.14 ± 0.60^{b}	83.85 ± 0.07^{d}
2	5.09 ± 0.09^{g}	100.73 ± 0.72^{a}	1.73 ± 0.05^{f}	2.67 ± 0.07^{f}	0.37 ± 0.01^{ef}	87.72 ± 0.11^{a}
3	5.39 ± 0.17^{f}	100.34 ± 0.72^{a}	1.88 ± 0.02^{e}	2.90 ± 0.01^{e}	0.14 ± 0.01^{g}	86.78 ± 0.24^{b}
4	5.08 ± 0.05^{g}	99.59 ± 0.69^{ab}	1.60 ± 0.03^{g}	2.63 ± 0.02^{f}	0.25 ± 0.00^{fg}	87.10 ± 0.41^{a}
5	5.82 ± 0.08^{f}	98.29 ± 0.70^{c}	0.90 ± 0.02^{i}	2.51 ± 0.03^{f}	1.22 ± 0.04^{d}	84.68 ± 0.07^{c}
6	8.69 ± 0.04^{c}	99.99 ± 0.69^{a}	3.05 ± 0.01^{b}	5.74 ± 0.07^{a}	0.27 ± 0.30^{fg}	79.99 ± 0.33^{f}
7	9.16 ± 0.17^{b}	98.70 ± 0.68^{c}	4.14 ± 0.06^{a}	4.50 ± 0.22^{b}	0.00 ± 0.00^{h}	81.13 ± 0.28^{f}
8	7.20 ± 0.19^{d}	99.60 ± 0.71^{b}	2.04 ± 0.03^{d}	3.55 ± 0.06^{d}	0.32 ± 0.12^{ef}	84.28 ± 0.32^{d}
9	7.87 ± 0.07^{a}	80.76 ± 0.45^{a}	2.52 ± 0.01^{c}	4.34 ± 0.05^{c}	2.21 ± 0.03^{c}	79.24 ± 0.69^{g}
10	5.27 ± 0.04^{e}	81.98 ± 0.83^{a}	0.16 ± 0.02^{j}	1.04 ± 0.00^{h}	4.41 ± 0.23^{a}	83.16 ± 0.56^{e}

Note: Each value is the mean ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

The concentration of all sugars was calculated in term of dry basis.

Volatile flavour compound

Volatile flavour compounds in palm sap, palm sugar syrup and palm sugar cake were investigated using Headspace Soild Phase Microextraction technique (HS-SPME) coupled with Gas chromatography-mass spectrometry (GC-MS). An example of chromatogram of colatile flavour compounds in palm sap was shown in Appendix Figure 4. Similar profiles of volatile flavour compounds from ten palm sap samples were obtained. Volatile flavour compounds were commonly found in all samples, consisting of 2 alcohols, 4 esters, 7 ketones, and 1 acid as shown in Table 12. Ethanol was mainly presented in all samples. Ethanol may produced by fermentation from carbohydrates with microorganisms. During the collecting process, it is highly susceptible to spontaneous yeast-lactic fermentation of the sugary sap. This process is reported to be rapid under sunlight. Sources of fermenting microorganisms are tapping implement (knife and bamboo tube) and air (Uzochukwu *et al.,* 1994; Borse *et al.,* 2007). In general, lactic acid bacteria produce very little ethanol (parts per million levels), and they use pyruvate as the principle final hydrogen receptor in metabolism. On the other hand, yeast produces ethanol as a major end product in metabolism (Lindsay, 1996). Additionally, Samarajeewa *et al.* (1981) reported that palm sap undergoes spontaneous two stages of fermentation. The first is lactic fermentation and subsequently fermented by yeasts to produce ethanol. Apart from ethanol, isomyl alcohol is also found in a product such as wine (Demyttenaere *et al.,* 2003). The level of isoamyl alcohol is influenced by natural of palm sap and the fermentation condition. Glucose was converted by yeast to isoamyl alcohol via pyruvate pathway (Hammond, 1993). Acetic acid was also found in palm sap. This indicated the existence of acetic acid bacteria such as *Acetobacter* sp. This acid is produced by two-step reactions. Firstly, oxidation of ethanol to acetaldehyde and secondly, oxidation of acetaldehyde to acetic acid. In addition, acetic acid is also produced by lactic acid bacteria through heterofermentation (Adams and Moss, 2000). Microbiological analysis at different stages of palm sap fermentation were done by Uzochukwu *et al.* (1991, 1994 and 1997) and reported that the microorganisms found in

palm sap in the early stage of fermentation (sugar 12%, pH 7-7.2) are mostly entirely Leuconostocs and Lactobacilli. Thus, the Leuconostocs and Lactobacilli produce glucans likely to be dextran. The fructose produced early in palm sap fermentation as a by product of dextran synthesis by lactic acid bacteria are likely to have been used in this way by the same bacteria to produce lactic and acetic acids. Flavour such as 3-hydroxy-2-butanone was reported to be responsible for sweet falvour (Cheetham, 2002). This volatile flavour compound in palm sap was reported by Taiapaiboon (2004). Moreover, some esters including isoamyl acetate, ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl-9-decanoate have been detected in palm sap. This result is similar to the result reported by Samarajeewa *et al.* (1981) and Uzochukwu *et al.* (1994, 1997 and 1999). Normally, many esters are formed during yeast fermentation. Isoamyl acetate in palm sap contributed to fruity and sweet flavour. This compound is derived from isoamyl alcohol and acetyl coenzyme A by alcohol acetyl transferase in yeast (Inoue *et al.*, 1994).

An example of chromatogram of colatile flavour compounds in palm sugar syrup and palm sugar cake was shown in Appendix Figure 5 and 6, respectively. Similar profiles of the volatile flavour compounds from ten palm sugar syrup samples and ten palm sugar cake samples were obtained. However, the amount of the volatile flavour compounds was different. The differences in the volatile components identified in palm sugar syrup samples are possibly due to differences in the property of palm sap and the heating conditions. A low pH and high total acidity of palm sap could promote an inversion reaction during heating, yielding high amounts of reducing sugars. Reducing sugars act as a substrate of the Maillard reaction, resulting in the forming of high amounts of pyrazines, furans and pyrroles (Purnomo, 2007). The differences in the volatile components identified in the palm sugar cake samples were possibly due to the difference in the property of the palm sugar syrup, the ingredients added and heating condition. Volatile flavour compounds were commonly found in all palm sugar syrup samples, consisting of 4 alcohols, 1 ester, 1 ketone, 1 acid, 1 pyrrole, 8 furans and 6 pyrazines. Volatile flavour compounds were commonly found in all palm sugar cake samples,

consisting of 2 alcohols, 1 ketone, 1 acid, 1 pyrrole, 11 furans and 6 pyrazines. Table 13 and 14 show the types and concentrations of volatile flavour compounds in ten palm sugar syrup samples and ten palm sugar cake, respectively. Ethanol was presented in all palm sugar syrup samples due to the fermentation of carbohydrates with microorganisms, particularly osmophilic yeast. Acetic acid can be found in palm sugar syrup and palm sugar cake. There are two possible pathways for acetic acid formation including osmophilic yeast metabolism and Maillard reaction. Acetic acid may be derived from isoamyl alcohol and acetyl coenzyme A by alcohol acetyl transferase in yeast (Inoue *et al.,* 1994). In addition, the α-dicarbonyl compounds are unstable and undergo a cleavage reaction (at the C-C bond), resulting in acetic acid during the Maillard reaction (Martins and Van Boekel, 2005). Phenethyl alcohol or benzene ethanol has been reported to possess a sweet or rose flavour (Soufleros *et al.,* 2004). It is derived from L-phenylalanine through the metabolic reaction of yeast (Soufleros *et al.,* 2004). The 3-hydroxy-2-butanone was also detected in all palm sugar syrup and palm sugar cake and it is a typical flavour in palm sap. This flavour is responsible for the sweet flavour (Cheetham, 2002). Higher amount of 3-hydroxy-2-butanone in palm sugar syrup sample was found when compared to palm sap due to the evaporation process during concentration. On the other hand, lower amount of 3-hydroxy-2-butanone in palm sugar cake sample was detected when compared to palm sugar syrup. This might be due to the palm sugar cake production has a longer heating time and its boiling point point (115^{o}C) of this flavour was lower than the processing temperature of palm sugar cake (120^{o}C, resulting in this flavour might be volatiled during the production. Moreover, ingredient added to produce palm sugar cake might reduce palm sap or palm sugar syrup in the formulation, resulting in the decrease in 3-hydroxy-2-butanone in palm sugar cake.

Volatie flavour compounds from the Maillard reaction and Caramelisation were found in palm sugar syrup and palm sugar cake including furans, pyrazines and pyrroles. These compounds have been reported in various plant syrups such as maple syrup, coconut syrup and birch syrup (Kallio, 1989; Akochi *et al.,* 1994; Akochi *et al.,* 1997; Purnomo, 2007). Furan derivatives such as 2-furanmethanol, 2-

furancarboxaldehyde, 2- furancarboxaldehyde, 2-acetylfuran, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2-methyl dihydro-3(2H)-furanone, 2-methyl dihydro-2(3H)-furanone and 2,3-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one were detected in all samples. These furans contribute to sweet, caramel, cooked sugar or burnt sugar (Ho *et al.,* 2007). Furans are the products of Maillard reactions and they account for the caramel-like odour of heated carbohydrates. The formation of furan derivatives can be formed through two ways including lipid peroxidation and degradation of carbohydrate involved in a Maillard reaction (Ho *et al.,* 2007). In this study, furan was known to be formed by the degradation of carbohydrate. Furfural is formed from the Amadori intermediate of the Maillard reaction by 1,2-enolization followed by deamination and dehydration and can be formed through Caramelisation (Nursten, 1980; Meynier and Mottram, 1995).

Pyrazine derivatives were detected in palm sugar syrup and palm sugar cake such as 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, methyl pyrazine, 2-methoxy-6-methylpyrazine and 2-ethyl-3,5-dimethylpyrazine. All these pyrazines are correlated with sensory attributes, such as nutty, sweet and roast-like flavour which consequently contribute to the typical flavour of palm sugar syrup (Ho *et al.,* 2007). The presence of pyrazines indicated that not only Caramelisation, but also a Maillard reaction took place during the production of palm sugar syrup and palm sugar cake (Apriyantono *et al.,* 2002). Normally, alkyl pyrazine are commonly found in heated foods. One of the main routes of pyrazines formation is via the Strecker degradation of amino acids in the presence of dicarbonyl compounds. In addition to the Strecker aldehyde, this reaction yields an aminocarbonyl compound, and the condensation of two of these molecules results in an alkyl pyrazine (Amrani-Hemaimi *et al.,* 1995; Hwang *et al.,* 1995; Meynier and Mottram, 1995). One pyrrole (acetylpyrrol) was identified in palm sugar syrup. This pyrrole tended to contribute to sweet or burnt sugar compounds and it has been reported in various heated foods such as birch syrup (Kallio, 1989). There are two pathways to forming pyrrole. The first is the interaction between an amino acid and a 3-dexoyhezosone through the Strecker degradation followed by dehydration and ring closure. The second pathway is the reaction of furans with amines or amino acids.

(Rizzie, 1974; Hwang *et al.,* 1995). As a result, higher amount of pyrazines, furans and pyrrole were detected in palm sugar cake when compared to palm sugar syrup. This probably due to palm sugar cake used higher heating temperature when compared to palm sugar syrup, resulting in the promotion of nonenzymatic browning reactions.

Table 12. Volatile flavour compounds of ten palm sap samples

RT [A]	RI [B]	Volatile flavour compounds	Attribute [C]	Sample No./Concentration (ppb)									
				1	2	3	4	5	6	7	8	9	10
		Alcohols											
2.18	1002	ethanol	alcoholic	16959	16672	7174	10386	16730	22863	18546	38943	32026	18654
4.98	1218	isoamyl alcohol	alcoholic	3430	6563	1634	3422	5085	1674	1556	1579	1687	5234
		Acid											
7.84	1473	acetic acid	sour	nd	2322	2352	nd	nd	2347	21567	1657	1779	nd
		Ketones											
6.00	1307	3-hydroxy-2-butanone	sweet	336	315	310	327	371	478	820	356	371	399
7.57	1196	1-hydroxy-2-propanone	citrus, herbal	250	276	nd	nd	nd	nd	nd	nd	nd	nd
9.28	1395	2-nonanone	fruity	nd	167	492	nd	nd	160	197	164	160	213
5.71	1095	2-heptanone	cheesy	nd	159	nd	nd	nd	nd	234	159	164	nd
12.60	2323	2-undecanone	fruity	nd	nd	712	nd	nd	nd	159	nd	nd	nd
8.71	1030	2,3-butanedione	powerful, buttery	397	nd	nd	389	471	nd	nd	250	309	nd
8.91	1041	2,3-pentanedione	fruity, sweet	163	336	712	nd	nd	nd	nd	156	nd	198
		Esters											
4.54	1059	isoamyl acetate	fruity, sweet	nd	712	nd	nd	157	323	302	273	167	nd
1.59	997	ethyl acetate	sweet, fruity	3976	2451	1677	3122	11765	3232	1191	3200	1433	7865
6.51	1136	ethyl hexanoate	fruity, sweet	nd	156	nd	nd	nd	712	519	162	163	nd
9.99	1439	ethyl octanoate	fruity, pineapple	633	786	nd	nd	478	781	478	1564	398	nd

Note: [A] RT refers to retention time (min); [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24})

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

nd refers to not detected.

Table 13. Volatile flavour compounds of ten palm sugar syrup samples

RT [A]	RI [B]	Volatile flavour compounds	Attribute [C]	Sample No./Concentration (ppb)									
				1	2	3	4	5	6	7	8	9	10
		Pyrazines											
5.81	1292	methyl pyrazine	nutty, roasty	174	224	381	184	335	133	149	155	174	666
6.84	1314	2,3-dimethylpyrazine	roasted nut, sweet	167	141	121	101	143	150	271	155	174	646
6.50	1309	2,5-dimethylpyrazine	sweet, roasty	109	119	132	147	153	103	164	115	115	164
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	2048	1830	1567	1664	1206	1096	2358	1717	1763	1666
7.82	1470	2-ethyl-3,5-dimethylpyrazine	roasty	110	91	90	107	597	110	323	131	167	645
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	117	147	204	176	167	121	215	309	154	619
		Furans											
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	743	780	645	716	634	1776	1758	2403	1829	3135
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	148	177	290	256	101	246	97	494	157	189
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	128	176	128	140	151	179	182	238	197	202
8.35	1537	2-acetylfuran	sweet, caramel	100	146	90	120	101	184	369	279	341	719
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	169	250	151	142	159	288	435	150	276	358
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	123	191	180	107	148	338	100	164	339	1168
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	130	187	188	158	321	288	277	377	411	332
11.72	2577	2,3-dihydro-3,5-dihydroxy-6M-pyran-4-one	caramel, sweet, burnt sugar	186	131	89	106	138	95	161	130	228	132

Note: [A] RT refers to retention time (min); [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24})

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

Table 13. Volatile flavour compounds of ten palm sugar syrup samples (continued)

RT [A]	RI [B]	Volatile flavour compounds	Attribute [C]	Sample No./Concentration (ppb)									
				1	2	3	4	5	6	7	8	9	10
		Alcohols											
2.18	1002	ethanol	alcoholic	39363	89599	79765	11366	11316	11547	131523	73014	138648	146889
4.98	1218	isoamyl alcohol	alcoholic	5253	7200	24391	3541	2576	1651	66993	6260	59981	70218
10.36	1951	phenethyl alcohol	sweet	501	963	702	969	1362	495	1089	508	4037	8701
8.71	1587	2,3-butanediol	fruity	1168	5172	4997	4735	3788	6134	5715	1727	5628	5252
		Acid											
7.84	1473	acetic acid	sour	11809	21698	19217	12785	11394	12654	24138	14138	21394	34008
		Ketone											
6.00	1307	3-hydroxy-2-butanone	sweet	1519	1251	1829	1832	1480	1377	1434	1807	1339	1168
		Ester											
1.86	997	ethyl acetate	fruity, sweet	6833	8682	7284	6134	10489	7745	7979	5311	6910	7538
		Pyrrole											
10.62	2023	acetylpyrrole	sweet, burnt sugar	128	139	180	177	176	99	134	176	138	236

Note: [A] RT refers to retention time (min); [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24})

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

Table 14. Volatile flavour compounds of ten palm sugar cake samples

RT [A]	RI [B]	Volatile flavour compounds	Attribute [C]	Sample No./Concentration (ppb)									
				1	2	3	4	5	6	7	8	9	10
		Furans											
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	6531	1793	2622	7744	5792	1576	3361	3911	4048	2630
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	2838	1662	2173	4970	1935	4704	6988	1698	3177	2099
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	514	500	690	571	503	698	709	304	535	548
12.88	2344	5-hydroxymethylfurfural	caramel	1179	1112	1156	8707	1114	2185	1351	1193	1184	1136
8.81	1608	5-methylfurfural	sweet, caramel, nutty	195	185	102	448	157	111	1374	330	102	133
8.35	1537	2-acetylfuran	sweet, caramel	345	386	342	1573	348	348	491	2013	344	258
		2-furan methane thiol	roasted coffee	629	499	105	550	252	113	296	247	100	181
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	1597	1391	1726	2372	1562	1654	1360	1274	1378	1238
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	104	179	141	251	123	125	504	681	209	116
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	1186	1146	1320	1482	1265	1111	1735	1113	1138	1228
11.72	2577	2,3-dihydro-3,5-dihydroxy-6M-pyran-4-one	caramel, sweet, burnt sugar	1223	1251	1770	2471	1759	1344	1527	1302	1709	1318

Note: [A] RT refers to retention time (min); [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24})

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

Table 14. Volatile flavour compounds of ten palm sugar cake samples (continued)

RT [A]	RI [B]	Volatile flavour compounds	Attribute [C]	Sample No./Concentration (ppb)									
				1	2	3	4	5	6	7	8	9	10
		Pyrazines											
5.81	1292	methyl pyrazine	nutty, roasty	409	879	874	479	887	814	3728	524	436	307
6.84	1314	2,3-dimethylpyrazine	roasted nut, sweet	364	377	325	475	306	381	364	330	381	257
6.50	1309	2,5-dimethylpyrazine	sweet, roasty	415	516	205	419	528	601	441	456	413	478
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	2259	4340	3137	7990	6490	9370	4120	5790	1908	1620
7.82	1470	2-ethyl-3,5-dimethylpyrazine	roasty	2690	1671	1820	1630	1880	2650	1430	5440	3380	1866
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	1156	1197	1171	1662	1511	1214	1154	1141	1181	1171
		Alcohols											
10.36	1951	phenethyl alcohol	sweet	237	145	176	498	215	125	144	141	227	159
		2,3-butanediol	fruity	109	219	132	303	455	321	467	358	943	327
		Acid											
7.84	1473	acetic acid	sour	9123	8153	7141	7749	9565	8523	9189	6440	8110	7714
		Ketone											
6.00	1307	3-hydroxy-2-butanone	sweet	379	219	874	295	387	297	324	383	392	307
		Pyrrole											
10.62	2023	acetylpyrrole	sweet, burnt sugar	808	504	244	332	320	364	533	613	224	135

Note: [A] RT refers to retention time (min); [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24})

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

Microbiological properties

Microbial loads of ten palm sap samples were shown in Table 15. Total microbial count, the yeast and mold count and the lactic acid bacteria count of all samples ranged from 1.2×10^7 to 6.7×10^8 cfu/g, 4.4×10^5 to 7.5×10^6 cfu/g and 9.5×10^6 to 2.1×10^8 cfu/g, respectively. The microorganisms can naturally contaminate in palm sap, especially during tapping process. Since typical tapping process is conducted in an open condition and the bamboo tube is reused to collect the palm sap. The bamboo tubes are not considered to be cleaned before and after usages. Moreover, most of the producers do not have good practice in sanitary during palm sap harvesting process (Phaichamnan *et al.,* 2010). Microorganisms presented in palm sap were responsible for the increase in total acidity and decrease in pH due to the fermentation of sugars. Additionally, ethanol was also produced during fermentation. The contamination and the growth of microorganisms in palm sap after harvesting is the main factor influencing on the property of palm sugar syrup afterward (Phaichamnan *et al.,* 2010).

In general, microbes reported to be found in sugar based product are yeasts and molds. Yeasts can grow under acid conditions and are not inhibited by sucrose. Osmophilic or sugar tolerant yeasts are a problem in syrup industry, because they can grow even at the limited level of water (Aw ranged from 0.65 to 0.80), high sucrose content and acid conditions. Microbial loads of ten palm sugar syrup samples are shown in Table 16. The total microbial count, the yeast and mold count and the osmophilic yeast count of all samples ranged from 2.6×10^3 to 4.7×10^5 cfu/g, 1.3×10^2 to 3.0×10^4 cfu/g and 3.5×10^2 to 4.6×10^5 cfu/g, respectively. As the Thai Industrial Standards Institute of the Ministry of Industry (2003) requires that total microbial and the yeast and mold counts in palm sugar syrup samples shall not be more than 5.0×10^2 cfu/g and 100 cfu/g, respectively. According to these criteria, all palm sugar samples in this study did not meet this standard. The microorganisms can naturally contaminate the raw material and the finished product, especially during harvesting and storage. In fact, when palm sugar syrup is produced under high temperature during evaporation in order to convert

palm sap to palm sugar syrup, substantial amounts of microorganisms are destroyed. However, a few microorganisms, especially osmophillic yeasts, are found to survive and grow after processing. Thus, the growth of osmophilic yeast is another factor affecting the decrease of pH values and increase in the total acidity and ethanol content of the syrup (Snowdon & Cliver, 1996; Phaichamnan *et al.*, 2010).

Table 15. Microbial load of ten palm sap samples

Sample/ Property	Total microbial count (cfu/g)	Yeast and mold (cfu/g)	Lactic acid bacteria (cfu/g)
1	2.3×10^{7}	5.8×10^{5}	9.8×10^{6}
2	4.5×10^{8}	5.2×10^{6}	5.9×10^{7}
3	1.6×10^{8}	4.3×10^{6}	4.4×10^{7}
4	1.2×10^{7}	4.4×10^{5}	9.5×10^{6}
5	1.6×10^{8}	3.6×10^{6}	2.1×10^{7}
6	2.0×10^{7}	2.1×10^{6}	1.3×10^{7}
7	6.7×10^{8}	7.5×10^{6}	2.1×10^{8}
8	2.3×10^{8}	7.2×10^{6}	9.6×10^{7}
9	4.8×10^{8}	7.3×10^{6}	9.9×10^{7}
10	5.7×10^{7}	5.9×10^{5}	1.5×10^{7}

Note: Each value is the mean of triplicate determinations.

Table 16. Microbial load of ten palm sugar syrup samples

Sample/ Property	Total microbial count (cfu/g)	Yeast and mold (cfu/g)	Osmophilic yeast (cfu/g)
1	7.6×10^3	3.5×10^3	1.8×10^3
2	1.2×10^5	1.0×10^4	1.5×10^5
3	8.5×10^3	2.8×10^3	1.4×10^3
4	4.4×10^3	3.0×10^2	3.5×10^2
5	8.0×10^3	4.0×10^3	2.1×10^3
6	2.6×10^3	1.3×10^2	4.2×10^2
7	7.9×10^4	1.8×10^4	2.5×10^5
8	8.3×10^3	4.5×10^3	2.5×10^3
9	5.8×10^4	2.2×10^4	2.1×10^5
10	4.7×10^5	3.0×10^4	4.6×10^5

Note: Each value is the mean of triplicate determinations.

Relationship among palm sap properties

Since, each palm sap sample contained large variation in physical and chemical properties. Each property was significantly different among palm sap samples ($P<0.05$). A principal component analysis (PCA) was used to explore relationships among data that measured on sixteen physical, chemical and microbiological properties from ten palm sap samples. Two principal components (PC1 and PC2) were calculated. They accounted for 78.35% of the variability in the original data as can be seen in Figure 6. The graphical PCA illustrated high positive relationship between L* value and turbidity (measured in terms of transmittance value). On the other hand, colour parameters, the negative correlation of L* with a* and b* was found, claiming on enzymatic browning of palm sap and pigment from Kiam wood caused in the decrease in L* value and increase in a* and b* values. Additionally, the pH value showed the negative relationship with total acidity, reducing sugars as well as fructose and glucose, total soluble solids, total sugars, total microbial, yeast and mold and lactic acid bacteria.

Total acidity was negatively correlated to pH value. A decrease in pH value could be due to organic acids production from microorganisms. When acids increase in solution, the pH will go down because the acids release hydrogen ions in the solution. The negative correlation of pH value with reducing sugar as well as fructose and glucose could be attributed to an inversion reaction induced the increment of fructose and glucose content. Furthermore, the negative correlation of pH value with total soluble solids and total sugars may presumably due to the growth of microorganisms. They used sugars in the sap as an energy source and produced organic acids via fermentation step. Moreover, two groups of samples were observed. Samples No. 1, No. 4, No. 5, No. 6 and No. 10 are located in the left part of the score plot, consequently these samples are well correlated with pH, L* and transmittance value. On the other hand, samples No. 2, No. 3, No. 7, No. 8 and No. 9 appeared on the right part of score plot are showing that these samples contained high reducing sugars as well as fructose and glucose, sucrose, total acidity, total sugars and total soluble solids.

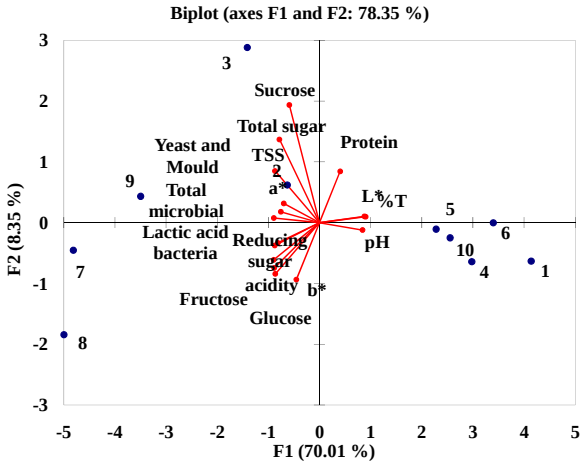


Figure 6. Biplot PC1 and PC2 of the properties of ten palm sap samples

Relationship among palm sugar syrup properties

Since the palm sugar syrup samples contained large variation of properties scores, all properties were significantly different ($P<0.05$). The data measured on eighteen physical and chemical properties from ten samples were also analyzed using multivariate technique-Principal Component Analysis (PCA). The first three principal components (PC1, PC2 and PC3) derived from PCA explain 76.12% of total variance of the original data. The PCA bi-plot which is composed from PC1 and PC2, explaining 66.88% of the data variance. As shown in Figure 7, the graphical PCA illustrates high positive relationship among L*, b*, pH and % Sucrose. It is indicating that nonenzymatic browning reactions took place and resulted in the decrease of L* and b* values. The pH value and sucrose content correlated well and showed negative relationship with reducing sugar as well as fructose and glucose contents, claiming on an inversion reaction induced the increment in fructose and glucose contents and a decrease in sucrose content due to this reaction greatly occurred in acid condition. The negative correlation between L* and b* value with HMF content and BI suggests accumulation of brown pigment from nonenzymatic brownings occurred in palm sugar syrup. The high correlation between physical properties such as L*, a* and BI with chemical properties such as pH, HMF, sucrose and reducing sugar as well as fructose and glucose refers to nonenzymatic browning reactions and inversion reaction. The L* and b* value decrease during nonenzymatic browning reactions occurred. These reactions influenced on the increase in BI and HMF content. Moreover, an inversion reaction occurred during the production of palm sugar syrup by the evidence of the increase in reducing sugar as well as fructose and glucose and decrease in sucrose under acid condition as shown in PC1. Thus, the nine indicators contributes greatly on PC1 could be called a group of nonenzymatic browning reactions and inversion reaction factors. As consider factor loading scores in PC2, there is a positive relationship between a* value and IBP, demonstrating similar changing pattern due to Maillard reaction took place. The negative correlation between a* and INB with protein and moisture contents also suggests that the decrease in protein content could be

related to Maillard reaction. In general, it appears that Maillard reaction is favored at an optimum moisture content corresponding to fairly low moisture content (Danehy, 1986). Thus, Maillard reaction is responsible for the factors highly loaded on PC2. There is a negative relationship among turbidity, TSS and total acidity when consider factor loading scores in PC3. The increase of TSS referred to higher concentration and caused the increase of undissolved particle, hence increase the turbidity. Furthermore, the increase in TSS is in accordance with the increase in organic acid (indicating by total acidity) since they was concentrated during heating process. From the factors grouped In PC3, it can be concluded as factors related to concentration effects. Additionally, samples No. 5 and No. 10 were found to be extremely different as shown in Figure 1. Sample No. 10 presented the highest dark colour, indicating by the lowest L* and b* values while sample No. 5 showed the highest light colour, indicating by the highest L* and b* values.

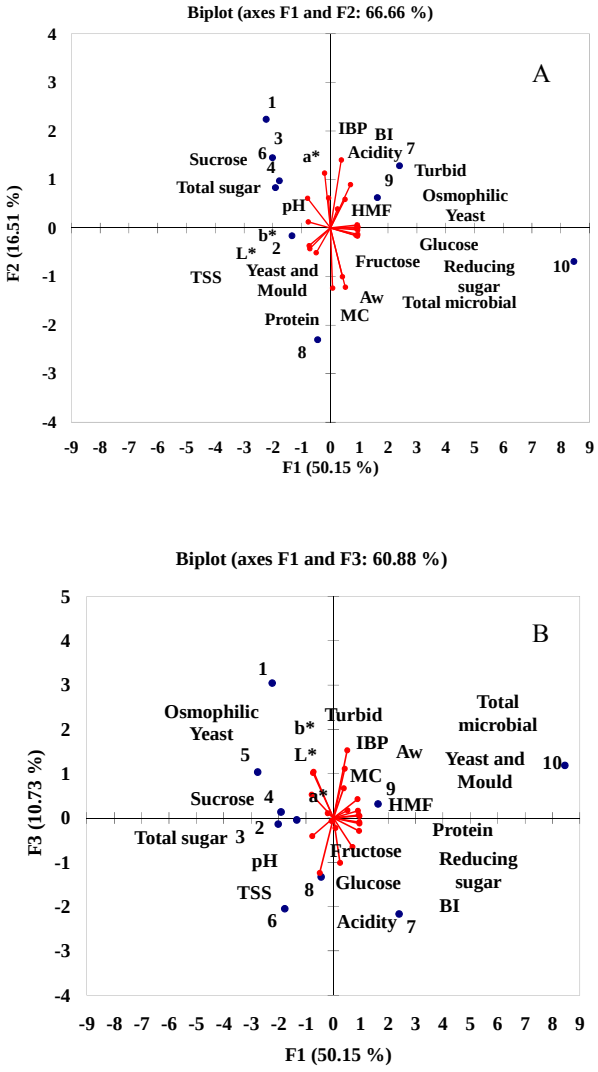


Figure 7. Biplot PC1 and PC2 (A) and biplot PC1 and PC3 (B) of the properties of ten palm sugar syrup samples

Relationship among palm sugar cake properties

Since the palm sugar cake samples contained large variations in the scores for properties, all properties were significantly different ($P<0.05$). A principal component analysis (PCA) was used to explore relationships among data that measured twenty two

physical and chemical properties from the ten palm sugar cake samples. The PCA bi-plot which is composed from the PC1 and PC2, explained 66.67% of the data variance (Figure 8). As shown in Figure 1, the graphical PCA illustrates a positive relationship between L* value, stickiness, maltose and moisture content. This suggests that the increase in maltose refers to the high glucose syrup content added during the production of palm sugar cake. Higher concentrations of glucose syrup caused an increment in the stickiness of the palm sugar syrup. Moreover, palm sugar syrup containing high moisture content also yields high stickiness. A positive relationship was found in the a* value, IBP, BI, HMF, fructose and glucose content. Browning parameters such as a* value, IBP, BI and HMF involve nonenzymatic browning reactions. The occurrence of nonenzymatic browning reactions is generally indicated by the increase in a* value, IBP, BI and HMF content and the decrease in L* value. This is made evident by a negative correlation between the L* value and browning parameters. Furthermore, a positive relationship between hardness, pH and sucrose content was found. This phenomenon could be attributed to the high sucrose content tending to increase the crystalline structure of palm sugar cake resulting in an increment in hardness. Moreover, a positive relationship of Tg, crystallinity and sucrose content was observed. These results could be explained by the high sucrose content increasing the Tg and crystallinity of the product.

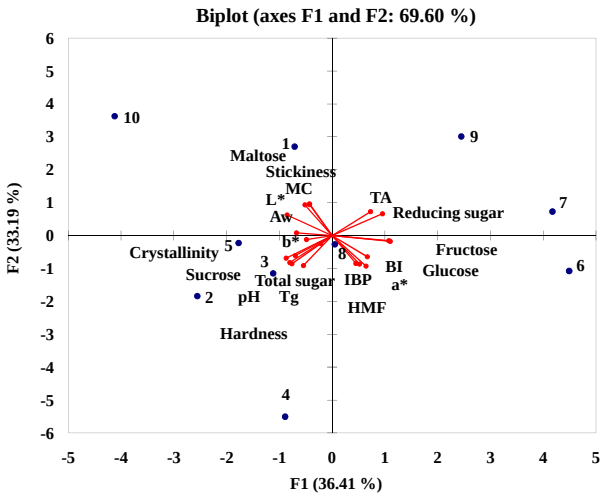


Figure 8. Biplot PC1 and PC2 of the properties of ten palm sugar cake samples

2.5 Conclusion

The physical, chemical and microbiological properties of palm sap, palm sugar syrup and palm sugar cake differed among samples since these products were locally produced by the producer experience and have not produced in commercial. In palm sap samples, microorganisms contaminated during tapping process affected their physical and chemical properties. Microorganisms can use sugar and produce organic acids and alcohol, mainly ethanol. These organic acids cause the decrease in pH and the inversion reaction, yielding high reducing sugar content. Ethanol is off-flavour in palm sap and may cause in unacceptable from consumers. On the other hand, the 3-hydroxy-2-butanone was detected in all samples and responsible for typical sweet flavour in palm sap.

The physical, chemical and microbiological properties of the palm sugar syrup, produced by traditional evaporating processes in an open pan differed among the samples. The TSS of two out of ten samples did not meet the requirements of the Thai

Ministry of Industry's Industrial Standards Institute. The steps of heating processe affected the properties of the palm sugar syrup. The BI and HMF contents of the samples produced by the one step of heating process were lower than those produced by the two steps of heating process due to using short heating time. The HMF content of three out of ten samples was higher than the maximum limited as recommend by the Codex Alimentarious (40 mg/kg). Low pH and high total acidity as affected by the fermentation of sugars by microorganisms can promote inversion reaction during the heating process, yielding a high content of reducing sugar. The dominant flavours, such as ethanol and acetic acid, were found in syrup as a result of microorganism activity. Flavours such as pyrazines and furans were mainly derived from nonenzymatic browning reaction during the heating process.

The physical and chemical properties of palm sugar cake also differed among the samples. The HMF content of eight out of ten samples was higher than the maximum limited as recommend by Codex Alimentarious (40 mg/kg). The properties of palm sugar cake may depend on the heating process, the property of raw material used either palm sap or syrup and other ingredients added. High heating temperature and long heating time can accelerate nonenzymatic browning reactions and cause a dark colour of the product. Low pH and high total acidity of palm sap or syrup can promote inversion reactions during the heating process, yielding high reducing sugar content. High amount of reducing sugar can react with protein through a Maillard reaction to form a dark colour. In addition, high reducing sugar content can retard the crystallisation of sugar, resulting in palm sugar cake with low hardness and high stickiness. Ingredients added, such as sucrose, also influenced on the crystallization, hardness and Tg of the palm sugar cake.

The evaporation to make a production of palm sugar syrup and palm sugar cake from palm sap was correlated well with the increase in IBP, BI, HMF, reducing sugar as well as glucose and fructose and sucrose content. This propably due to the nonenzymatic browning reaction took place during heating.

CHAPTER 3

EFFECT OF HARVESTING TIME OF PALM SAP AND STORAGE CONDITION ON THE PROPERTIES CHANGES OF PALM SUGAR SYRUP

3.1 Abstract

The aim of this study was to investigate the changes in physical and chemical properties during the production of palm sugar syrup by palm sap at different harvesting times (6 h, 12 h, 18 h and 24 h) and its changes during storage under temperature at 4^{o}C and 30^{o}C for 12 months. During production, samples were collected at 15 min intervals until the final TSS reached 70^{o}Brix. It was found that the properties of each sample during heating were significantly different ($P<0.05$). The results showed that L*, transmittance value, moisture content and FAG content decreased with heating time in all samples. On the other hand, a* value, b* value, IBP, BI, HMF, TSS, and reducing sugar contents as well as glucose and fructose increased with heating time in all samples. The highest nonenzymatic browning reactions and inversion reaction were found in palm sugar syrup sample which was taken from palm sap which was collected after 24 h. This result was indicated by the highest IBP, BI, reducing sugar as well as fructose and glucose and HMF contents and the lowest L* value, sucrose and free amino group contents. HMF, a possible mutagen formed by nonenzymatic browning reactions during the heating of sugar based products was also determined. It was found that only the sample that was collected at 24 h contained higher amount of HMF (113.07 mg/kg) than the permitted maximum limit (40 mg/kg) as recommend by the Codex Alimentarious.

During storage, temperature and time had a significant effect ($P<0.05$) on the properties of palm sugar syrup. Maillard reaction took place in all samples that stored under 30^{o}C higher than those that stored under 4^{o}C as evidenced by higher a* value, IBP, BI and HMF content and lower L* value, fructose, glucose and free amino group content

($P<0.05$). In addition, an increase in IBP, BI, HMF content and a decrease in L*, free amino group content were found with increasing storage time in all samples ($P<0.05$). The highest HMF content at 12 months of storage (160.23 mg/kg) of sample stored under 30^{o}C was found in palm sugar syrup that produced by palm sap after harvesting time for 24 h that higher than the standard as setted by Codex alimentarious. Only, sample that produced by palm sap after collection time for 6 h (stored under 4^{o}C and 30^{o}C) and 12 h (stored under 4^{o}C) had HMF in agreement with the standard during storage for 12 months. Thus, suitable collection time of palm sap for palm sugar syrup production that stored for 12 months was 6 h after harvesting. Moreover, storage under low temperature (4^{o}C) can benefit for reduce dark colour and HMF formation in palm sugar syrup sample.

3.2 Introduction

Palm sugar syrup is one of the local delicacies widely consumed by Asians and used as an ingredient in making cakes, desserts, food coating or drinks. Palm sugar syrup produced from sap derived from the tropical palm tree called Palmyra palm (*Borassus flabellifer* Linn.) (Apriyantono *et al.,* 2002; Ho *et al.,* 2008). Palm sap is used as a raw material for the production of palm sugar syrup. The composition and quality of palm sap is found to vary with place and duration of tapping or collection time. Generally, fresh sap is sweet, oyster white colour and translucent, with nearly neutral pH (Lasekan *et al.,* 2007). The sap, itself is sterile while flowing in Palmyra inflorescences. However, microorganisms are found in the sap coming from an environment during collecting process. Microbes are contaminated into the sap by unsanitary tapping procedures and unsanitary collection. The microorganism is responsible for the fermentation of palm sap and results in low quality of palm sugar syrup. Since palm sap is rich in sugars (10-15%). The fermenting organisms are dominated by yeasts, particularly *Saccharomyces cerevisiae* and lactic acid bacteria. These microorganisms converted sugars to acids and alcohols (Sanni, 1993; Iwuoha and Eke, 1996; Ezeronye and Okerentugba, 2000; Lasekan *et al.*, 2007; Stringini *et al.*, 2009).

Generally, palm sap was collected from its tree after 12 h of tapping. Sap was collected twice a day from each inflorescence either in the morning or the evening. Palm sap collected in the morning was immediately subjected to heat for palm sugar syrup production. However, palm sap collected in the evening was not produced palm sugar syrup in the same day. It was placed at room temperature and mixed with palm sap collected in the next day to produce palm sugar syrup. Thus, palm sap that was collected in the evening was left at room temperature for at least 12 h. This phenomenon results in the increase in microbial load and causes in the increase in total acidity and decrease in pH. When this sap was produced to be palm sugar syrup, the dark colour of syrup was obtained. Additionally, some palm trees gave a little yield of palm sap during collection. Therefore, producers used a longer harvesting time such as 18 h or 24 h for harvesting palm sap to obtain high yield of palm sap. Harvesting time also affected the property of palm sap especially, pH and its reducing sugar content. Long harvesting time also induced in the increase of microorganisms. The microorganisms use sugars in the sap as an energy source and produce organic acids. Thus, the changes in chemical properties may affect the quality of palm sugar syrup afterward. The formation of nonenzymatic browning reactions was taken place during the production of palm sugar syrup. These reactions mainly the Maillard reaction and Caramelisation are greatly influenced by temperature, pH and concentration of a substrate (Ajandouz and Puigserver, 1999; Oh *et al.*, 2006). The different harvesting time of palm sap showed a difference of pH and affected the property of palm sugar syrup.

Since quality is supremely important in food, thermal deterioration has to be controlled during processing and storage. Nonenzymatic browning reactions may cause unacceptable nutritional and sensory effects in sugar based food products and may be a limiting factor in the shelf life of products. Storage temperature and storage time generally influenced the browning of sugar based products during storage (Buedo *et al.*, 2000; Burdurlu and Karadeniz, 2003). So far, there is no information regarding the effect of harvesting time of palm sap and storage condition on the property of palm sugar syrup. Therefore, the aim of this study was to monitor the physical and chemical properties

changes during the production of palm sugar syrup by palm sap at different harvesting times. Additionally, the effect of storage temperature (4^{o}C and 30^{o}C) and storage time (0-12 months) on the properties changes of palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h was also investigated.

3.3 Materials and Methods

Chemicals

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). 2,4,6-Trinitrobenzenesulfonic acid (TNBS), L-leucine, hydroxymethylfurfural and thiobarbituric acid were purchased from Sigma-Aldrich (St.Louis. MO, USA).

Production of palm sugar syrup

Palm sap was collected at different harvesting times including 6 h, 12 h, 18 h and 24 h. Harvesting time refers to the starting time of hanging the bamboo tube on the infloresences of palmyra palm until reached the cycle of each harvesting process. Palm sap was kept in an icebox during transportation (30 min) to Department of Food Technology, Prince of Songkla University, Hat Yai Campus. The palm sap was concentrated to 70^{o}Brix by conventional method (an open pan at temperature approximately 110^{o}C) to obtain palm sugar syrup. Temperature of a sample was monitored during heating process at 15 min interval. During heating process, sample was collected at 15 min interval until the total soluble solid reach 70^{o}Brix. Immediately after

production, the physical and chemical properties of sample were determined. In addition, each palm sugar syrup sample was stored under 4^{o}C and 30^{o}C in a closed plastic cup for 12 months. The physical and chemical properties of each sample were determined at one month interval. Microbial loads were analysed at six months interval.

Measurement of colour

The colour measurements of the samples were carried out using a Hunter Lab Colourflex colourimeter. A colourimeter was adjusted for reflectance, illuminant D 65, and angle of 10^{o}. Instrumental colour data was provided in accord with the CIE system in terms of L* (lightness), a* (redness and greenness) and b* (yellowness and blueness).

Measurement of clarity

The clarity of samples was estimated by measuring the transmittance at 650 nm using a spectrophotometer as describe by Taipaiboon (2004) and expressed in term of percentage.

Measurement of intermediate browning product and browning intensity

Intermediate browning product (IBP) and browning intensity (BI) of palm sugar syrup were determined by monitoring the absorbance at 280 and 420 nm, respectively. The absorbance was measured by using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan), as described by Takano (2005) and Kawai *et al.* (2005). Appropriate dilution (8-fold for IBP and 4-fold for BI) was made using distilled water to obtain a reliable absorbance reading.

Determination of pH

The pH value was measured at ambient temperature with a pH meter (Satorious, USA) which calibrated at pH 4.0 and 7.0.

Determination of total acidity

The sample was diluted with distilled water and titrated with 0.01 N NaOH using a few drops of 1% phenolphthalein solution as an indicator. The sample was transferred as a measured quantity (1-3 ml) into approximately 10 ml of distilled water. The result was calculated as a percentage of lactic acid (Rangana, 1986).

Determination of total soluble solid

The total soluble solid (TSS) content of sample was determined as degree Brix using a hand refractometer.

Determination of moisture content

The moisture content of palm sugar syrup was measured using a vacuum oven. About 2-5 g of the sample was placed in a pre-dried aluminum dish and dried in a vacuum oven at 60^{o}C (pressure<70mmHg) for 6 h. The dried sample was placed in a desiccator and cooled for 0.5 h to room temperature. The weight was recorded, and the percentage moisture based on the initial wet weight was calculated.

Determination of total sugar and reducing sugar

The total sugar and reducing sugar content were quantified by the Lane and Eynon Volumetric method using titration with Fehling's reagents. The results were expressed as grams of glucose per 100 g of sample (Rangana, 1986).

Determination of type and concentration of sugars

The type and concentration of sugar was determined using HPLC (Shimadzu, CR6A Chromatopac) with a Hypersil NH_2 column and refractive index detector. The mobile phase was the solution of acetonitrile and water (80:20), pumped at a flow rate of 1.5 ml/min and an injection volume of 20 µl. The samples were prepared by making appropriate dilutions with distilled water. All sample solutions were passed through a 0.45 µm nylon syringe filter to remove particulates prior to HPLC analysis. D-glucose, D-fructose, maltose and sucrose were used as the external standards. The calibration curve of each sugar was plotted between peak areas and concentrations (Stuckel and Low, 1996).

Determination of free amino group content

Free amino group content was determined according to the method of Benjakul and Morrissey (1997). Firstly, the sample was diluted through appropriate dilution. Then 125 µl of the sample was mixed with 2.0 ml of 0.21 M phosphate buffer, pH 8.2, and 1.0 ml of 0.01% TNBS solution was then added. The solutions were mixed thoroughly and placed in a temperature-controlled water bath (Memmert, Bavaris, Germany) at 50^{o}C for 30 min in dark. The reaction was terminated by adding 2.0 ml of 0.1 M sodium sulfite. The mixtures were cooled at room temperature for 15 min. The blank was prepared in the same manner as the samples except that distilled water was

used instead of 0.01% TNBS. The absorbance was measured at 420 nm. The free amino group content was expressed in terms of L-leucine.

Determination of 5-Hydroxymethylfurfural content

The palm sugar syrup (5-10 g) was dissolved in deionized water and made up to 50 ml with deionized water. After that it was centrifuged at 5,000 rpm for 15 min. The supernatant was used to measure 5-hydroxymethylfurfural (HMF) content. To determine the HMF content, 2 ml of supernatant was introduced into the tube. Two ml of 12% trichloroacetic acid and 2 ml of 0.025 M thiobabituric acid were subsequently added and mixed thoroughly. The tube with the sample was then placed in a water bath at 40^{o}C. After incubating for 50 min, the tube was cooled immediately, using water, and the absorbance was measured at 443 nm. A calibration curve of HMF was utilised to quantify the HMF concentration (Rattanathanalerk *et al.*, 2005).

Determination of volatile flavour compounds

Volatile flavour compounds were analysed using the HS-SPME-GC-MS technique. A 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber was used (Supelco, Bellefonte, PA, USA). Each sample (2 ml) was added in a 12 ml headspace vial and then an internal standard 2-methyl-3-heptanone was spiked into the sample. After that, the vial was sealed tightly with a crimp cap and a PTFE/silicone septum and equilibrated at 50^{o}C for 30 min in a water bath. A manual SPME holder containing fiber was inserted into the vial and exposed to the sample headspace at 50^{o}C for 15 min. The fiber was then transferred directly into the injector port of the GC-MS system. Thermal desorption of analytes from the fiber in the GC injector port was carried out with an SPME inlet liner (0.75 mm i.d., Supelco) in the splitless mode at a desorption temperature of 240^{o}C. The SPME fiber was conditioned at 250^{o}C for 10 min before starting the first measurement. It was then left in the injection

port for re-conditioning during the whole GC run before taking the next sample. GC-MS analysis was conducted using the Agilent 6890 Plus GC/HP 5973 MSD, equipped with an HP-FFAP column (25 m×0.32 mm i.d.×25 µm film thickness). The injector temperature was 240^{o}C. The GC oven temperature was programmed from 40 to 230^{o}C at the rate of 8^{o}C/min and held at 230^{o}C for 10 min. The carrier gas was ultra high purity helium at a constant flow of 1.5 ml/min. The mass spectrometer condition was as follows: MSD capillary direct-interface temperature was 280^{o}C; the Ionization energy was 70 eV; and the mass range was between 20-450 a.m.u. Positive identification of a component was performed by comparison of the mass spectrum. Tentatively identified compounds were specifically identified in the basis of the mass spectra from the Wiley 275.L mass spectra database (Hewlett-Packard Co.). The integration of peaks was done on HP chemstation software (Hewlett-Packard Co.). The minimum peak area for detection was 10,000 counts.

In this study, the given concentration values were noted as equivalents to the internal standard. The relative concentrations of the investigated compounds (IC) were calculated by relating the areas of the internal standard (IS) to the areas of the compounds of interest.

$$\text{Relative concentration} = \frac{\text{(Peak area of IC)}}{\text{(Peak area of IS)}} \times \text{Concentration of IS}$$

Determination of microbial load

Microbial loads of palm sugar syrup including total microbial count, yeast and mold count and osmophilic yeast were analysed at six months interval. At the specified time intervals, samples were aseptically taken and serially diluted in 0.1 g/100 g peptone water for microbial counts. Pour plating on Plate Count Agar (Merck KGaA, Darmstadt, Germany) was performed for the total microbial count, overlaid with the same medium, and the plates incubated at 35-37^{o}C for 1-2 days. Spread plating on Potato Dextrose Agar acidified with 10 g/100 g tartaric acid (Merck KGaA, Darmstadt,

Germany) was performed for yeast and mold count and the plates incubated at 20-25^{o}C for 5 days. The osmophilic yeast count was also analysed using the spread plate technique on osmophilic potato dextrose agar and the plates were incubated at 37^{o}C for 3 days. The results are means of measurements in triplicate (Kiss, 1984).

Statistical analysis

All analysis and measurements were performed in triplicates. During the production, the effect of harvesting time of palm sap (6 h, 12 h, 18 h and 24 h) on a given data within each heating time and the effect of heating time (0-195 min) on a given data within each harvesting time of palm sap were determined using a completely randomized design (CRD). During storage, the effect of storage temperatures (4^{o}C and 30^{o}C) on a given data within each harvesting time of palm sap was run using paired test of the difference. The effect of storage times (0-12 months) on a given data within each harvesting time of palm sap and storage temperature was run using CRD. Data was subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test (Steel and Torrie, 1980). Analysis was performed using a SPSS package (SPSS 6.0 for windows, SPSS Inc, Chicago, IL).

3.4 Results and Discussion

Changes in physical properties during the production of palm sugar syrup produced by palm sap at different harvesting times

Changes in colour values (L*, a* and b*) during the production

Colour is one of the most important parameters for justment of buying. Figure 9 shows the changes in L*, a* and b* values during the production of palm sugar syrup by palm sap at different harvesting times. Appendix Figure 7 shows the samples of

palm sugar syrup that produced by palm sap at different harvesting times. Harvesting time of palm sap influenced the L*, a* and b* ($P<0.05$). The L* values of palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h were 78.21, 76.33, 71.30 and 59.59, respectively. The a* values of palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h were 2.35, 2.44, 2.54 and 2.70, respectively. The b* values of palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h were 18.11, 16.91, 13.79 and 12.51, respectively. The increase in a* values and decrease in L* and b* values with increasing harvesting time were probably due to enzymatic browning reaction taking place during collecting of palm sap. Polyphenol oxidaes is responsible for this reaction (Taipaiboon, 2004; Loetkitsomboon, 2004). This enzyme catalyzes the hydroxylation of monophenols (from metabolite of plant and Kiam wood) to *o*-diphenols and oxidation of *o*-diphenols to *o*-quinones. Quinones are very reactive compounds which strongly interact with other molecules, leading to a large pigment of high molecular weight with very red to brown colour (Eskin, 1990).

The heating time had a significant effect ($P<0.05$) on the change in L*, a* and b* values of all samples. The L* values decreased with heating time in all samples which reduced to 46.16 , 55.33, 47.00 and 45.41 at the end of heating process (195 min) for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. The a* values increased with heating time in all samples. At the end of heating process (195 min), it was observed that a* values of finished samples were 24.44, 24.35, 24.32 and 33.21 for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. The b* values also increased with heating time in all samples which increased to 73.50 , 86.41, 74.56 and 60.11 at the end of heating process for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. The results showed that with increasing heating time, samples became dark. Similar results were obtained by various researchers and they have been reported that the decrease in L* values and increase in a* values correlated well with the increase in the nonenzymatic browning reactions of food products (Ibarz *et al.,* 1999; Rattanathanakerk *et al.,* 2005; Damasceno *et al.,* 2008; Rao

et al., 2009). The major reactions occurred during the heating process of sugar riched products are nonenzymatic browning reactions including Maillard reaction and Caramelisation (Phaichamnan *et al.,* 2010).

Generally, it is well known that nonenzymatic browning reactions are very much dependent on temperature, pH and concentration of a reactant (Ajandouz and Puigserver, 1999; Oh *et al.,* 2006; Kim and Lee, 2008). In this research work, processing temperature (approximately 110^{o}C) was monitored and constant in all treatments. Hence, the different properties of palm sugar syrup samples may mainly depend on pH and concentration of reactant. The pH of system significantly influences both of the reaction rate and the type of product formed. At high pH, more colour intensity was produced by both Maillard reaction and Caramelisation (Oh *et al.,* 2006). The amount of unprotonated amino group, which is considered to be the reactive species, increases obviously with increasing pH. Furthermore, pH has an effect on the reactant sugar. First, the open chain form of the sugar is considered to be the reactive species, and the amount of open chain increases with pH (Yayalayan *et al.,* 1993; Martins and Van Boekel, 2005). Moreover, Caramelisation is responsible for the brown colour of syrup. The rate of Caramelisation as well as the rate of ring-sugar aldehyde transformation increases rapidly with increasing pH because the bond between the oxygen and hydrogen attached to C-1 is slightly ionic and is weakened as pH increases (Buera *et al.,* 1987). From this results, palm sugar syrup that produced by palm sap after harvesting time for 6 h presented lower L* values and higher a* values than those that produced by palm sap after harvesting time for 12 h and 18 h ($P<0.05$). This is probably due to the effect of pH. The effect of pH of palm sap in ranged of 4.54-6.57 (pH of palm sap after harvesting time for 6-18 h) was a dominant factor for browning colour formation in palm sugar syrup afterward. As high pH value can promote nonenzymatic browning reactions, resulting in high brown colour formation in a sample that produced by palm sap after harvesting time for 6 h when compared to 12 h and 18 h.

On the other hand, palm sugar syrup that produced by palm sap after harvesting time for 24 h presented the lowest L* and highest a* value ($P<0.05$) although

the pH of this palm sap is the lowest. Long harvesting time of palm sap can promote the decrease in pH and increase in total acidity. The increase in total acidity and decrease in pH may contribute to the activity of microorganisms. When this palm sap was subjected to heat to produce palm sugar syrup, high inversion of sucrose greatly took place due to high acid condition. Inversion reaction caused an increase in reducing sugar content. Reducing sugar can act as a substrate of Maillard reaction and resulting in the highest brown colour formation of palm sugar syrup. Thus, in palm sap, high concentration of substrate (reducing sugar) can be a dominant factor to promote nonenzymatic browning reactions during heating when compared to the effect of pH.

Changes in clarity during the production

The clarity was determined by measuring the percentage of light transmittance at 650 nm. High transmittance value presents more clarified juice. Changes in transmittance values of palm sugar syrup were presented in Figure 9D. Harvesting time also affected the transmittance value of palm sap ($P<0.05$). The transmittance value decreased with increasing of harvesting time. Initially, transmittance values of palm sap were 62.71%, 60.34%, 57.24% and 48.66% for palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. Normally, palm sap is transparent. The growth of microorganisms can increase during delay time of collection palm sap. The fermented palm sap contains a heavy suspension of yeast and bacteria, giving a milky white appearance (Uzochukwu *et al.*, 1999; Lasekan *et al.*, 2007). This phenomenon is responsible for the decrease in transmittance value of palm sap.

Heating time affected the transmittance value of all samples ($P<0.05$). The transmittance values of all palm sugar syrup samples decreased with heating times ($P<0.05$). At the end of heating process (195 min), the transmittance values were 40.42%, 50.39%, 38.40% and 36.83% for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. The decrease of transmittance value during heating may due to undissolved particles, the protein content from sap itself

and the polyphenolic compounds, which was contaminated from Kiam wood (*Cotylelobium lanceotatum* craih.) during the collecting process as well as insoluble complexes caused by the interaction of protein and polyphenol (Kermasha *et al.*, 1995; Siebert *et al.*, 1996; Phaichamnan *et al.*, 2010). Moreover, the colloid particles from brown pigment that were concentrated during heating are responsible for the decrease in clarity of palm sugar syrup (Takano, 2005).

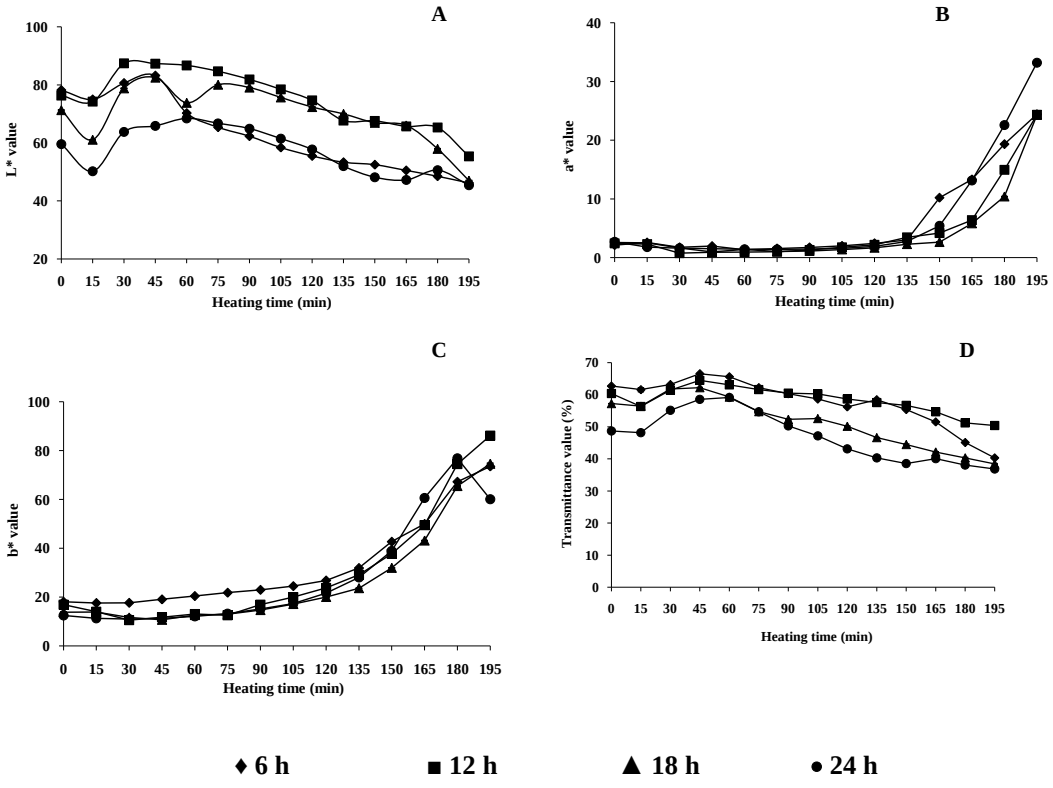


Figure 9. Changes in L* (A), a* (B), b* (C) and transmittance values (D) during the production of palm sugar syrup produced by palm sap at different harvesting times

Changes in intermediate browning product and browning intensity during the production

The IBP and BI tended to increase with increasing of harvesting time ($P<0.05$). Figure 10A and 10B depicts the changes in IBP and BI during the production of palm sugar syrup that produced by palm sap at different harvesting times. BI of palm sap was 0.03, 0.04, 0.05 and 0.06 for palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. The increase in BI might due to enzymatic browning reaction took place during harvesting.

Heating time also affected the IBP and BI ($P<0.05$). The IBP and BI of all palm sugar syrup samples increased with increasing heating time ($P<0.05$). The IBP and BI of all palm sugar syrup samples slightly increased within the first 90 min ($P<0.05$). After that, IBP and BI increased sharply with increasing time till 195 min ($P<0.05$). The IBP of palm sugar syrup samples was 0.42, 0.40, 0.46 and 0.53 in samples that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively while the BI was 0.61, 0.45, 0.61 and 0.75 for samples that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. The increase in IBP and BI were due to nonenzymatic browning reactions including the Maillard reaction and Caramelisation taking place during heating. During the production of palm sugar syrup, the Maillard reaction had more effect on browning development than Caramelisation. This probably due to Caramelisation greatly progresses at temperature approximately $120^{o}C$ or above (Kroh, 1994; Eskin, 1990). Moreover, the decrease in free amino group and reducing sugar during heating can be confirmed the formation of brown colour via the Maillard reaction. The highest IBP and BI were found in palm sugar syrup that produced by palm sap after harvesting time for 24 h ($P<0.05$). This is probably due to this sample contain the highest reducing sugar content that it can act as a substrate for the IBP and BI formation.

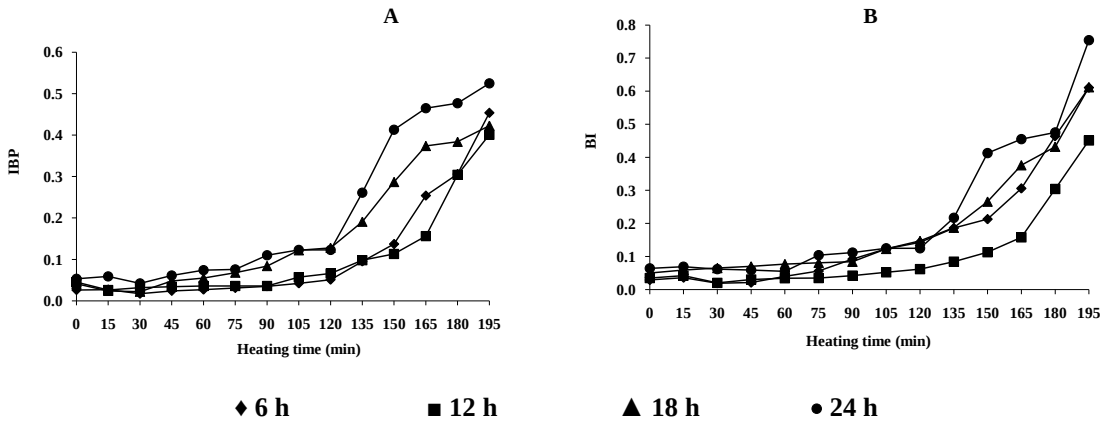


Figure 10. Changes in IBP (A) and BI (B) during the production of palm sugar syrup produced by palm sap at different harvesting times

Changes in chemical properties during the production of palm sugar syrup produced by palm sap at different harvesting times

Changes in total soluble solid and moisture content during the production

Changes in total soluble solid (TSS) and moisture contents (MC) during heating process are shown in Figure 11 ($P<0.05$). The TSS was 13.00^{o}Brix, 12.03^{o}Brix, 10.57^{o}Brix and 10.00^{o}Brix for palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. There was a decrease in TSS as harversting time increased ($P<0.05$) due to microorganism used sugars in the sap as an energy source. The TSS of palm sap after harvesting time for 24 h was out of range of the pH of palm sap that was harvested in the commercial (as mentioned in chapter 2). In addition, no change in MC of palm sap was found with increasing harvesting time.

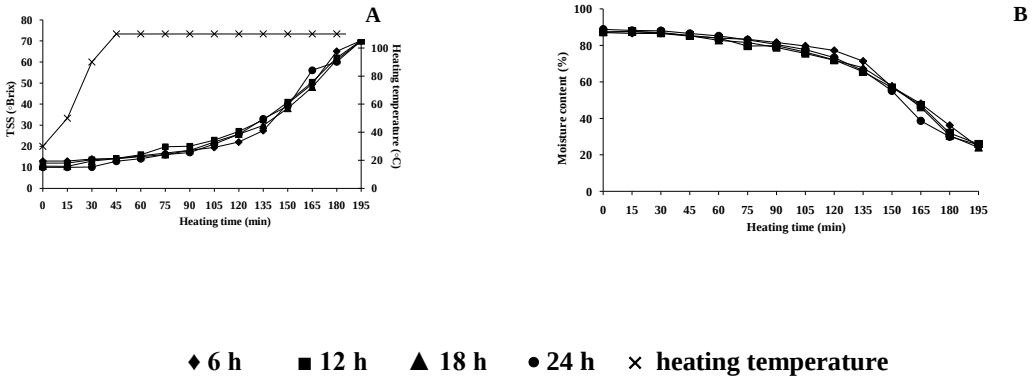


Figure 11. Changes in total soluble solid (A) and moisture content (B) during the production of palm sugar syrup produced by palm sap at different harvesting times

Heating time had a significant effect on TSS and MC ($P<0.05$). During heating process, an increase in TSS and decrease in MC was found in all samples ($P<0.05$). The increase in TSS and decrease in MC could be attributed to the evaporation of water from palm sap during the increasing heating temperature (Akochi-K *et al.*, 1997; Rao *et al.*, 2009). The decrease in MC and increase in TSS were not significant effect by harvesting time of palm sap ($P\geq0.05$).

Changes in pH and total acidity during the production

The pH value and total acidity were monitored during heating process as presented in Figure 12A and 12B, respectively. Harvesting time affected the pH and total acidity of palm sap ($P<0.05$). The pH of sample decreased with increasing harvesting times while the total acidity increased ($P<0.05$). Initially, the pH of palm sap was 6.57, 5.00, 4.54 and 4.07 in palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. The total acidity of palm sap was 0.01%, 0.05%, 0.10% and 0.22% in palm

sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. The decrease in pH and increase in total acidity during harvesting of palm sap might be contributed to the activity of microorganisms (Phaichamna, 2009). Normally, the pH of natural palm sap was at neutral pH approximately 7 and total acidity was approximately 0.006% as reported by Jitbunjerdkul (1989) and Lasekan *et al.* (2007). Hence, a high percentage of total acidity and low pH indicates the initial fermentation step in palm sap occurs. It was notices that the pH of palm sap after harvesting time for 24 h was out of range compared to commercial. Moreover, a low pH of palm sap as influenced by harvesting time can promote inversion reaction during collection and heating process, resulting in high nonenzymatic browning reactions take place.

Heating time had a significant ($P<0.05$) effect on total acidity of all samples. An increase in total acidity of all samples was found with increasing heating time ($P<0.05$). The total acidity slightly increased during 135 min and rapidly increased to 0.17%, 0.25%, 0.35% and 0.55% at the end of heating process (195 min) in palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively ($P<0.05$). The increase in total acidity was due to the concentration of organic acid during heating process.

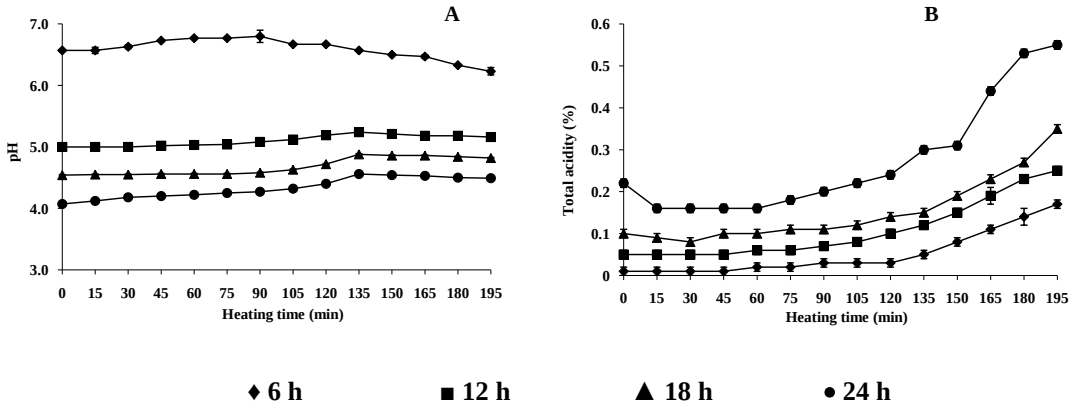


Figure 12. Changes in pH (A) and total acidity (B) during the production of palm sugar syrup produced by palm sap at different harvesting times

Changes in sugar content during the production

Sugar is a major component in palm sap and palm sugar syrup. Changes in total sugar and reducing sugar contents at different harvesting timers during heating process are shown in Figure 13. Initial reducing sugar content of palm sap was 0.74%, 0.99%, 1.99% and 5.01% in palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. Initial total sugar content of palm sap was 12.04%, 10.92%, 10.38% and 9.20% for palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. Moreover, fructose content was 3.00%, 3.22%, 8.09% and 20.34%, glucose content was 3.05%, 3.35%, 8.06% and 20.69% and sucrose content was 93.42%, 90.05%, 80.45% and 58.14% in palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. Harvesting time of palm sap affected the total sugar, reducing sugar, fructose, glucose and sucrose contents ($P<0.05$). There was an increase in reducing sugar including fructose and glucose and a decrease in sucrose content as harvesting time increased ($P<0.05$). This might be due to inversion caused by invertase activity and acid condition. The occurrence of invertase in palm sap was due to its present naturally and also synthesized by microorganisms. The primary sources of invertase are certain yeasts such as *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* (Pancoast and Junk, 1980; Taipaiboon, 2004; Takano, 2005). The invertase can be converted sucrose to glucose and fructose and finally to organic acids and alcohols. Furthermore, an increase in total acidity and decrease in pH during harvesting can accelerate inversion reaction (Wiene and Shallenberger, 1988). A decrease in total sugar content of all palm sap samples when harvesting time increased was found ($P<0.05$). This is probably due to the fermentation of sugars by microorganisms.

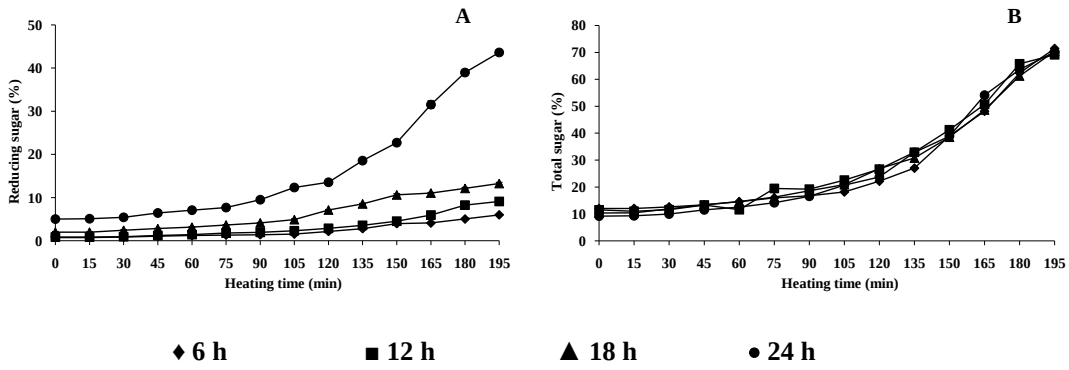


Figure 13. Changes in reducing sugar (A) and total sugar (B) contents during the production of palm sugar syrup produced by palm sap at different harvesting times

Heating time had a significant effect on the total sugar, reducing sugar, fructose, glucose and sucrose content ($P<0.05$). An increase in reducing sugar content such as fructose and glucose content and a decrease in sucrose content were observed during heating process in all samples ($P<0.05$) (Figure 14). The fructose, glucose and sucrose contents of palm sugar syrup were 5.49%, 5.44%, 85.32% for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 6.36%, 6.43%, 83.03% for palm sugar syrup that produced by palm sap after harvesting time for 12 h, 11.35%, 11.35%, 72.26% for palm sugar syrup that produced by palm sap after harvesting time for 18 h and 28.47%, 29.32% and 37.42% for palm sugar syrup that produced by palm sap after harvesting time for 24 h. The increase in reducing sugar including fructose and glucose and the decrease in sucrose content can be indicated that inversion reaction took place during heating. The highest inversion reaction (sucrose decreases higher than 50%) was found in palm sugar syrup that produced by palm sap after harvesting time for 24 h as evidenced by this sample contained the highest reducing sugar as well as fructose and glucose content and the lowest sucrose content ($P<0.05$). This probably due to this sap presented the lowest pH, resulting in the promotion of inversion reaction during heating.

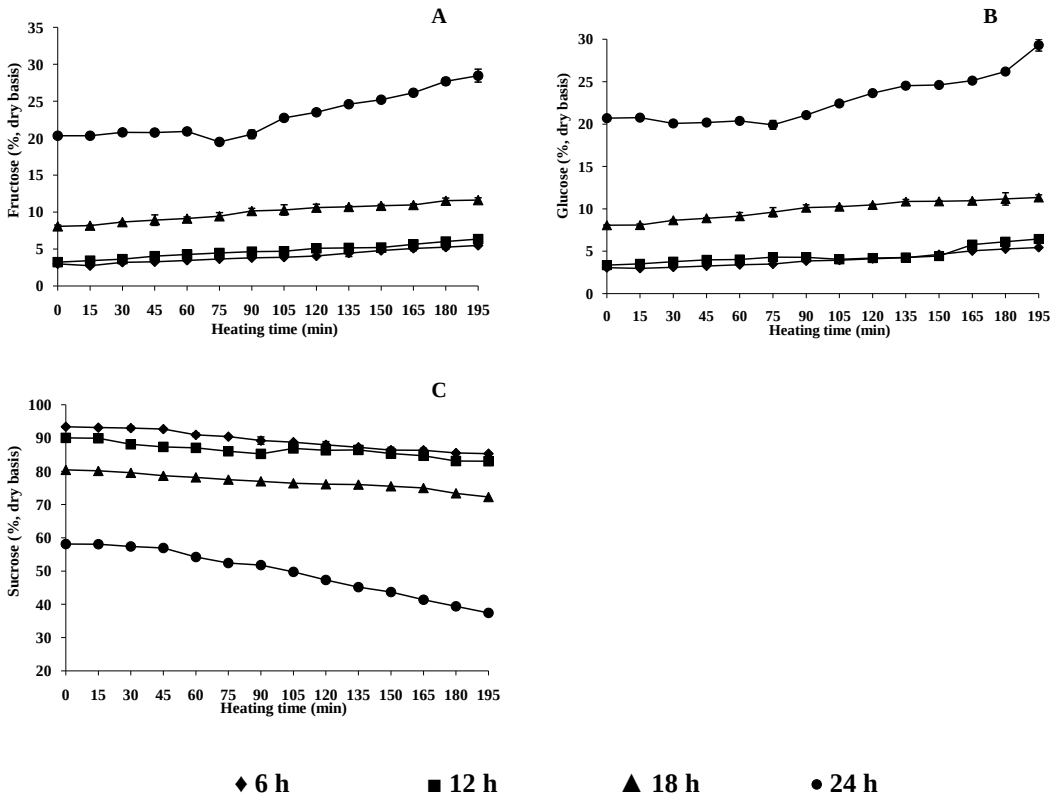


Figure 14. Changes in fructose (A), glucose (B) and sucrose (C) contents during the production of palm sugar syrup produced by palm sap at different harvesting times

Changes in free amino group content during the production

As mentioned previously, Maillard reaction is the main reaction and responsible for dark colour of syrup. Monitoring free amino group content can be used to indicate the Maillard reaction during heating due to it acts as a substrate of this reaction. The free amino group content was not significant effect by harvesting time of palm sap ($P \geq 0.05$). Free amino group content initially was 7.06 mg/g, 7.09 mg/g, 6.94 mg/g and 6.79 mg/g in palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively.

Heating time had a significant effect on the free amino group content ($P<0.05$). Free amino group content decreased in all samples with increasing heating time as shown in Figure 15A. At the end of heating process, free amino group content of palm sugar syrup decreased to 3.20 mg/g, 4.05 mg/g, 3.08 mg/g and 1.57 mg/g for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. As a result, Maillard reaction took place more effectively at high pH, as evidenced by lower free amino group content remaining in palm sugar syrup that produced by palm sap after harvesting time for 6 h when compared with 12 h ($P<0.05$). Palm sugar syrup that produced by palm sap after harvesting time for 24 h presented the lowest free amino group content ($P<0.05$). This indicated that palm sugar syrup that produced by palm sap after harvesting time for 24 h took place the highest Maillard reaction due to this sap contained high reducing sugar content.

Changes in HMF content during the production

HMF is used as an indicator of heat stress for sugar based foods such as honey and syrup because of its toxicological status (Cammerer *et al.*, 1999). The dehydration of carbohydrates, especially hexose, causes the formation of HMF greatly taking place in acid medium (Kus *et al.* 2005; Risner *et al.* 2006). Additionally, the Maillard reaction can also take place, giving rise to Amadori compounds during the first step of the reaction, and HMF as a consequence of further reaction. In addition, it is well known that HMF is a precursor of coloured compounds in the Caramelisation reaction (Kroh, 1994). Changes in HMF content during the production of palm sugar syrup that produced by palm sap at different harvesting times were presented in Figure 15B. The HMF content was not significant effect by harvesting time of palm sap ($P\geq0.05$).

Heating time had a significant effect on the HMF content ($P<0.05$). A significant increase in HMF content as heating time increased was observed in all samples ($P<0.05$). At the end of heating process, HMF content of palm sugar syrup was

18.93 mg/kg, 22.23 mg/kg, 34.12 mg/kg and 113.07 mg/kg for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. The highest HMF content was found in a sample produced by palm sap after harvesting time for 24 h ($P<0.05$). This probably due to high acid and high reducing sugar content of this sap can promote the HMF formation during heating. HMF content of palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h and 18 h was lower than the permitted maximum limit of 40 mg/kg as recommended by the Codex Alimentarius (Turhan *et al.*, 2007). However, HMF content was found higher than 40 mg/kg in palm sugar syrup that produced by palm sap after harvesting time for 24 h. This indicated that palm sap after harvesting time for 24 h was improper to produce palm sugar syrup.

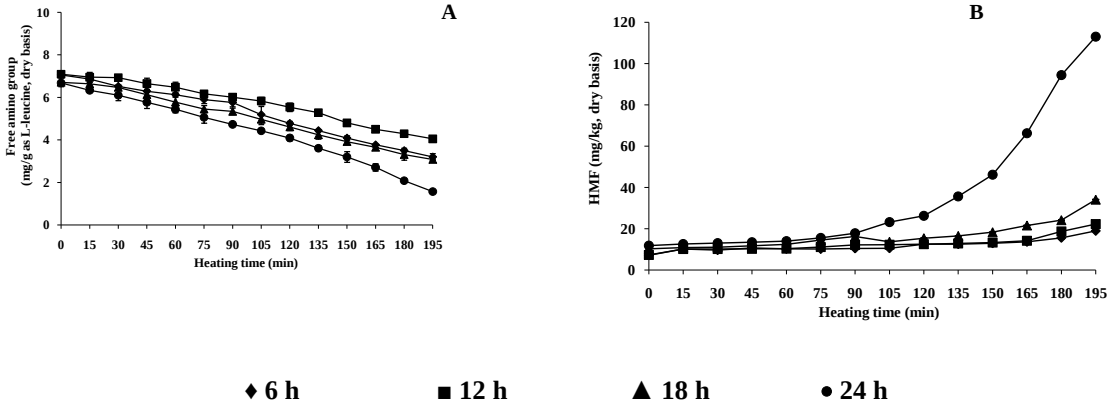


Figure 15. Changes in free amino group (A) and HMF (B) contents during the production of palm sugar syrup produced by palm sap at different harvesting times

From the result, it can be seen that the harvesting time of palm sap affected the properties of palm sugar syrup, especially colour and HMF content. Palm sap after 6-18 h of harvesting time can still be used to produce palm sugar syrup due to the HMF in palm sugar syrup was lower than the requirement of standard. On the other hand, palm sap after harvesting time for 24 h was fermented by microorganisms as indicated by

the lowest pH and the highest total acidity. When this sap was produced to palm sugar syrup, the highest nonenzymatic browning reactions took place resulting in the highest dark colour formation and the highest HMF content. The HMF content of a sample that produced by palm sap after harvesting time for 24 h was higher than the requirement of standard. This indicated that palm sap after harvesting time for 24 h is not sutitable for using as a raw material to produce palm sugar syrup, although this syrup still presented the typical flavour from nonenzymatic browning reaction.

Changes in physical properties during storage of palm sugar syrup produced by palm sap at different harvesting times

Normally, syrup with concentration approximately 70^{o}Brix is stable to store under room temperature for one year (Potter and Hotchkiss, 1995). The most common chemical reactions that influence on the property of syrup during storage are inversion reaction and Maillard reaction (Akochi-K *et al.,* 1997; Apriyantono *et al.,* 2002; Ho *et al.,* 2007; Perkin and Van den Berg, 2009). In this study, the effect of storage temperature (4^{o}C and 30^{o}C) and storage time (0-12 months) on the properties changes of palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h was investigated. Appendix Figure 8 shows the samples of palm sugar syrup that produced by palm sap at different harvesting times during storage.

Changes in colour values (L*, a* and b*) of palm sugar syrup during storage

The colour of palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h during storage at two temperatures (4^{o}C and 30^{o}C) for 12 months was also observed by using the CIE colour system. The L* values of palm sugar syrup was monitored during storage as depicted in Figure 16. Storage temperature and storage time had a significant effect on L*, a* and b* values ($P<0.05$). The L* values were significantly decreased with increasing storage temperature and time in each palm

sugar syrup sample that produced by different harvesting times ($P<0.05$). Initial L* values were found to be 46.16, 55.33, 47.00 and 45.41 for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. L* values decreased to 29.69, 39.94, 31.99 and 22.98 for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h and stored under 4^{o}C until 12 months of storage, respectively. Additionally, L* values also reduced to 10.05, 12.28, 7.29 and 2.01 for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h and stored under 30^{o}C until 12 months of storage, respectively. The results indicated that decreasing of these values giving the colour of palm sugar syrup changed to brown and dark, especially at higher temperature and longer time during storage.

Changes in a* values of palm sugar syrup during storage are shown in Figure 16. The a* values were significantly increased with increasing storage temperature and time in each palm sugar syrup sample that produced by palm sap at different harvesting times ($P<0.05$). The a* value increased during storage from an initial value of 24.44, 24.35, 24.32 and 33.21 to 38.17, 34.31, 37.74 and 38.59 for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h and stored under 4^{o}C until 12 months of storage, respectively. Moreover, a* value increased to 35.67, 38.74 and 37.80 within the first 8 months and then decreased to 27.93, 32.00 and 26.74 for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h and 18 h and stored under 30^{o}C until 12 months of storage, respectively. The a* value of sample that produced by palm sap after harvesting time for 24 h and stored under 30^{o}C tended to increased to 35.73 within the first 5 months and then decreased to 14.59 at 12 months during storage.

Changes in b* value of palm sugar syrup during storage are presented in Figure 16. The b* values were significantly decreased with increasing storage temperature and time in each palm sugar syrup sample that produced by palm sap at different harvesting times ($P<0.05$). Initial b* value was found to 73.50, 86.14, 74.65 and 60.11 and then decreased to 50.84, 67.14, 56.84 and 40.41 for palm sugar syrup that

produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h and stored under 4^{o}C until 12 months of storage, respectively. The b* value reduced to 15.82, 20.84, 11.17 and 4.41 for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h and stored under 30^{o}C until 12 months of storage, respectively. The greatest decrease in b* value during storage occurred in palm sugar syrup stored under 30^{o}C, followed by those stored under 4^{o}C.

Generally, L* and b* value decrease while a* value increases during browning. It indicated that the colour of palm sugar syrup became darker with more red component and less yellow component (Krapfenbauer *et al.,* 2006). Changes in colour parameters as mentioned previously are responsible for dark colour and it may contribute to the nonenzymatic browning (Maskan, 2006; Ibarz, 1999; Rattanathanalerk *et al.,* 2005; Damasceno *et al.,* 2008). During storage of sugar based products, Maillard reaction in sugar based product can also take place during storage. Meanwhile, Caramelisation can not occur since this reaction effective at temperature 120^{o}C or above. Therefore, only Maillard reaction caused the browning of palm sugar syrup at ambient and low temperature during storage (Eskin, 1990; Coultata, 1993). The rate of Maillard reaction also increased with increasing temperature and time (Martins *et al.,* 2001). Temperature affects the activities of the reducing sugars. The active form of sugar is considered to be opened chain, which is formed markedly with increasing temperature (Van Boekel and Martins, 2002).

Changes in clarity of palm sugar syrup produced during storage

Clarity of palm sugar syrup can be measured by transmittance value at 650 nm. Changes in transmittance values in palm sugar syrup during storage under 4^{o}C and 30^{o}C for 12 months are shown in Figure 16. Storage temperature and storage time had a significant effect on the transmittance values ($P<0.05$). The transmittance values were significantly decreased with increasing storage temperature and time in each palm sugar syrup sample that produced by palm sap at different harvesting times ($P<0.05$). Initial

transmittance values of palm sugar syrup were 40.32%, 50.39%, 38.40% and 36.83% for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. At 12 months of storage, transmittance value declined to 30.91%, 38.86%, 22.91% and 20.03% for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h and stored under 4^{o}C, respectively. Palm sugar syrup that stored under 30^{o}C presented lower transmittance value than those that stored under 4^{o}C, which was 9.56%, 13.86%, 6.57% and 2.70% for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. The clarity of palm sugar syrup depends greatly on its protein concentration and the polyphenol compounds, which is dissolved from Kiam wood and as presented in natural of palm sap itself. The complex between protein and polyphenol can be induced and therefore, a large colloid size or haze can be developed (Kermasha *et al.,* 1995, Siebert *et al.,* 2006). The transmittance decreased slightly under lower storage temperature (4^{o}C), and more dramatically under 30^{o}C for all palm sugar syrup samples. At higher temperature, molecular mobility is higher thus, allowing more interactions to take place polyphenol and protein interaction (Calderon *et al.,* 1986; Lee *et al.,* 2007). Moreover, the Maillard reaction taking place during storage might affect the decrease in clarity of palm sugar syrup due to the formation of the colloid particles (brown polymer), resulting higher light scattering (Takano, 2005).

Changes in intermediate browning product and browning intensity of palm sugar syrup during storage

As Maillard reaction caused of colour change in palm sugar syrup during storage. Figure 17 shows the changes in IBP and BI of palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h. Temperature and time during storage had a significant effect on IBP and BI ($P<0.05$). The IBP and BI of all samples were significantly increased with increasing storage temperature in each harvesting time of palm sap ($P<0.05$). Additionally, IBP and BI of all palm sugar syrup samples were

also significantly increased with increasing storage temperature and time in each harvesting time of palm sap ($P<0.05$).

At the beginning, IBP values were 0.45, 0.40, 0.42 and 0.53 for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. At 12 months of storage under 4^{o}C, IBP increased to 1.02, 0.88, 1.02 and 1.27 for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. An increase of IBP was also found higher in all samples stored under 30^{o}C than under 4^{o}C in all treatments. The increase in IBP was in accordance with BI. Initial BI of palm sugar syrup was 0.61, 0.45, 0.61 and 0.75, and then increased to 0.90, 0.68, 0.86 and 1.16 for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h at 12 months of storage under 4^{o}C, respectively. At 12 months of storage, samples that stored under 30^{o}C presented higher BI than those that stored under 4^{o}C in all treatments. The increasing in IBP and BI during storage was affected by Maillard reaction. These browning developments during storage were greatly enhanced by storing palm sugar syrup at 30^{o}C and were suppressed at 4^{o}C due to high storage temperature can promote Maillard reaction (Eskin, 1990; Martins *et al.*, 2001). In addition, a similar relationship between IBP and BI suggested that some intermediate products might undergo conversion to the brown compounds, while some intermediates are still being generated during storage.

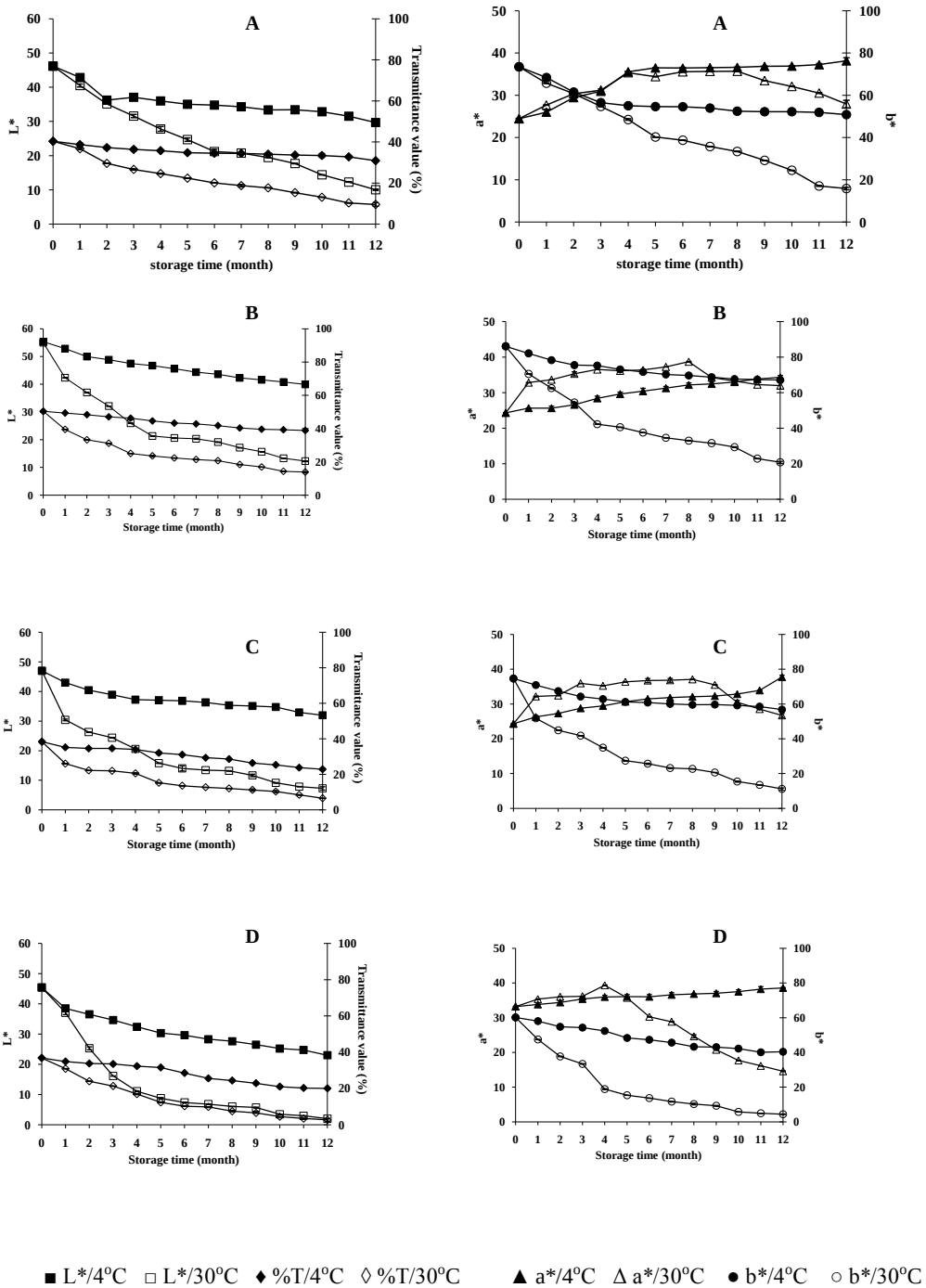


Figure 16. Changes in L*, a*, b* and transmittance values during storage of palm sugar syrup produced by palm sap after harvesting time for 6 h (A), 12 h (B), 18 h (C) and 24 h (D)

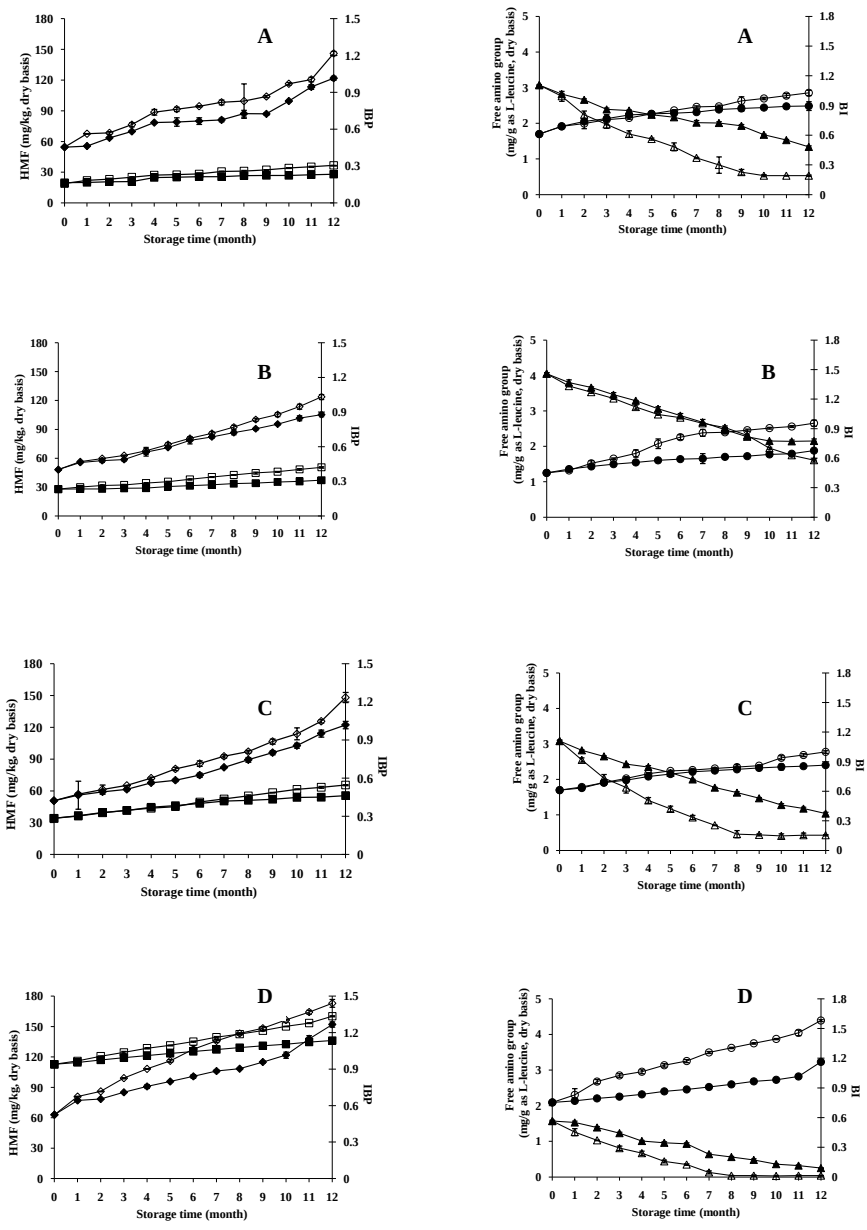


Figure 17. Changes in HMF, IBP, free amino group and BI during storage of palm sugar syrup produced by palm sap after harvesting time for 6 h (A), 12 h (B), 18 h (C) and 24 h (D)

Changes in chemical properties during storage of palm sugar syrup produced by palm sap at different harvesting times

Changes in total soluble solid of palm sugar syrup during storage

Initially, TSS of all palm sugar syrup samples was approximately 70^{o}Brix. The TSS of all palm sugar syrup samples remained constant during 12 months of storage either 4^{o}C or 30^{o}C. Temperature and time during storage had not a significant effect on TSS ($P \geq 0.05$). According to the Thai Industrial Standards Institute Ministry of Industry (2003) stated that the standard of TSS in palm sugar syrup shall not be less than 65^{o}Brix in order to prevent the microorganisms growth during storage under room temperature.

Changes in moisture content and water activity of palm sugar syrup during storage

The moisture content (MC) and water activity (Aw) are highly important for the shelf life of syrup during storage (De Rodriguez *et al.*, 2004). Initially, MC and Aw of all palm sugar syrup samples were approximately 25% and 0.80, respectively. The MC and Aw of all palm sugar syrup samples remained constant during 12 months of storage either 4^{o}C or 30^{o}C. Storage temperature and storage time had not a significant effect on MC and Aw ($P \geq 0.05$).

Changes in pH and total acidity of palm sugar syrup during storage

Changes in pH and total acidity were observed during 12 months of storage as shown in Figure 18. Temperature and time during storage had a significant effect on pH and total acidity ($P < 0.05$). The pH values of all samples were significantly decreased with increasing storage temperature in each harvesting time of palm sap

($P<0.05$). Additionally, the pH values of all samples were also significantly decreased with increasing storage time in each harvesting time of palm sap and storage temperature ($P<0.05$). The initial pH value was found to 6.23, 5.16, 4.82 and 4.49 and then decreased to 6.04, 5.00, 4.67 and 4.36 for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h and stored under 4^{o}C until 12 months of storage, respectively. The pH values of all samples stored under 30^{o}C greater decreased than those stored under 4^{o}C. The total acidity of all samples were significantly increased with increasing storage temperature in each harvesting time of palm sap ($P<0.05$). Additionally, the total acidity of all samples were also significantly increased with increasing storage time in each storage temperature ($P<0.05$). The total acidity increased during storage from an initial value of 0.17%, 0.25%, 0.35% and 0.55% to 0.26%, 0.38%, 0.50% and 0.65% for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h and stored under 4^{o}C until 12 months of storage, respectively. Moreover, total acidity increased to 0.37%, 0.47%, 0.59% and 0.72% for palm sugar syrup that produced by palm sap after harvesting time of 6 h, 12 h, 18 h and 24 h and stored under 30^{o}C until 12 months of storage, respectively. Results suggest that, the decrease in pH value and increase in total acidity was probably due to chemical reaction and the growth of microorganism. The reduction in pH value and increase in total acidity occurring in Maillard reaction was due to the formation of organic acids such as formic acid and acetic acid (Ames, 1998; Brands and Van Boekel, 2002; Lertittikul *et al.*, 2007).

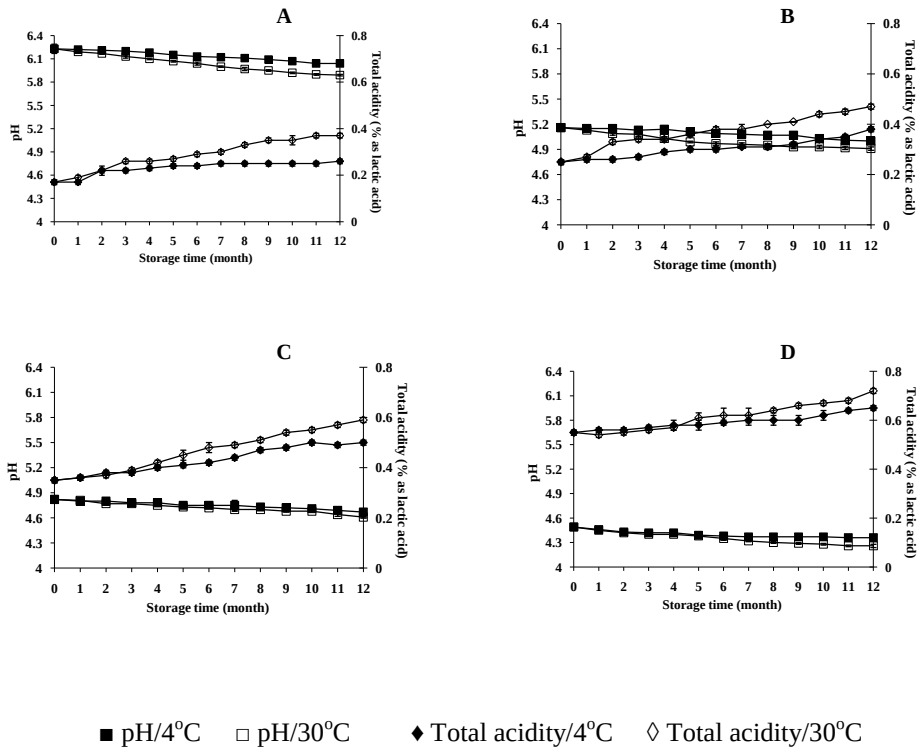


Figure 18. Changes in pH and total acidity during storage of palm sugar syrup produced by palm sap after harvesting time for 6 h (A), 12 h (B), 18 h (C) and 24 h (D)

Changes in HMF content of palm sugar syrup during storage

Temperature and time during storage can induce an increase in HMF content of palm sugar syrup ($P<0.05$). Changes in HMF content during storage of all palm sugar syrup samples were presented in Figure 17. HMF content of all samples were significantly increased with increasing storage temperature and time in each harvesting time of palm sap ($P<0.05$). Initial HMF contents were 13.83 mg/kg, 26.05 mg/kg, 34.12 mg/kg and 112.74 mg/kg for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. At 12 months of storage, HMF content increased to 28.00 mg/kg, 37.15 mg/kg, 55.59 mg/kg and 136.27 mg/kg for palm sugar

syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h and stored under 4^{o}C, respectively. Palm sugar syrup that stored under 30^{o}C showed higher HMF content than those that stored under 4^{o}C, which was 36.70 mg/kg, 50.58 mg/kg, 65.55 mg/kg and 160.23 mg/kg for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h, respectively. Initial HMF content of palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h and 18 h was lower than the permitted maximum limit of 40 mg/kg as recommended by the Codex Alimentarius (Turhan *et al.,* 2008). However, initial HMF content was found higher than 40 mg/kg in palm sugar syrup that produced by palm sap after harvesting time for 24 h. HMF greatly took place in highly acidic medium (Kus *et al.,* 2005) as in agreement with the result of the highest HMF content was found in palm sugar syrup that produced by palm sap after harvesting time for 24 h. Furthermore, HMF can be formed during storage of palm sugar syrup as indicated from the increase in HMF content with storage time. The in agreement of HMF with the standard until 12 months of storage was found in palm sugar syrup that produced by palm sap after harvesting time for 6 h that stored under both 4^{o}C and 30^{o}C and palm sugar syrup that produced by palm sap after harvesting time for 12 h that stored under 4^{o}C. In addition, initial HMF content (112.74 mg/kg) of palm sugar syrup that produced by palm sap after harvesting time for 24 h was higher than the allowed maximum limit (40 mg/kg). This result indicated that palm sap after harvesting time for 24 h was not suitable to produce palm sugar syrup. Low temperature can be used to retard the increase in HMF content in palm sugar syrup during storage.

Changes in free amino group content of palm sugar syrup during storage

At early stage of Maillard reaction, terminal α-amino groups of peptide and ε-NH_2 groups of lysine react with the carbonyl function of reducing sugars present in the reaction medium. Thus, the loss of available primary amino groups can be used to evaluate Maillard reaction in palm sugar syrup during storage. The free amino group

content in palm sugar syrup was monitored during storage as shown in Figure 17. Temperature and time during storage had a significant effect on free amino group content ($P<0.05$). Free amino group content of all samples were significantly decreased with increasing storage temperature and time in each harvesting time of palm sap ($P<0.05$). Initial free amino group content was 3.70 mg/g, 4.02 mg/g, 3.08 mg/g and 1.57 mg/g and then decreased to 1.34 mg/g, 2.15 mg/g, 1.04 mg/g and 0.25 mg/g for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h and stored under 4^{o}C until 12 months of storage, respectively. The free amino group content reduced to 0.53 mg/g, 1.61 mg/g, 0.43 mg/g and 0.04 mg/g for palm sugar syrup that produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h and stored under 30^{o}C until 12 months of storage, respectively. The reduction of free amino group content during storage was due to it loss during Maillard reaction. In addition, all palm sugar syrup samples that stored under 30^{o}C contained lower free amino group content than those that stored under 4^{o}C. This result could be explained as a promotion of Maillard reaction caused by high temperature during storage.

Changes in sugar of palm sugar syrup during storage

Sugar is a major component in palm sap and palm sugar syrup. Figure 19 showed the changes in total sugar and reducing sugar content of palm sugar syrup during storage. Changes in fructose, glucose and sucrose content of palm sugar syrup are shown in Figure 20. Storage temperature and storage time had a significant effect on total sugar, reducing sugar as well as fructose and glucose and sucrose contents ($P<0.05$). Initally, the fructose, glucose and sucrose contents of palm sugar syrup were found to be 5.49%, 5.44%, 85.32% for 6 h, 6.36%, 6.43%, 83.03% for 12 h, 11.36%, 11.35%, 72.42% for 18 h and 28.47%, 29.32%, 37.42% for 24 h. A significant decrease in fructose and glucose content as storage time increased was observed in all palm sugar syrup samples stored under 4^{o}C ($P<0.05$). In addition, there was a continuous decrease in sucrose content of all samples stored under either at 4^{o}C or at 30^{o}C during 12 months of storage. During storage

for 12 months, the highest decrease in sucrose content was found in samples that produced by palm sap after harvesting time for 24 h stored under both storage temperatures. Results suggested that, the decrease in fructose and glucose contents during storage was due to they acted as a substrate for Maillard reaction. Moreover, an increase in fructose and glucose contents of palm sugar syrup that produced by palm sap after harvesting time for 24 h and stored under 30^{o}C during storage after 8 months was found. These results indicated that the reaction rate of sucrose inversion was greater than that of Maillard reaction formation since low amount of FAG content reacted with reducing sugar. Additionally, a decrease in sucrose can be used to confirm the inversion of sucrose occurred during storage. The sucrose content of samples that stored under 30^{o}C greater decreased than that stored under 4^{o}C.

Accroding to the physical and chemical properties point view, it can be noticed that intermediate products were formed during storage and further converted to brown pigment as indicated by the increase in IBP, BI and HMF content.

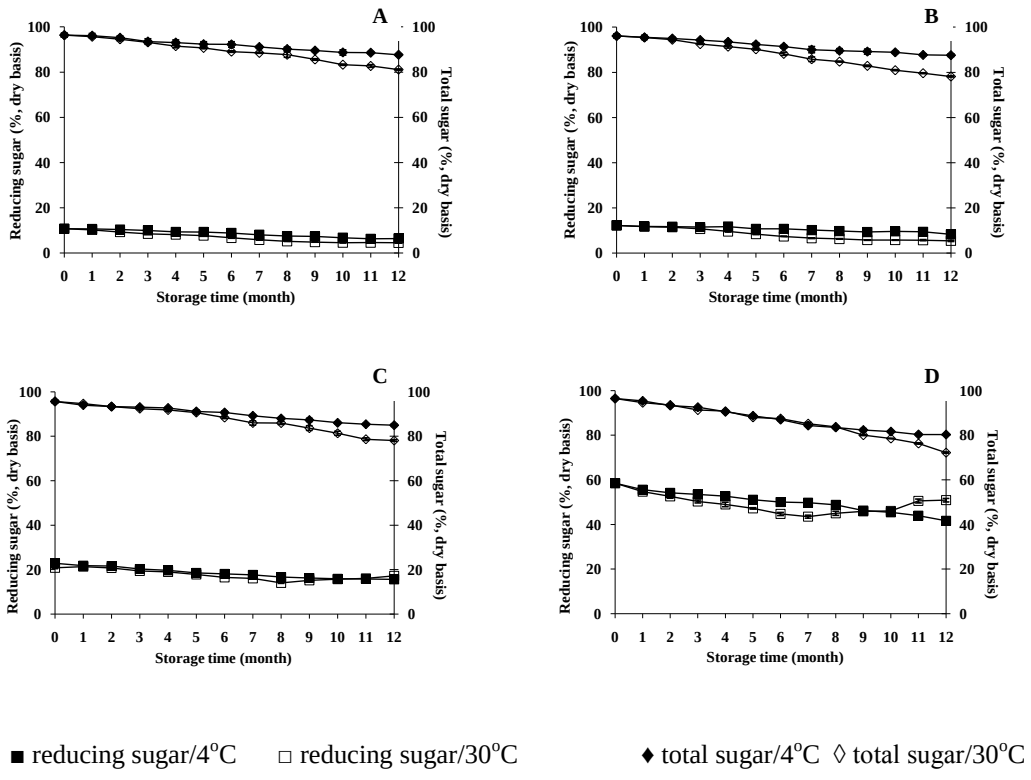


Figure 19. Changes in reducing sugar and total sugar during storage of palm sugar produced by palm sap after harvesting time for 6 h (A), 12 h (B), 18 h (C) and 24 h (D).

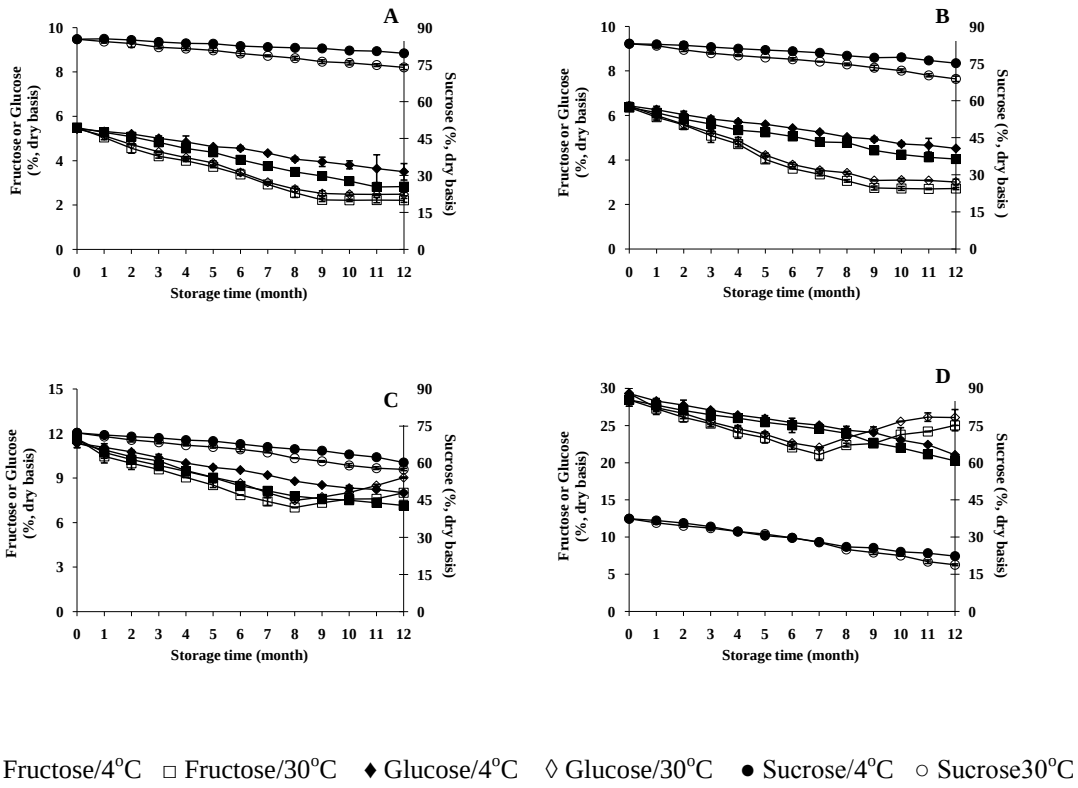


Figure 20. Changes in fructose, glucose and sucrose content during storage of palm sugar produced by palm sap after harvesting time for 6 h (A), 12 h (B), 18 h (C) and 24 h (D).

Changes in volatile flavour compound of palm sugar syrup during storage

Changes in volatile flavour compounds of palm sugar syrup produced by using palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h during storage were analysed using Headspace Soild Phase Microextraction technique (HS-SPME) coupled with Gas chromatography-mass spectrometry (GC-MS) as shown in Table 17, 18, 19 and 20, respectively. Similar profiles of volatile flavour compounds from all palm sugar syrup samples were obtained. Volatile flavour compouds were commonly found in all samples, consisting of 2 alcohols, 1 ester, 1 ketone, 1 acid, 1 pyrrole, 8 furans and 4 pyrazines.

Volatile flavour compounds from Maillard reaction and Caramelisation were found in palm sugar syrup including furans, pyrazines and pyrrole. These compounds have been reported as a typical flavour in syrup and found in various plant syrup such as maple syrup, coconut syrup and birch syrup (Kallio, 1989; Akochi *et al.*, 1994; Akochi *et al.*, 1997; Purnomo, 2007). Furan derivatives such as 2-furanmethanol, 2-furancarboxaldehyde, 3-furancarboxaldehyde, 2-acetylfuran, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2-methyl dihydro-3(2H)-furanone, 2-methyl dihydro-2(3H)-furanone and 2,3-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one were detected in all samples. These furans contribute to sweet, caramel, cook sugar or burnt sugar flavour (Ho *et al.*, 2007). High temperature during storage (30^{o}C) had more effect on the formation of furan derivatives than low temperature during storage (4^{o}C). Generally, furans are the products of Maillard reaction and they account for the caramel-like flavour of carbohydrates. The formation of furan derivative can be formed through two pathways including lipid peroxidation and degradation of carbohydrate involved Maillard reaction. In this study, furan is known to be formed by the degradation of carbohydrate. Furfural is formed from the Amadori intermediate of the Maillard reaction by 1,2-enolization followed by a deamination and dehydration and can be formed via Caramelisation (Nursten, 1980; Meynier and Mottram, 1995). This tautomerism is believed to be favoured by lower pH, while 2,3-enolization is more dependent on higher pH (Nursten, 1980). Therefore, the highest amount of furan derivatives was found in sample that produced by palm sap after harvesting time for 24 h.

Pyrazine derivatives were detected in palm sugar syrups including 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, methyl pyrazine, 2-methoxy-6-methylpyrazine and 2-ethyl-3,5-dimethylpyrazine. All these pyrazines are correlated with sensory attributes, such as nutty, sweet and roasty flavour which consequently contribute to the typical flavour of palm sugar syrup (Ho *et al.*, 2007). The presence of pyrazines in palm sugar syrup indicated that not only Caramelisation, but also the Maillard reaction took place during preparation of palm sugar syrup (Apriyantono *et al.*, 2002). Normally, alkyl pyrazine are commonly found in heated food. Meynier and

Mottram (1995) reported that an increase in pH can promote pyrazine formation in the Maillard reaction, which may be explained by decreased reactivity of the amino group at lower pH due to its protonation. This is in agreement with the decrease in pyrazine formation with increasing harvesting time of palm sap. High temperature during storage (30^{o}C) had more effect on the formation of pyrazine derivatives than low temperature during storage (4^{o}C). One of the main routes of pyrazine formation is via the Strecker degradation of amino acids in the presence of dicarbonyl compounds. In addition to the Strecker aldehyde, this reaction yields an aminocarbonyl compounds, and the condensation of two of these molecules results in an alkyl pyrazine (Amrani-Hemaimi *et al.,* 1995; Hwang *et al.,* 1995; Meynier and Mottram, 1995). One pyrrole (acetylpyrrol) was identified in palm sugar syrup. This pyrrole tends to contribute to sweet or burnt sugar flavour and it has been reported in various heated foods. There are two pathways to form pyrrole. First pathway is the interaction between an amino acid and a 3-dexoyhezosone through the Strecker degradation followed by dehydration and ring closure. The second pathway is the reaction of furans with amines or amino acids (Rizzie, 1974; Hwang *et al.,* 1995).

Phenethyl alcohol or benzene ethanol had been reported to possess a sweet or rose flavour. It is derived from L-phenylalanine through metabolic reaction of yeast (Soufleros *et al.,* 2004). Phenethyl alcohol increased with increasing storage temperature and time. The 3-hydroxy-2-butanone was reported to be responsible for sweet flavour (Cheetham, 2002). It is the main volatile flavour compounds in palm sap as reported by Taipaiboon (2004). A decrease in 3-hydroxy-2-butanone was found during storage in all samples. Acetic acid also increased with increasing storage temperature and time. There are two possible pathways for acetic acid formation including osmophilic yeast metabolism and Maillard reaction. Acetic acid may be derived from isoamyl alcohol and acetyl coenzyme A by alcohol acetyl transferase from yeast (Inoue *et al.,* 1994). In addition, the α-dicarbonyl compounds are unstable and undergo a cleavage reaction (at the C-C bond), resulting in acetic acid during Maillard reaction (Martins and Van Boekel, 2005).

Changes in microbiological properties during storage of palm sugar syrup produced by palm sap at different harvesting times

In general, microorganisms reported to be found in sugar based product are yeasts and molds. Osmophilic or sugar tolerant yeasts are a problem in syrup industry, because they can grow even at the limited level of water (Aw ranged from 0.65 to 0.80), high sucrose content and acid condition. Heat from heating process can destroy the microorganisms presented in palm sap. However, microorganisms especially osmophilic yeasts may be found to survive and grow after process due to sugar can protect their spores (Dumont *et al.*, 1993; Snowdon and Cliver, 1996; Phaichamnan, 2009). According to The Thai Industrial Standards Institute Ministry of Industry (2003) indicates that the total microbial count and the yeast and mold counts in palm sugar syrup shall not be more than 5×10^2 CFU/g and 100 CFU/g, respectively. Total microbial count and yeast and mold count of all samples were lower than the maximum limit value ruled by The Thai Industrial Standards Institute Ministry of Industry (Table 21-23). Low temperature during storage (4^oC) restricted or delayed the growth of osmophilic yeasts and, thus reduced a risk of acid formation and a product spoilage. The growth of osmophilic yeasts is another factor affecting the decrease of pH value and increase in total acidity of syrup during storage.

Table 17. Changes in volatile flavour compounds (ppb) of palm sugar syrup produced by palm sap after harvesting time for 6 h during storage under 4°C and 30°C for 12 months

RT[A]	RI[B]	Volatile flavour compounds	Attribute[C]	Storage temperature (°C)/Storage time (month)								
				0	3 months		6 months		9 months		12 months	
				month	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C
		Pyrazines										
5.81	1292	methyl pyrazine	nutty, roasty	1203	1208	1354	1202	1497	1185	1501	1164	1635
6.84	1314	2,3-dimethylpyrazine	roasted nut, sweet	1437	1432	1568	1420	1599	1381	1623	1368	1658
6.50	1309	2,5-dimethylpyrazine	sweet, roasty	975	956	1002	922	1184	901	1298	885	1347
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	5073	5114	5236	5182	5296	5221	5341	5264	5498
7.82	1470	2-ethyl-3,5-dimethylpyrazine	roasty	1484	1492	1523	1507	1558	1487	1604	1496	1701
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	2205	2208	2314	2285	2486	2256	2667	2391	2647
		Furans										
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	387	354	396	331	458	298	527	250	598
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	147	132	189	122	194	114	200	105	217
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	125	120	198	102	256	115	374	100	425
8.35	1537	2-acetylfuran	sweet, caramel	83	85	100	75	156	70	214	61	204
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	81	96	100	75	189	65	241	58	326
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	454	439	504	421	598	400	687	384	778
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	1124	1104	1258	1087	1345	1082	1459	1048	1600
11.72	2577	2,3-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one	caramel, sweet, burnt sugar	169	157	204	141	289	124	356	100	457
		Pyrrole										
10.62	2023	acetylpyrrole	sweet, burnt sugar	225	245	298	258	324	238	339	288	400
		Alcohols										
10.36	1951	phenethyl alcohol	flora	121	111	13	98	158	134	178	139	201
8.81	1587	2,3-butanediol	fruity	1067	1089	1147	1097	1258	1125	1347	1185	1407
		Acid										
7.84	1473	acetic acid	sour	13366	13569	14789	13998	15987	14258	17002	14987	19547
		Ketone										
6.00	1307	3-hydroxy-2-butanone	sweet	2256	2224	2258	2200	2274	2158	2358	2104	2389
		Ester										
1.86	997	ethyl acetate	fruity, sweet	8551	8500	8475	8487	8400	8457	8357	8400	8304

Note: [A] RT refers to retention time (min). [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24}).

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

Table 18. Changes in volatile flavour compounds (ppb) of palm sugar syrup produced by palm sap after harvesting time for 12 h during storage under 4°C and 30°C for 12 months

RT[A]	RI[B]	Volatile flavour compounds	Attribute[C]	Storage temperature (°C)/Storage time (month)							
				0 month	3 months		6 months		9 months		12 months	
					4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C
		Pyrazines										
5.81	1292	Methyl pyrazine	nutty, roasty	644	657	687	637	748	627	847	600	899
6.84	1314	2,3-dimethylpyrazine	roasted nut, sweet	995	975	1025	947	1189	927	1287	887	1369
6.50	1309	2,5-dimethylpyrazine	sweet, roasty	884	856	912	875	936	841	998	800	1005
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	3657	3625	3698	3611	3874	3587	3784	3566	3996
7.82	1470	2-ethyl-3,5-dimethylpyrazine	roasty	1044	1002	1125	987	1254	956	1369	944	1440
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	1501	1489	1589	1456	1621	124	1785	1204	1897
		Furans										
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	508	489	548	471	602	447	689	402	784
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	234	224	296	204	341	185	411	154	487
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	332	321	397	300	456	266	587	244	661
8.35	1537	2-acetylfuran	sweet, caramel	112	100	159	86	187	88	204	68	300
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	98	84	152	77	127	87	205	66	298
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	728	700	789	675	854	645	952	603	1002
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	2534	2501	2596	2475	2687	2423	2774	2369	2896
11.72	2577	2,3-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one	caramel, sweet, burnt sugar	357	325	425	300	418	284	487	257	523
		Pyrrole										
10.62	2023	Acetylpyrrole	sweet, burnt sugar	220.53	211	256	189	304	167	389	144	447
		Alcohols										
10.36	1951	Phenethyl alcohol	flora	172.57	178	189	196	217	200	228	215	269
8.81	1587	2,3-butanediol	fruity	2393	2347	2441	2456	2499	2489	2554	2541	2966
		Acid										
7.84	1473	Acetic acid	sour	23569	24478	25631	25876	26471	26874	27846	26991	28996
		Ketone										
6.00	1307	3-hydroxy-2-butanone	sweet	2425	2400	2584	2347	2574	2345	2411	2301	2311
		Ester										
1.86	997	Ethyl acetate	fruity, sweet	8084	8076	7956	8002	7968	7984	7844	7900	7800

Note: [A] RT refers to retention time (min). [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24}).

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

Table 19. Changes in volatile flavour compounds (ppb) of palm sugar syrup produced by palm sap after harvesting time for 18 h during storage under 4ºC and 30ºC for 12 months

RT[A]	RI[B]	Volatile flavour compounds	Attribute[C]	Storage temperature (ºC)/Storage time (month)								
				0 month	3 months		6 months		9 months		12 months	
					4ºC	30ºC	4ºC	30ºC	4ºC	30ºC	4ºC	30ºC
		Pyrazines										
5.81	1292	Methyl pyrazine	nutty, roasty	210	200	198	185	156	165	144	144	100
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	1624	1600	1587	1587	1504	1546	1475	1402	1400
7.82	1470	2-ethyl-3,5-dimethylpyrazine	roasty	81	75	78	81	60	65	55	60	50
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	429	422	400	418	385	397	367	342	357
		Furans										
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	798	745	806	700	818	678	824	657	839
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	431	415	521	385	611	357	687	300	721
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	1225	1214	1269	1200	1347	1185	1478	1145	1552
8.35	1537	2-acetylfuran	sweet, caramel	157	142	187	122	256	102	347	97	411
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	102	91	156	75	213	66	296	51	314
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	953	941	996	917	1024	856	1125	811	1247
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	2011	1997	2148	1985	2254	1902	2317	1845	2456
11.72	2577	2,3-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one	caramel, sweet, burnt sugar	441	423	513	401	648	385	785	378	821
		Pyrrole										
10.62	2023	Acetylpyrrole	sweet, burnt sugar	208	225	285	247	315	259	400	300	511
		Alcohols										
10.36	1951	Phenethyl alcohol	flora	212	223	257	239	304	256	328	301	398
8.81	1587	2,3-butanediol	fruity	4359	4387	4487	4412	4587	4571	4687	4612	4758
		Acid										
7.84	1473	Acetic acid	sour	34885	35214	36987	35987	38542	36974	39745	37421	40125
		Ketone										
6.00	1307	3-hydroxy-2-butanone	sweet	2151	2015	2089	1997	2004	1904	2063	1875	1987
		Ester										
1.86	997	Ethyl acetate	fruity, sweet	7531	7500	7485	7463	7406	7413	7364	7395	7218

Note: [A] RT refers to retention time (min). [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24}).

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

Table 20. Changes in volatile flavour compounds (ppb) of palm sugar syrup produced by palm sap after harvesting time for 24 h during storage under 4ºC and 30ºC for 12 months

RT[A]	RI[B]	Volatile flavour compounds	Attributes[C]	Storage temperature (ºC)/Storage time (month)								
				0 month	3 months		6 months		9 months		12 months	
					4ºC	30ºC	4ºC	30ºC	4ºC	30ºC	4ºC	30ºC
		Pyrazines										
5.81	1292	Methyl pyrazine	nutty, roasty	163	143	133	133	127	105	94	95	85
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	457	450	436	445	418	435	387	415	356
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	148	142	130	130	120	102	90	91	80
		Furans										
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	1094	1085	1128	1062	1287	1038	1396	1010	1569
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	503	496	586	482	648	468	857	441	958
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	7513	7489	7596	7456	7698	7412	7825	7314	7941
8.35	1537	2-acetylfuran	sweet, caramel	402	385	489	367	574	325	624	307	789
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	124	112	189	98	256	75	357	55	411
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	1015	1000	1125	985	1239	975	1387	958	1496
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	2583	2514	2679	2475	2814	2401	2963	2347	3001
11.72	2577	2,3-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one	caramel, sweet, burnt sugar	635	614	698	600	752	587	841	562	947
		Pyrrole										
10.62	2023	Acetylpyrrole	sweet, burnt sugar	124	146	187	158	243	196	315	215	323
		Alcohols										
10.36	1951	Phenethyl alcohol	flora	662	684	701	702	745	756	784	789	801
8.81	1587	2,3-butanediol	fruity	5135	5187	5214	5214	5245	5278	5374	5299	5400
		Acid										
7.84	1473	Acetic acid	sour	51415	52103	53461	52569	54774	53476	55876	54002	56897
		Ketone										
6.00	1307	3-hydroxy-2-butanone	sweet	2084	2005	1997	1987	1875	1923	1756	1892	1744
		Ester										
1.86	997	Ethyl acetate	fruity, sweet	5105	5091	4974	5045	4756	4956	4563	4875	4471

Note: [A] RT refers to retention time (min). [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24}).

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

Table 21. Changes in total microbial count (cfu/g) of palm sugar syrup produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h during storage under 4°C and 30°C for 12 months

Storage time (month)	Collection time of palm sap/Storage temperature (°C)							
	6 h		12 h		18 h		24 h	
	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C
0	<10	<10	<10	<10	<10	<10	<10	<10
6	<10	<10	<10	<10	<10	<10	<10	<10
12	<10	<10	<10	<10	<10	<10	<10	<10

Table 22. Changes in yeast and mold count (cfu/g) of palm sugar syrup produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h during storage under 4°C and 30°C for 12 months

Storage time (month)	Collection time of palm sap/Storage temperature (°C)							
	6h		12h		18h		24h	
	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C
0	<10	<10	<10	<10	<10	<10	<10	<10
6	<10	<10	<10	<10	<10	<10	<10	<10
12	<10	<10	<10	<10	<10	<10	<10	<10

Table 23. Changes in osmophilic yeast (cfu/g) of palm sugar syrup produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h during storage under 4°C and 30°C for 12 months

Storage time (month)	Collection time of palm sap/Storage temperature (°C)							
	6h		12h		18h		24h	
	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C
0	<10	<10	<10	<10	<10	<10	<10	<10
6	30	40	30	40	40	50	50	70
12	40	70	40	70	50	80	70	90

3.5 Conclusion

The properties of raw material influenced the browning development of palm sugar syrup during processing and storage. Harvesting time showed a pronounced effect on the properties of palm sap especially, pH, total acidity and reducing sugar content. The pH decreased while total acidity and reducing sugar content increased during harvesting time due to the activity of microorganisms. The browning development of palm sugar syrup during heating greatly took place in a sample that produced by palm sap after harvesting time for 6 h (short harvesting time). However, the concentration of reactant (reducing sugar) that increased with increasing harvesting time presented a dominate effect when pH of palm sap decrease to 4.07 (at harvesting time for 24 h). HMF content was found higher than 40 mg/kg in palm sugar syrup that produced by palm sap after harvesting time for 24 h. This indicated that palm sap after harvesting time for 24 h was improper to produce palm sugar syrup. Thus, suitable harvesting time of palm sap for the production of palm sugar syrup was not longer than 18 h of collection. Moreover, heating time also influenced the dark colour formation due to nonenzymatic browning reactions as indicated by the increase in IBP, BI and HMF content in all samples during heating. During the production of palm sugar syrup, the Maillard reaction has higher effect on the brown colour formation than the Caramelisation.

Temperature and time during storage showed a pronounced effect on the properties changes of palm sugar syrup. Low temperature during storage (4^{o}C) can retard the browning development of palm sugar syrup. In addition, longer time during storage will accelerate the nonenzymatic browning reactions. The Maillard reaction is responsible for the brown colour formation during storage of palm sugar syrup.

CHAPTER 4

EFFECT OF PROCRSSING METHOD AND STORAGE CONDITION ON THE PROPERTIES CHANGES OF PALM SUGAR SYRUP

4.1 Abstract

The aim of this study was to investigate the changes in physical, chemical properties and antioxidant activity during the production of palm sugar syrup by an open pan (110^{o}C) and a vacuum evaporator (70^{o}C and 80^{o}C) and the effect of storage temperatures (4^{o}C and 30^{o}C) and storage times on the properties changes of palm sugar syrup produced by an open pan and a vacuum evaporator. During the production, samples were collected at 15 min (for an open pan) and 10 min (for a vacuum evaporator) intervals until TSS reached 70^{o}Brix. Each sample was determined for physical, chemical properties and antioxidant activity. The properties of each sample during heating process were significantly different ($P<0.05$). The results showed that L*, transmittance value, moisture content and free amino group content decreased with heating time in each heating process. On the other hand, a*, b*, IBP, BI, HMF content, TSS, and reducing sugar as well as glucose and fructose increased with heating time in each heating process. The increase in IBP, BI and HMF with heating time was concomitant with the increase in DPPH radical scavenging activity, FRAP and reducing power in each heating process. Among all heating processes, palm sugar syrup that produced by an open pan rendered the highest browning development and antioxidant activity. Hence, heating temperature also showed a pronounced effect on the browning development and antioxidant activity during the production of palm sugar syrup. During storage, temperature and time had a significant effect ($P<0.05$) on the properties of palm sugar syrup. Low temperature (4^{o}C) during storage can be retarded the Maillard reaction. This was shown by lower a* value, IBP, BI and HMF content, and higher L* value, fructose, glucose and free amino group

content during storage for 12 months ($P<0.05$). In addition, an increase in IBP, BI, HMF content and a decrease in L*, free amino group content were found with increasing storage time in all samples that stored under either $4^{o}C$ or $30^{o}C$ ($P<0.05$). HMF, a possible mutagen formed by nonenzymatic browning reactions during the heating and storage of sugar based products was monitored. Only the sample that produced by an open pan and stored under $30^{o}C$ contained HMF content (50.58 mg/kg) higher than the permitted maximum limit (40 mg/kg as recommend by the Codex Alimentarious). While, other samples still contained HMF in agreement with this standard.

4.2 Introduction

Palmyra palm (*Borassus flabellifer* Linn.) can be found in tropical countries such as Thailand, Malaysia, Indonesia, India, Myanmar, Sri Lanka and Cambodia. In Thailand, palmyra palms are crowded in southern part of Thailand from Phetchaburi to Songkhla provinces. The most important product of palmyra palm is the sap or juice. Palm sap represents a solution in which sucrose is the major component. However, other compounds like organic acids, minerals and phenolic compounds have been reported (Lasekan *et al.,* 2007; Naknean *et al.,* 2010). Phenolic compounds are widely distributed in plants and contributed to the sensory properties associated with food quality such as colour and flavour. These compounds may have a potential health benefits, including reduction of cancer risk (Macheix *et al.,* 1990).

Deslauriers (2000) and Cote (2003) reported that phenolic compounds in plant sap such as maple sap are consisted of free phenolic and bound phenolic. In general, phenolic compounds in plants can be found in combined forms (with sugar), as they are aroma precursors that can be released during processing by heating or acid (Amiot *et al.,* 1997). Phenolic compounds belong to two different classes, hydroxybenzoic acids (HBA) and hydroxycinamic acids (HCA) which are derived from two nonphenolic molecules, benzoic and cinamic acids, respectively (Macheix and Fleuriet, 1998). Deslauriers (200) and Cote (2003) reported that phenolic compounds such as HBA derivatives, HCA

derivatives and flavonoid derivatives were found in maple sap. HBA and HCA can occur as a free form after hydrolysis but frequently they are presents as derivatives such as glycoside (glycosylated phenolic compound) (Macheix *et al.,* 1990). Glycosides are carbohydrate acetals in which the hemiacetal or the reducing group of a sugar (the glycone) link through a hydroxyl group of nonsugar component such as flavour and phenolic (aglycone) (Whistler and James, 1996). Glycosidic links are stable under ambient condition but they are readily hydrolysed in heat condition, acid condition or in the presence of appropriate hydrolytic enzyme (Buttery *et al.,* 1990; Salles *et al.,* 1991). Hydrolysis of glycosides leads to the liberation of aglycone (Williams, 1982; Gunata *et al.,* 1985).

Since palm sap is rich in sugars (10-17%) and, unless it is collected under hygienic conditions, rapidly fermentation and conversion reactions to acids and alcohols occur (Iwuoha and Eke, 1996; Lasekan *et al.,* 2007). To prevent fermentation, Kiam wood (*Cotylelobium lanceotatum* carih.) is commonly added because it has phenolic compounds and it can delay the spoilage in palm sap by reducing microbial populations as well as keeping the quality of a product (Department of Agricultural extension Thailand, 2001; Chanthachum and Beuchat, 1997). Balange and Benjakul (2009) reported that the total phenolic content in Kiam wood (intact form) as extracted by water contained tannin 29.33 mg/g of dry Kiam wood.

Normally, palm sap is a raw material to produce palm sugar syrup. Palm sugar syrup is obtained by heating fresh palm sap until it is concentrated. Traditionally, palm sugar syrup is produced by evaporating palm sap in an open pan, and heated, using a wood fired stove, until it becomes concentrated (Phaichumnan *et al.,* 2010). However, a traditional production uses high temperature (approximately 110-120^{o}C) for a long time. It has been reported that many reactions can take place during thermal processing that affect on the quality of a product, especially colour, flavour and nutritional values. Among them, the most common reactions that influence on the quality of syrup are inversion reaction, Maillard reaction and Caramelisation (Akochi-K *et al.,* 1997; Apriyantono *et al.,* 2002; Ho *et al.,* 2007; Phaichamnan, 2010). To reduce thermal

degradation during evaporation, it is necessary to minimize heating temperature and time. An alternative way, vacuum evaporation under low temperature, can be used to reduce the dark colour formation during heating (Potter and Hotchkiss, 1995; Heldman and Hartel, 1998).

As mentioned previously, nonenzymatic browning reaction including Maillard reaction and Caramelisation took place during heating process of palm sugar syrup. Maillard reaction products (MRPs) and Caramelisation products (CPs) have been found to exhibit antioxidative activity due to radical scavenging activity (Marales and Jimenez-Perez, 2001; Yen and Hsieh, 1995), reducing power (Matmaroh *et al.*, 2006).

Since quality is supremely important in food, thermal deterioration has to be controlled during processing and storage. Nonenzymatic browning reactions including Maillard reaction and Caramelisation may cause unacceptable nutritional and sensory effects in sugar based food products and may be a limiting factor in the shelf life of products. However, fundamental studies on the effect of processing method and storage condition on the properties changes during the production and storage of palm sugar syrup and antioxidant activity of palm sap and its changes during the production are lacking. Thus, the aim of this study was to monitor the physical, chemical properties and antioxidant activity changes during the production of palm sugar syrup by an open pan and a vacuum evaporator. Additionally, the effect of storage temperature (4^{o}C and 30^{o}C) and storage time (0-12 months) on the properties changes of palm sugar syrup produced by an open pan and a vacuum evaporator was also investigated.

4.3 Materials and Methods

Chemicals

D-glucose, D-fructose, sucrose were purchased from Fluka (Messerchmittstr, Switzerland). Acetonitrile, methanol, acetic acid, hydrochloric acid and water (HPLC grade) were obtained from Prolabo (Paris, France). Sodium hydroxide,

copper sulfate pentahydrate, trichloroacetic acid, potassium ferricyanide, sodium acetate trihydrate, ferric chloride hexahydrate and sodium sulfite were obtained from Merck (Darmstadt, Germany). Potassium sodium tartrate, lead acetate and potassium oxalate were obtained from Riedel-deHaen (Seelze, Germany). 2,4,6-Trinitrobenzenesulfonic acid (TNBS), L-leucine, hydroxymethylfurfural, thiobarbituric acid, 2,2-diphenyl-2-picryl-hydrazil (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-Tripyridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich (St.Louis. MO, USA).

Raw material

Palm sap was collected from contact farm, a farmer in Songkhla province. Two treatments were done including palm sap with added Kiam wood (3 g) and harvested after 12 h of collection and palm sap without added Kiam wood and harvested after 2 h of collection. After collection, palm sap was kept in an icebox (4^{o}C) during transportation (30 min) to the department of Food Technology, Prince of Songkla University, Hat Yai Campus. The sample was filtrated by sheet cloth at room temperature and stored at 4^{o}C. The chemical and antioxidant activity were determined within a day of collection.

Production of palm sugar syrup

Palm sap added Kiam wood after 12 h of collection was taken from contact farm, a farmer in Songkhla province. Two methods were used to concentrate palm sap either by an open pan or a vacuum evaporator. Palm sap (15 liters) was concentrated by an open pan (at approximately 110^{o}C) and a vacuum evaporator (at 70^{o} and 80^{o}C) until its total soluble solid reached 70^{o}Brix to obtain palm sugar syrup. During the heating process, samples were collected at 15 min (for an open pan) and 10 min (for

vacuum evaporator) intervals until the end of a process. Immediately after production, the physical, chemical properties and antioxidant activity of each sample were determined. In addition, each palm sugar syrup sample was stored under 4^{o}C and 30^{o}C in a closed plastic cup for 12 months. The physical and chemical properties of each sample were determined at one month interval. Microbial loads were analysed at six months interval.

Measurement of colour

The colour measurements of the samples were carried out using a Hunter Lab Colourflex colourimeter. A colourimeter was adjusted for reflectance, illuminant D 65, and angle of 10^{o}. Instrumental colour data was provided in accord with the CIE system in terms of L* (lightness), a* (redness and greenness) and b* (yellowness and blueness).

Measurement of clarity

The clarity of samples was estimated by measuring the transmittance at 650 nm using a spectrophotometer as describe by Taipaiboon (2004) and expressed in term of percentage.

Measurement of intermediate browning product and browning intensity

Intermediate browning product (IBP) and browning intensity (BI) were determined by monitoring the absorbance at 280 and 420 nm, respectively. The absorbance was measured by using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan), as described by Takano (2005) and Kawai *et al.* (2005). Appropriate dilution (8-fold for IBP and 4-fold for BI) was made using distilled water to obtain reliable absorbance readings.

Determination of pH

The pH value was measured at ambient temperature with a pH meter (Satorious, USA) which calibrated at pH 4.0 and 7.0.

Determination of total acidity

The sample was diluted with distilled water and titrated with 0.01 N NaOH using a few drops of 1% phenolphthalein solution as an indicator. The sample was transferred as a measured quantity (1-3 g) into approximately 10 ml of distilled water. The result was calculated as a percentage of lactic acid (Rangana, 1986).

Determination of total soluble solid

The total soluble solid (TSS) content of palm sap and palm sugar syrup was determined as degree Brix using a hand refractometer.

Determination of moisture content

The moisture content was measured using a vacuum oven. About 2-5 g of the sample was placed in a pre-dried aluminum dish and dried in a vacuum oven at 60^{o}C (pressure<70mmHg) for 6 h. The dried sample was placed in a desiccator and cooled for 0.5 h to room temperature. The weight was recorded, and the percentage moisture based on the initial wet weight was calculated.

Determination of water activity

Water activity was measured at room temperature using a water activity meter (Novasina, Thermostanter). The sample was inserted into a sample cup and another

water activity measurement was made immediately to restrict moisture transfer from the air to the samples.

Determination of total sugar and reducing sugar

The total sugar and reducing sugar content were quantified by the Lane and Eynon Volumetric method using titration with Fehling's reagents. The results were expressed as grams of glucose per 100 g of sample (Rangana, 1986).

Determination of type and concentration of sugars

The type and concentration of sugar was determined using HPLC (Shimadzu, CR6A Chromatopac) with a Hypersil NH_2 column and refractive index detector. The mobile phase was the solution of acetonitrile and water (80:20), pumped at a flow rate of 1.5 ml/min and an injection volume of 20 µl. The samples were prepared by making appropriate dilutions with distilled water. All sample solutions were passed through a 0.45 µm nylon syringe filter to remove particulates prior to HPLC analysis. D-glucose, D-fructose and sucrose were used as the external standards. The calibration curve of each sugar was plotted between peak areas and concentrations (Stuckel and Low, 1996).

Determination of free amino group content

Free amino group content was determined according to the method of Benjakul and Morrissey (1997). First, the sample was diluted through appropriate dilution. Then 125 µl of the sample was mixed with 2.0 ml of 0.21 M phosphate buffer, pH 8.2, and 1.0 ml of 0.01% TNBS solution was then added. The solutions were mixed thoroughly and placed in a temperature-controlled water bath (Memmert, Bavaris, Germany) at 50^{o}C for 30 min in dark. The reaction was terminated by adding 2.0 ml of

0.1 M sodium sulfite. The mixtures were cooled at room temperature for 15 min. The blank was prepared in the same manner as the samples except that distilled water was used instead of 0.01% TNBS. The absorbance was measured at 420 nm. The free amino group content was expressed in terms of L-leucine.

Determination of 5-hydroxymethylfurfural content

Palm sugar syrup (5-10 g) was dissolved in deionized water and made up to 50 ml with deionized water. After that it was centrifuged at 5,000 rpm for 15 min. The supernatant was used to measure 5-hydroxymethylfurfural (HMF) content. To determine the HMF content, 2 ml of supernatant was introduced into the tube. Two ml of 12% trichloroacetic acid and 2 ml of 0.025 M thiobabituric acid were subsequently added and mixed thoroughly. The tube with the sample was then placed in a water bath at 40^{o}C. After incubating for 50 min, the tube was cooled immediately using water and the absorbance was measured at 443 nm. A calibration curve of HMF was utilized to quantify the HMF concentration (Rattanathanalerk *et al.*, 2005).

Determination of volatile flavour compounds

Volatile flavour compounds were analyzed using HS-SPME-GC-MS technique. A 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber was used (Supelco, Bellefonte, PA, USA). Each sample (2 ml) was added in a 12 ml headspace vial and then an internal standard, 2-methyl-3-heptanone was spiked into the sample. After that, the vial was sealed tightly with a crimp cap and a PTFE/silicone septum and equilibrated at 50^{o}C for 30 min in a water bath. A manual SPME holder containing fiber was inserted into a headspace vial and exposed to the sample headspace at 50^{o}C for 15 min. The fiber then was transferred directly into the injector port of the GC-MS system. Thermal desorption of analytes from the fiber in the GC injector port was carried out with an SPME inlet liner (0.75 mm i.d., Supelco) in the

splitless mode at a desorption temperature of 240^{o}C. The SPME fiber was conditioned at 250^{o}C for 10 min before starting the first measurement and left in the injection port for re-conditioning during the whole GC run before taking the next sample. GC-MS analysis was conducted using the Agilent 6890 Plus GC/HP 5973 MSD, equipped with an HP-FFAP column (25 m×0.32 mm i.d.×25 µm film thickness). The injector temperature was 240^{o}C. The GC oven temperature was programmed from 40 to 230^{o}C at the rate of 8^{o}C/min and hold at 230^{o}C for 10 min. The carrier gas was ultra high purity helium at a constant flow of 1.5 ml/min. Mass spectrometer condition was as follows: MSD capillary direct-interface temperature was 280^{o}C. Ionization energy was 70 eV. Mass range was between 20-450 a.m.u. Positive identification of a component was performed by comparison of mass spectrum. Tentatively identified compounds were uniquely identified in the basis of the mass spectra from the Wiley 275.L mass spectra database (Hewlett-Packard Co.). The integration of peaks was done on HP chemstation software (Hewlett-Packard Co.). The minimum peak area for detection is 10,000 counts.

In this study, given concentration values were noted as equivalents to the internal standard. The relative concentrations of the investigated compounds (IC) were calculated by relating the areas of the internal standard (IS) to the areas of the compounds of interest.

$$\text{Relative concentration} = \frac{\text{(Peak area of IC)}}{\text{(Peak area of IS)}} \times \text{Concentration of IS}$$

Determination of DPPH radical scavenging activity

DPPH radical-scavenging activity was determined by DPPH assay, as described by Binsan *et al.* (2008) with a slight modification. Sample (1.5 ml) was added to 1.5 ml of 0.15 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 60 min. The absorbance of the resulting solution was measured at 517 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The blank was prepared in the same manner, except that distilled water was used instead of the sample. A standard curve was

prepared using Trolox in the range of 10–60 μM. The activity was expressed as μmol Trolox equivalents (TE)/g sample.

Determination of ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) was assayed according to the method of Benzie and Strain (1996). Stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$. A working solution was prepared freshly by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3.6H_2O$ solution. The mixed solution was incubated at 37^oC for 30 min and was referred to as FRAP solution. A sample (150 μl) was mixed with 2850 μl of FRAP solution and kept for 30 min in the dark. The ferrous tripyridyltriazine complex (coloured product) was measured by reading the absorbance at 593 nm. The standard curve was prepared using Trolox ranging from 50 to 600 μM. The activity was expressed as μmol TE/g sample.

Determination of reducing power

Reducing power of a sample was measured as described by Matmaroh *et al.* (2006). 0.5 ml of each sample (appropriate dilution) was mixed with 0.5 ml of 0.2 M sodium phosphate buffer, pH 6.6 and 0.5 ml of potassium ferricyanide. The mixture was incubated at 50^oC for 20 min and 0.5 ml of 10% (w/v) TCA was then added. Thereafter, 1 ml of distilled water and 200 μl of 0.1% (w/v) ferric chloride were added to the mixture. The absorbance was measured at 700 nm. Any increase in absorbance at 700 nm indicated an increased reducing power.

Determination of microbial load

Microbial loads of palm sugar syrup including total microbial count, yeast and mold count and osmophilic yeast were analysed at 6 months interval. At the specified time intervals, samples were aseptically taken and serially diluted in 0.1 g/100 g peptone water for microbial counts. Pour plating on Plate Count Agar (Merck KGaA, Darmstadt, Germany) was performed for the total microbial count, overlaid with the same medium, and the plates incubated at 35-37^{o}C for 1-2 days. Spread plating on Potato Dextrose Agar acidified with 10 g/100 g tartaric acid (Merck KGaA, Darmstadt, Germany) was performed for yeast and mold count and the plates incubated at 20-25^{o}C for 5 days. The osmophilic yeast count was also analysed using the spread plate technique on osmophilic potato dextrose agar and the plates were incubated at 37^{o}C for 3 days. The results are means of measurements in triplicate (Kiss, 1984).

Statistical analysis

All analysis and measurements were performed in triplicates. Data was subjected to analysis of variance (ANOVA). During the production, the effect of heating time on the properties changes during each processing method was determined using a completely randomized design (CRD). During storage, the effect of storage temperatures (4^{o}C and 30^{o}C) on a given data within each processing method was determined using paired test of the difference. The effect of storage times (0-12 months) on a given data within each processing method and storage temperature was determined using CRD. Comparison of means was carried out by Duncan's multiple-range test (Steel and Torrie, 1980). Analysis was performed using a SPSS package (SPSS 6.0 for windows, SPSS Inc, Chicago, IL).

4.4 Results and Discussion

Changes in physical properties during the production of palm sugar syrup produced by an open pan and a vacuum evaporator

Changes in colour values (L*, a* and b*) during the production

Figures 21 and 22 show the changes in L*, a* and b* values during the production of palm sugar syrup by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C. Appendix Figure 4 shows the samples that were taken during heating intervals by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C. During heating time in each method, a significant decrease in L* and increase in a* and b* values were found ($P<0.05$). The L* values decreased with heating times in all processing methods which reduced from 76.33 to 60.00, 58.64 and 57.78 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. Since the L* value is a measurement of the colour in the light-dark axis, this falling value indicates that samples were turning darker. Similar results were obtained by various investigators and they have been reported that decreases in L* values correlated well with increases in the browning of food products (Ibarz *et al.,* 1999; Rattanathanakerk *et al.,* 2005; Damasceno *et al.,* 2008; Rao *et al.,* 2009).

The a* values increased during the heating process in all palm sugar syrup samples. Initially a* value of palm sugar syrup was 2.70. It was also observed that the finished samples were 24.35, 9.96 and 13.88 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. An increase in b* values was also observed during heating process either palm sugar syrup that produced by an open pan or vacuum evaporator. The b* value was changed from 16.91 to 86.14, 49.19 and 56.95 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. There were slowly changes in the a* and b* values in the first 120, 20 and 10 min of heating for palm sugar syrup that produced by an

open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. Further heating, the a* and b* values continued to change, and the colour of each sample changed towards orange-yellow, indicating the onset of Caramelisation and Maillard reaction. When the total soluble solids content reached approximately 40^{o}Brix (at 135 min for an open pan process, 30 min for a vacuum evaporator at 70^{o}C and 20 min for a vacuum evaporator at 80^{o}C), there were rapidly changes in a* and b* values, confirming the Caramelisation and Maillard reaction took place (Rao *et al.*, 2009). Moreover, there was a higher change in a* and b* values for syrup that produced by an open pan than the syrup which was produced by a vacuum evaporator. This is probably due to palm sugar syrup that produced by an open pan using higher heating temperature and longer heating time than syrup produced by a vacuum evaporator. Generally, the chemical reaction rate increases with increasing temperature and time (Martins *et al.*, 2001). The decrease of L* values and increase a* values may contribute to the nonenzymatic browning reactions during heating process.

Changes in clarity during the production

Clarity was determined by measuring the percentage of light transmittance at 650 nm. This value can be used to evaluate the clarity in fruit juice. High transmittance value shows the juice more clarified than low transmittance value. Changes in transmittance values of palm sugar syrup were presented in Figure 21. The transmittance values of all palm sugar syrup samples decreased with heating times ($P<0.05$). The transmittance values decreased from 60.34% to 50.39%, 44.42% and 47.16% for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. The decrease of transmittance value may due to the concentration effect. The undissolved particles were concentrated during heating process, caused decreasing of transmittance value. Moreover, the clarity of palm sugar syrup depended greatly on its protein content and the polyphenolic compounds, which was contaminated from Kiam wood (*Cotylelobium lanceotatum* craih.) during the collecting process of the palm sap.

The interaction of protein and polyphenol can form insoluble complexes and could grow to a large colloid size or haze (Kermasha *et al.*, 1995; Siebert *et al.*, 1996; Paichamnan *et al.*, 2010). Moreover, the colloid particles from brown pigment that were concentrated during heating are responsible for the decrease in clarity of palm sugar syrup (Takano, 2005).

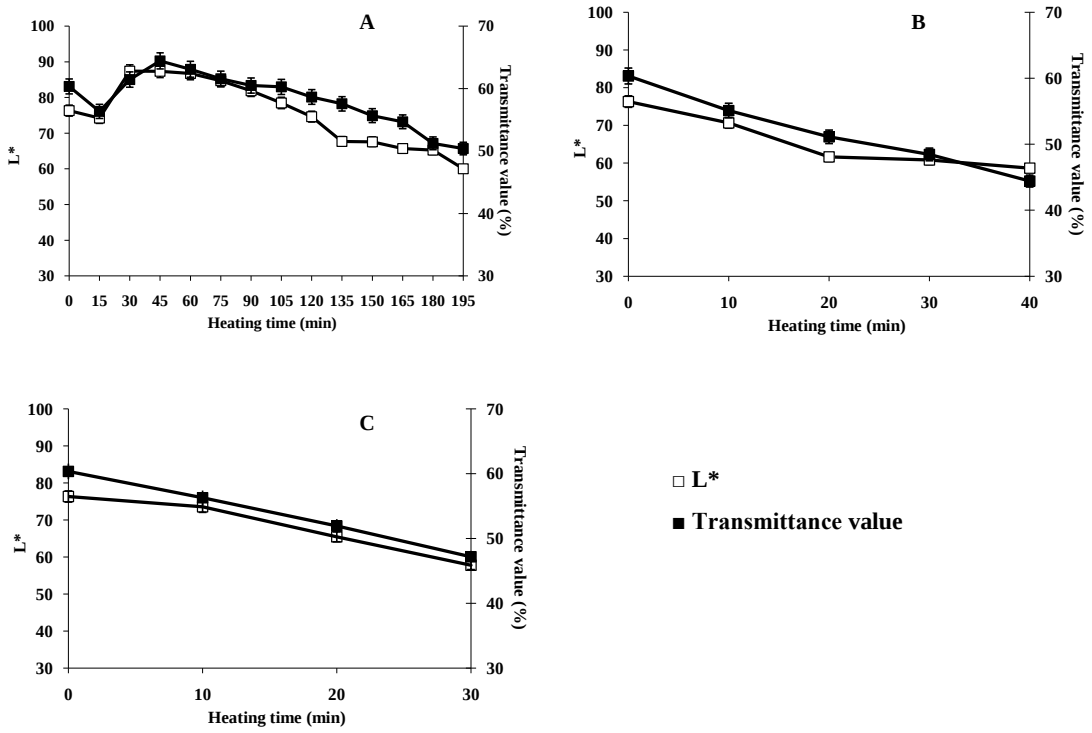


Figure 21. Changes in L* and transmittance values during the production of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70^{o}C (B) and 80^{o}C (C)

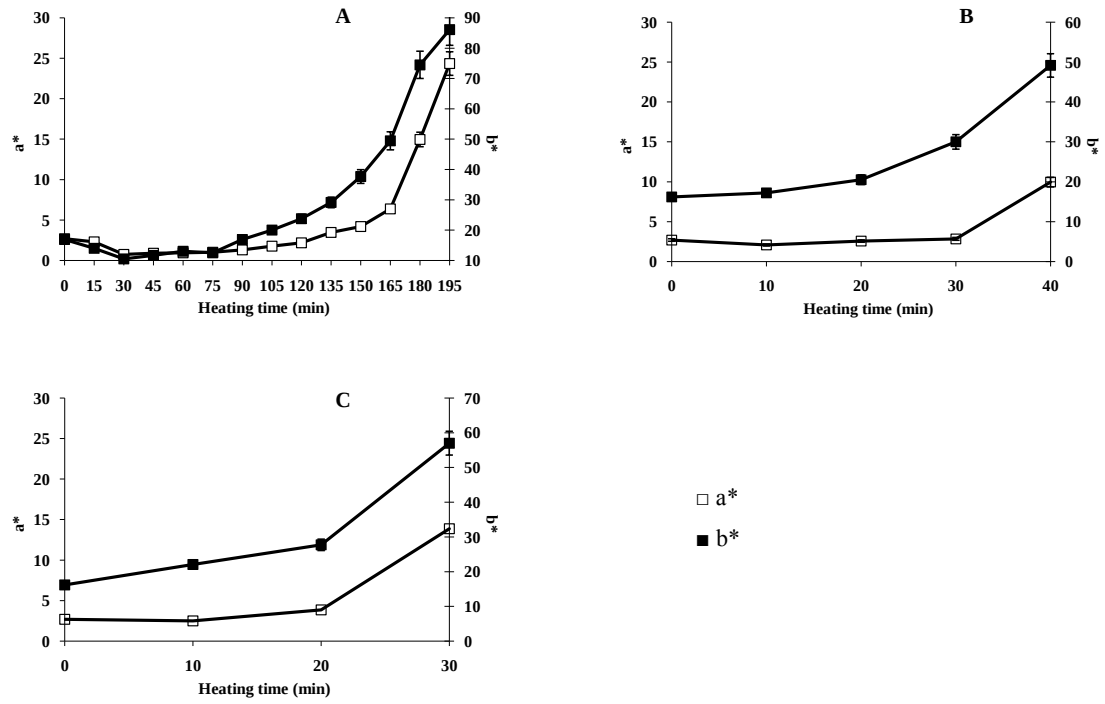


Figure 22. Changes in a* and b* values during the production of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70°C (B) and 80°C (C)

Changes in intermediate browning product and browning intensity during the production

The browning intensity is one of the parameter that indicates the nonenzymatic browning. Browning development was measured as changes in absorbance values of palm sugar syrup at 280 nm for intermediate browning product (IBP) and 420 nm for browning intensity (BI) as a function of heating times. Maillard reaction development is generally monitored by the increase in the absorbance value either at 280 (early MRPs), which is considered to indicate the formation of furfural compounds (presumably HMF) (Maio and Roos, 2006; Flink, 1983) and is used to detect products in

early stage of browning (Wijewikreme *et al.,* 1997; Billuad *et al.,* 2004). Moreover, the degree of browning, usually measured via absorbance value at 420 nm, is often used to follow the extent of Maillard reaction. This absorbance can be used to monitor the final stage of browning reaction. The browning development of Caramelization products from sugar also monitored by increasing in absorbance at 420 nm (Phongkanpai *et al.*, 2006) as same as Maillard reaction. Changes in IBP and BI in palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C were shown in Figure 23A, 23B and 23C, respectively. The IBP and BI of all palm sugar syrup samples increased by increasing heating times ($P<0.05$). The browning development either IBP or BI of palm sugar syrup that produced by an open pan slightly increased within the first 90 min ($P\geq0.05$). After that, browning development increased sharply with increasing time till 195 min ($P<0.05$). The browning development of palm sugar syrup that produced by a vacuum evaporator was changed in the same manner with sample that produced by an open pan but the IBP and BI of palm sugar syrup that produced by a vacuum evaporator increased rapidly after 20 min and 10 min for a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. The highest values of IBP and BI were observed in palm sugar syrup that produced by an open pan (0.40 for IBP and 0.45 for BI) followed by palm sugar syrup that produced by a vacuum evaporator at 80^{o}C (0.28 for IBP and 0.31 for BI) and palm sugar syrup that produced by a vacuum evaporator at 70^{o}C (0.21 for IBP and 0.19 for BI). These results indicated that during heating process the nonenzymatic browning reactions including Maillard reaction and Caramelisation took place. Maillard reaction occurs between reducing sugars and amino acids or protein (Sapers, 1993; Danehy, 1986). Palm sap contains abundant sucrose and polar side chain amino acids especially aspargine and glutamine that can react via Maillard reaction during heating process (Ho *et al.*, 2007). Sucrose can be hydrolysed during heating to obtain reducing sugars including glucose and fructose. Reducing sugars act as a substrate of Maillard reactions. Reactive intermediates are formed by a variety of pathways yielding brown nitrogenous compounds of higher molecular weight called melanoidin pigments (Akochi-K *et al.,* 1997). Moreover, nonenzymatic browning reactions greatly occur when temperature

increases. The increase in IBP and BI is corresponding to the decrease in L* value and increase in a* and b*value as mentioned previously. During the production of palm sugar syrup, the Maillard reaction had higher effect on the browning development than the Caramelisation. This probably due to Caramelisation is promoted under temperature approximately 120^{o}C or above (Kroh, 1994; Eskin, 1990). On the other hand, the production of palm sugar syrup by an open pan (110^{o}C) and a vacuum evaporator (70^{o}C and 80^{o}C) was used the processing temperature lower 120^{o}C. Moreover, the decrease in free amino group and reducing sugar during heating can be confirmed the formation of brown colour via the Maillard reaction.

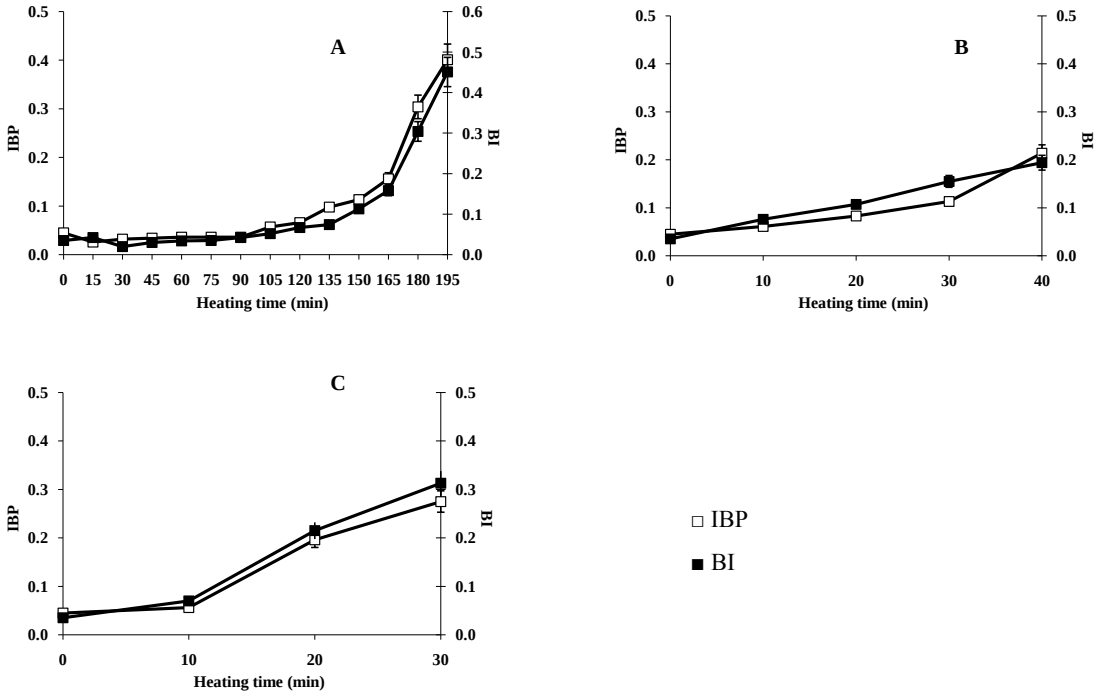


Figure 23. Changes in IBP and BI during the production of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70^{o}C (B) and 80^{o}C (C)

Changes in chemical properties during the production of palm sugar syrup by an open pan and a vacuum evaporator

Changes in total soluble solid and moisture content during the production

Changes in total soluble solid (TSS) contents and moisture content (MC) during heating process are shown in Figure 24 ($P<0.05$). Heating time had a significant effect on TSS and MC ($P<0.05$). The results showed that the TSS contents of palm sugar syrup that produced by an open pan increased slowly within the first 90 min (increase from 12.03^{o}Brix to 20.03^{o}Brix) ($P<0.05$). Subsequently, a sharply increase was observed ($P<0.05$), which obtained the final TSS content was 70.23^{o}Brix. For palm sugar syrup produced by a vacuum evaporator, the TSS increased from 12.03^{o}Brix to 18.13 ^{o}Brix (for a vacuum evaporator at 70^{o}C) and 18.57 ^{o}Brix (for vacuum evaporator at 80^{o}C) during the first 10 min of heating time. This was followed by a rapid increase in TSS contents to approximately 70^{o}Brix, which used the total heating times were 40 and 30 min for vacuum evaporation at 70^{o}C and 80^{o}C, respectively. Moisture content (MC) of all samples decreased continuously during heating process until the end of each process. The increase in TSS contents and decrease in MC could be attributed to rapid evaporation of water from palm sap during heating (Akochi-K *et al.*, 1997; Rao *et al.*, 2009).

Changes in pH and total acidity during the production

The pH values and total acidity were monitored during heating process as presented in Figure 25. A slightly change in pH was found in all heatng process. While total acidity slightly increased from 0.05% to 0.12% during 135 min and rapidly increased to 0.25% at the end of process (195 min) ($P<0.05$). The increase in total acidity was due to the concentration of organic acid during heating process. Moreover, the increase in total acidity occurring in Maillard reaction was due to the formation of

organic acids such as formic acid and acetic acid (Ames, 1998; Brands and Van Boekel, 2002; Lertittikul *et al.*, 2007).

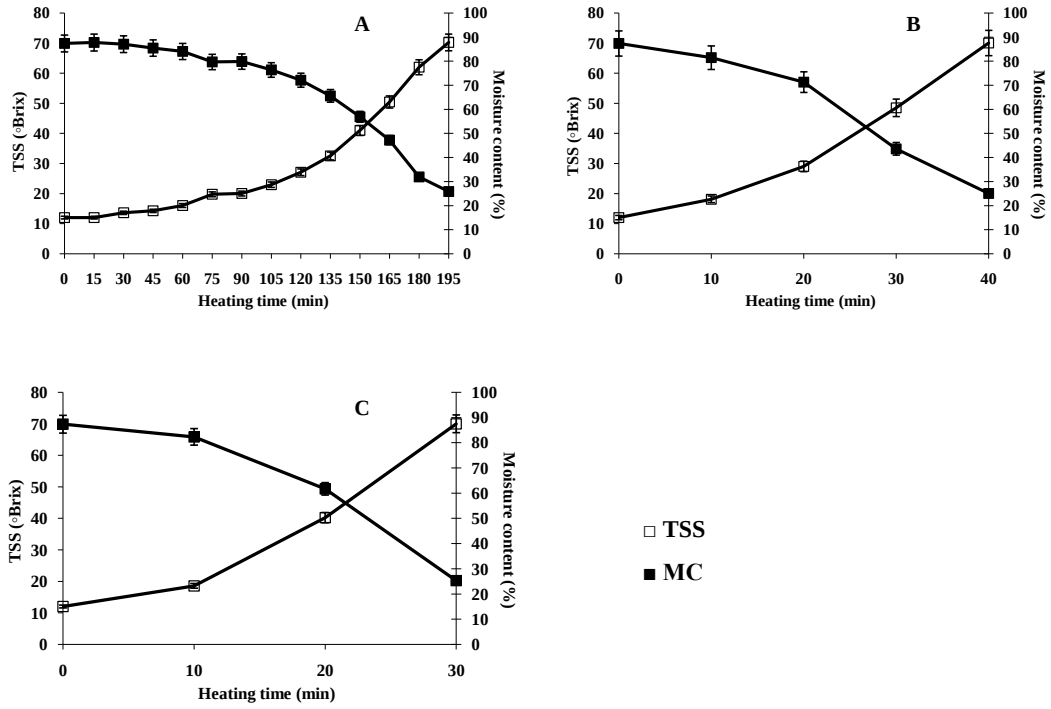


Figure 24. Changes in total soluble solid (TSS) and moisture content during the production of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70°C (B) and 80°C (C)

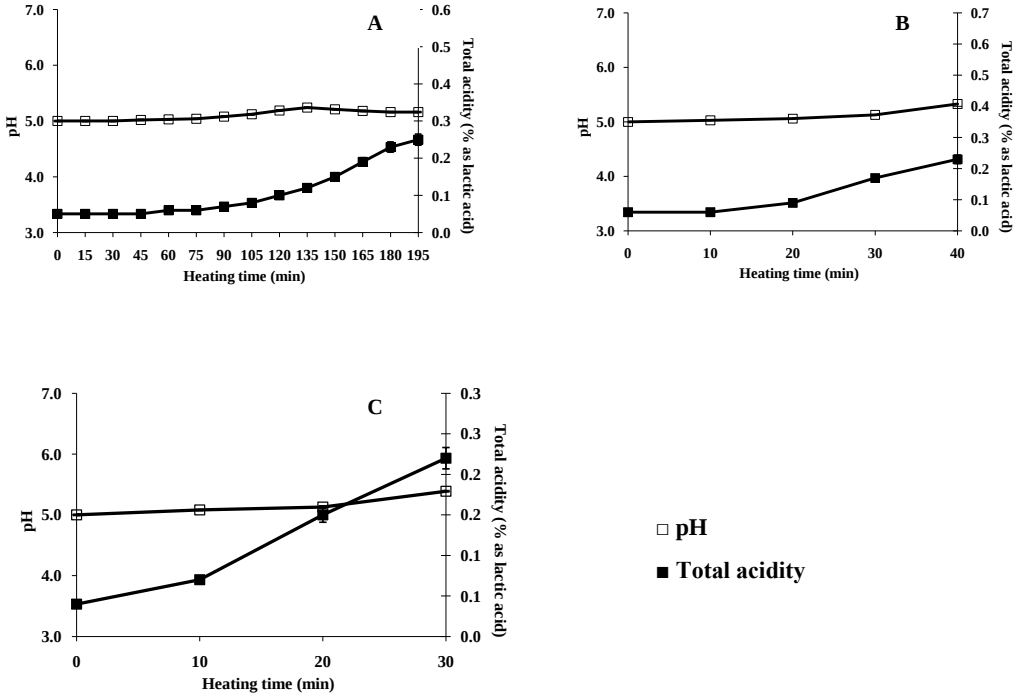


Figure 25. Changes in pH and total acidity during the production of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70^{o}C (B) and 80^{o}C (C)

Changes in sugar content during the production

Sugar is a major component in palm sap and palm sugar syrup. Changes in total sugar and reducing sugar contents during heating process are shown in Figure 26. Total sugar contents and reducing sugar contents of all samples increased with increasing heating times ($P<0.05$). Initially, all samples contained 0.99% of reducing sugar content and 10.92% of total sugar content. At the end of process, palm sugar syrup presented reducing sugar 9.11% (for an open pan), 5.00% (for a vacuum evaporator at 70^{o}C) and 6.10% (for a vacuum evaporation at 80^{o}C) and total sugar 69.14% (for an open pan), 71.13% (for a vacuum evaporator at 70^{o}C) and 70.11% (for a vacuum evaporator at

80^{o}C). The results are in accordance with the increase in fructose and glucose contents during heating process ($P<0.05$) (Figure 27). Initial fructose, glucose and sucrose contents of palm sap were 3.22%, 3.35% and 90.05%, respectively. The fructose, glucose and sucrose contents in finished palm sugar syrup were 6.36%, 6.43%, 83.03% for an open pan, 3.54%, 3.59%, 88.92% for a vacuum evaporator at 70^{o}C and 3.59%, 3.63% and 88.00% for a vacuum evaporator at 80^{o}C.

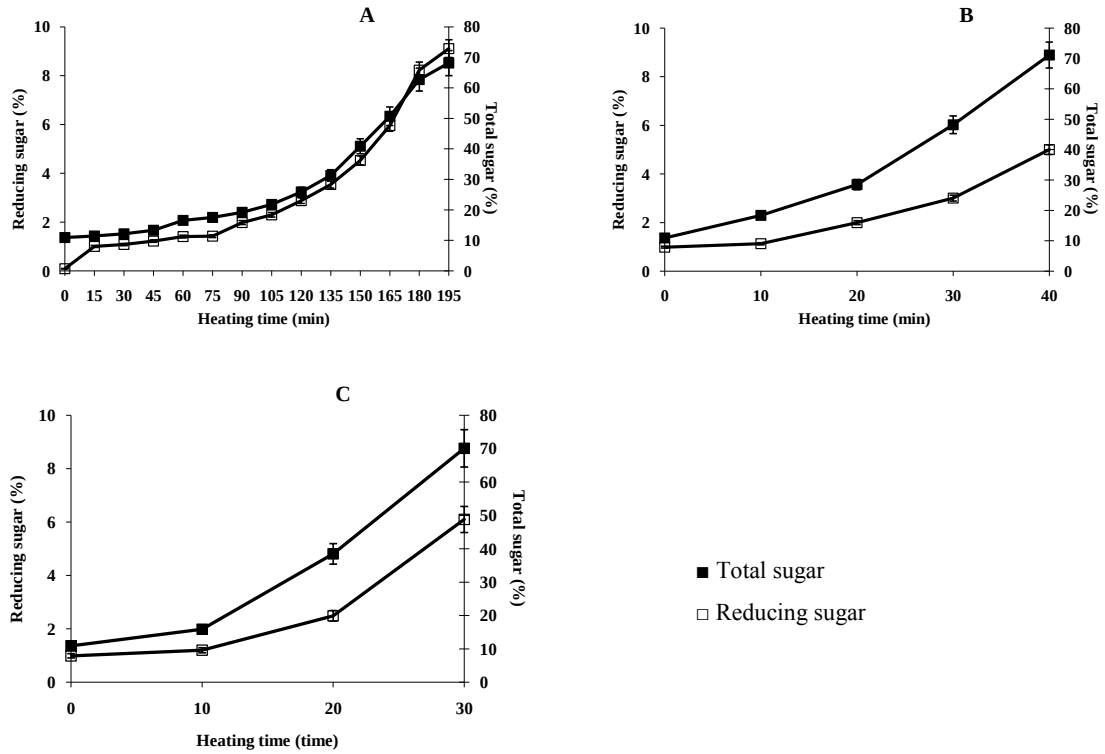


Figure 26. Changes in reducing sugar and total sugar during the production of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70^{o}C (B) and 80^{o}C (C)

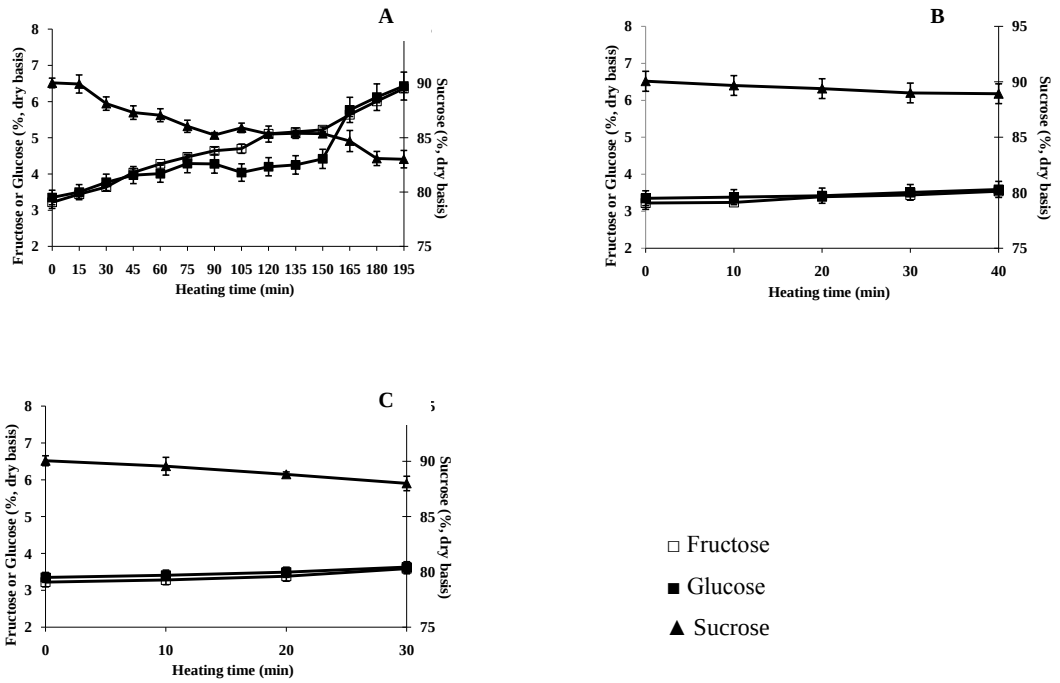


Figure 27. Changes in fructose, glucose and sucrose content during the production of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70^{o}C (B) and 80^{o}C (C)

The sucrose content in all samples decreased with increasing heating time ($P<0.05$). No change in fructose and glucose content of sample that produced by a vacuum evaporator at 70^{o}C and 80^{o}C during heating ($P\geq0.05$). However, fructose and glucose content of palm sugar syrup that produced by an open pan tended to increase until the end of process ($P<0.05$). The unconformity of fructose and glucose was caused sucrose inversion that occurred as heating temperature and heating time increased as evidenced by the reduction of sucrose and the increment of glucose and fructose during heating for palm sugar syrup that produced by an open pan. Sucrose content in samples that produced by a vacuum evaporator slightly decreased during heating while sucrose content in samples produced by an open pan sharply decreased. This probably due to

vacuum evaporation process used lower temperature and shorter time. Thus, vacuum evaporation process can minimize sucrose inversion, therefore lowering the reducing sugar content compared with the process of palm sugar syrup that produced by an open pan. The reducing sugar content is an important parameter that affects the properties of palm sugar syrup during storage since it can act as a substrate of Maillard reaction.

Changes in free amino group content during the production

As mentioned previously, Maillard reaction is the main reaction and responsible for dark colour of syrup. Monitoring free amino group content can be used to indicate the Maillard reaction during heating due to it acts as a substrate of this reaction. Free amino group content of palm sap initially was 7.09 mg/g. The decrease in free amino group content with heating time was observed in each heating process ($P<0.05$) as shown in Figure 28. At the end of heating process, free amino group content of palm sugar syrup was 4.05 mg/g, 6.18 mg/g and 5.61 mg/g for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. As a result, free amino group content in palm sugar syrup that produced by a vacuum evaporator was higher than that produced by an open pan. This indicated that high heating temperature and long time enhanced interaction between free amino group of proteins or peptides and reducing sugar via glycation process (Lertitikul *et al.*, 2007). The decrease in free amino group content was in accordance with the increase in IBP, BI and HMF content.

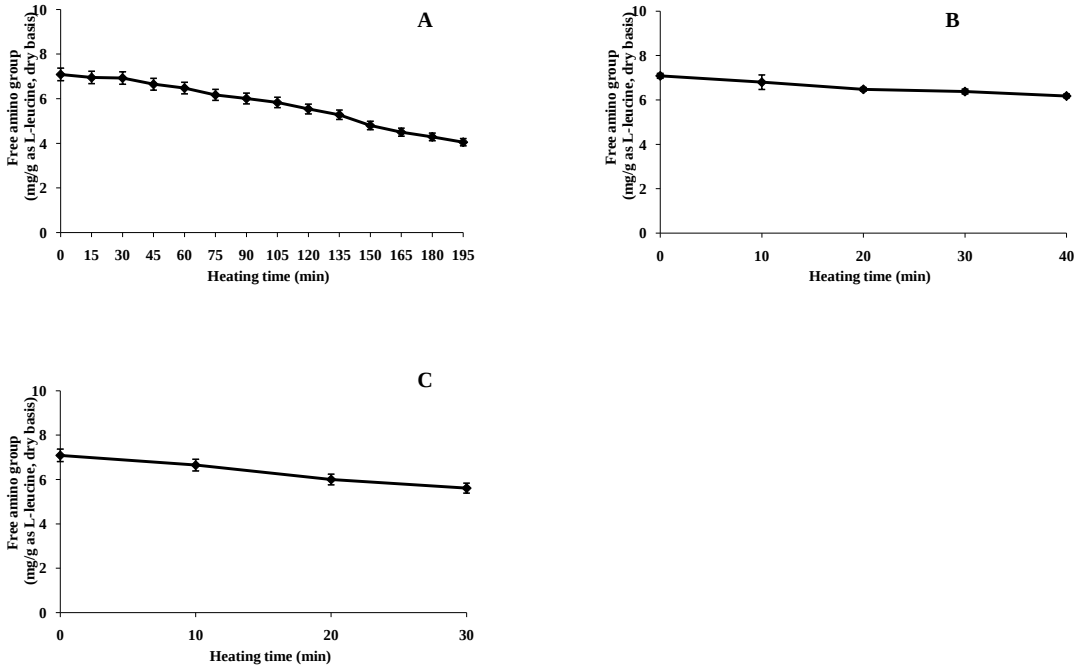


Figure 28. Changes in free amino group content during the production of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70^{o}C (B) and 80^{o}C (C)

Changes in HMF content during the production

Changes in HMF content during heating are shown in Figure 29. HMF can be formed during the production of palm sugar syrup by heating process. In the acid medium of this product, the dehydration of carbohydrates, especially hexose, causes the formation of HMF. Additionally, the Maillard reaction can also take place, giving rise to Amadori compounds during the first step of the reaction, and HMF as a consequence of further reaction. In addition, it is well known that HMF is a precursor of colored compounds in the Caramelization reaction (Kroh, 1994). Therefore, the considerable

variations of HMF found in samples may be used as an indication of overheating during the production of palm sugar syrup. Moreover, HMF is used as an indicator of heat stress for sugar based foods such as honey and syrup because of its toxicological status.

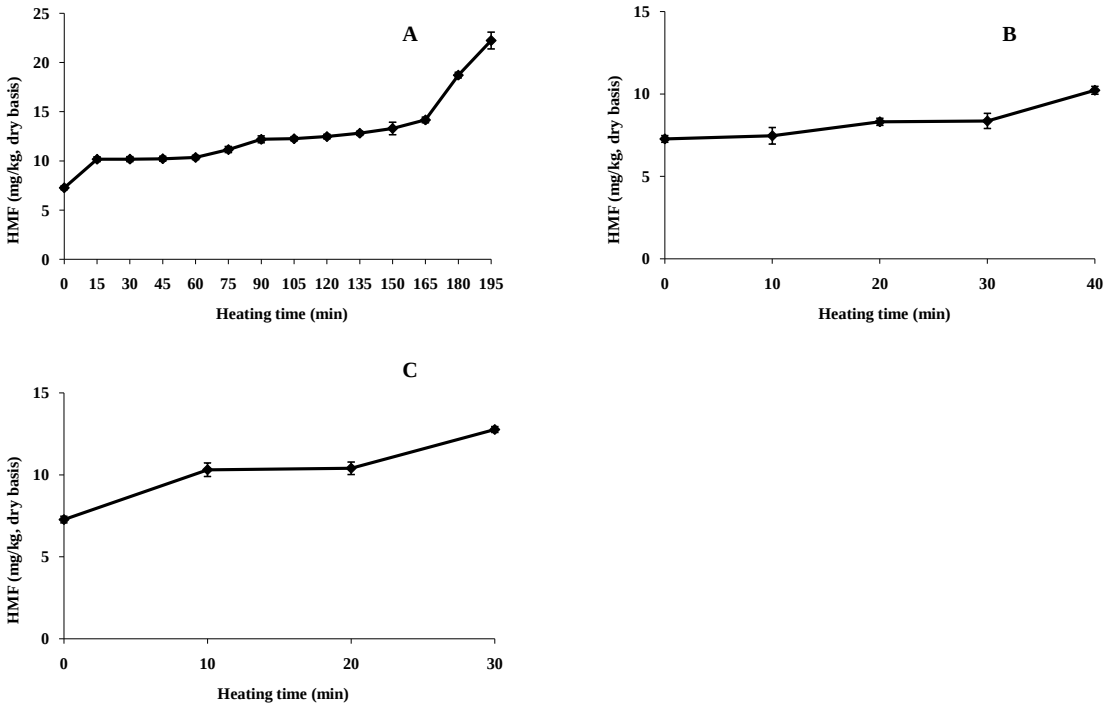


Figure 29. Changes in HMF content during the production of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70^{o}C (B) and 80^{o}C (C)

A significant increase in HMF content as heating time increased was observed in all processing methods ($P<0.05$). This indicated that heating temperature and time influenced the HMF formation. The HMF in fresh palm sap is approximately 7.20 mg/kg. At the end of each process, the HMF in the palm sugar syrup was approximately 22.33 mg/kg, 10.22 mg/kg and 12.76 mg/kg for sample that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. As a result, HMF content in palm sugar syrup that produced by an open pan was higher than that produced by a vacuum evaporator. Result suggested that, an open pan process used high heating temperature and

long time, resulting in a promotion of nonenzymatic browning reactions. Moreover, the HMF content of all samples was lower than the permitted maximum limit of 40 mg/kg as recommended by the Codex Alimentarius (Turhan *et al.*, 2008).

Changes in DPPH radical scavenging during the production

DPPH is a chromogen-radical-containing compound that can directly react with antioxidants. When the DPPH radical is scavenged by antioxidants through the donation of hydrogen to form stable DPPH-H molecule, the colour is changed from purple to yellow (Shon *et al.*, 2003). Stable radical DPPH has been widely used for the determination of primary antioxidant, that is, the free radical scavenging activities of pure antioxidant compounds, plants, fruit extracts and food materials (Shih *et al.*, 2006). In this study, palm sap with and without Kiam wood added were determined for DPPH radical scavenging activity. It was found that DPPH radical scavenging activity in palm sap were 2.42 μmol TE/g sample and 1.55 μmol TE/g sample for palm sap with and without Kiam wood added, respectively. DPPH radical scavenging activity in palm sap added Kiam wood was higher than that no Kiam wood added. This result was due to Kiam wood contained phenolic compound that can dissolve to the palm sap. The antioxidant activity of phenolic compounds is clearly related to free radical-scavenging and hydrogen-donation ability (Kucuk *et al.*, 2007). In addition, changes in DPPH radical scavenging activity during the production of palm sugar syrup that produced by an open pan and a vacuum evaporation were shown in Figure 30. Initally, there was slowly increase in DPPH radical scavenging activity in the first 135 of heating for palm sugar syrup that produced by an open pan. Thereafter, a rapid increase in DPPH radical scavenging activity was noticed until the end of heating process. While a continuous increase in DPPH radical scavenging activity was found in samples produced by a vacuum evaporator at 70^{o}C and 80^{o}C. The increase in DPPH radical scavenging activity was probably due to the increase in Maillard reaction products (MRPs) and Caramelisation products (CPs). DPPH radical scavenging activity in sample that

produced by an open pan was higher than those that produced by a vacuum evaporator. Results suggested that, this process took place the highest nonenzymatic browning reactions, resulting in the highest MRPs and CPs were formed. CPs and MRPs had generally DPPH radical scavenging activity due to they were able to reduce the DPPH radical to the yellow-coloured diphenylpicrylhydrazine (Morales and Jimenez-Perez, 2001; Benjakul *et al.*, 2005; Pongkapai, 2005; Lertittikul *et al.*, 2007). Either intermediate degradation products or brown polymers had an antioxidant activity (Lee and Lee, 1997). Rhee and Kim (1975) reported that the intermediate products at the earlier stage of Caramelisation reaction had an antioxidant activity. Moreover, Benjakul *et al.* (2005), Siddhuraju and Becker (2007) and Binson *et al.* (2008) reported that MRPs possessed hydrogen-donating ability, suggesting potency to react with free radical.

Changes in ferric reducing antioxidant power during heating

Antioxidant potential of palm sap and palm sugar syrup was estimated from their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II) complex (Siddhuraju and Becker, 2007). The ferric reducing antioxidant power (FRAP), generally measures the antioxidant effect of any substance in the reaction medium as its reducing ability. This assay is also commonly used for the routine analysis of single antioxidant or total antioxidant (Kim and Lee, 2009). In this study, palm sap with and without Kiam wood added were determined for FRAP. It was found that FRAP in palm sap were 4.84 μmol TE/g sample and 2.27 μmol TE/g sample for palm sap with and without Kiam wood added, respectively. FRAP in palm sap added Kiam wood was higher than that no Kiam wood added. FRAP in palm sap added Kiam wood was higher than that no Kiam wood added. This result was due to Kiam wood contained phenolic compound that can dissolve to the palm sap. The antioxidant activity of phenolic compounds is clearly related to free radical-scavenging and hydrogen-donation ability (Kucuk *et al.*, 2007). The changes in FRAP during the production of palm sugar syrup that produced by an open pan and a vacuum evaporation were shown in Figure 31. A continuous increase in FRAP was found

in all samples with heating time. Initally, there was slowly increase in FRAP in the first 120, 10 and 10 of heating for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. Thereafter, a rapid increase in FRAP was observed until the end of each process. These results are in agreement with those obtained for the antioxidant activity determined by the DPPH radical scavenging assay (Figure 31). Rufian-Henares and Morales (2007) and Kim and Lee (2009) pointed out that the ferric reducing ability of melanoidins was in parallel with the data from DPPH method. Compounds responsible for reducing activity are formed during the thermolysis of Amadori products in the primary phase of Maillard reaction (Hwang *et al.,* 2001) or they could be formed by heterocyclic compounds of Maillard reaction or Caramelisation of sugars (Charurin *et al.,* 2002). The results revealed that the MRPs and CPs could function as electron doners. The hydroxyl groups of MRPs or CPs play an important role in reducing activity (Yoshimura *et al.,* 1997). Additionally, the intermediate reductone compounds of MRPs were reported to break the radical chain by donation of hydrogen atom (Eichner, 1981). Furthermore, higher FRAP was found in sample that produced by an open pan compared to a vacuum evaporator due to heating by an open pan process had high rate of nonenzymatic browning reactions, resulting in high MRPs and CPs were formed.

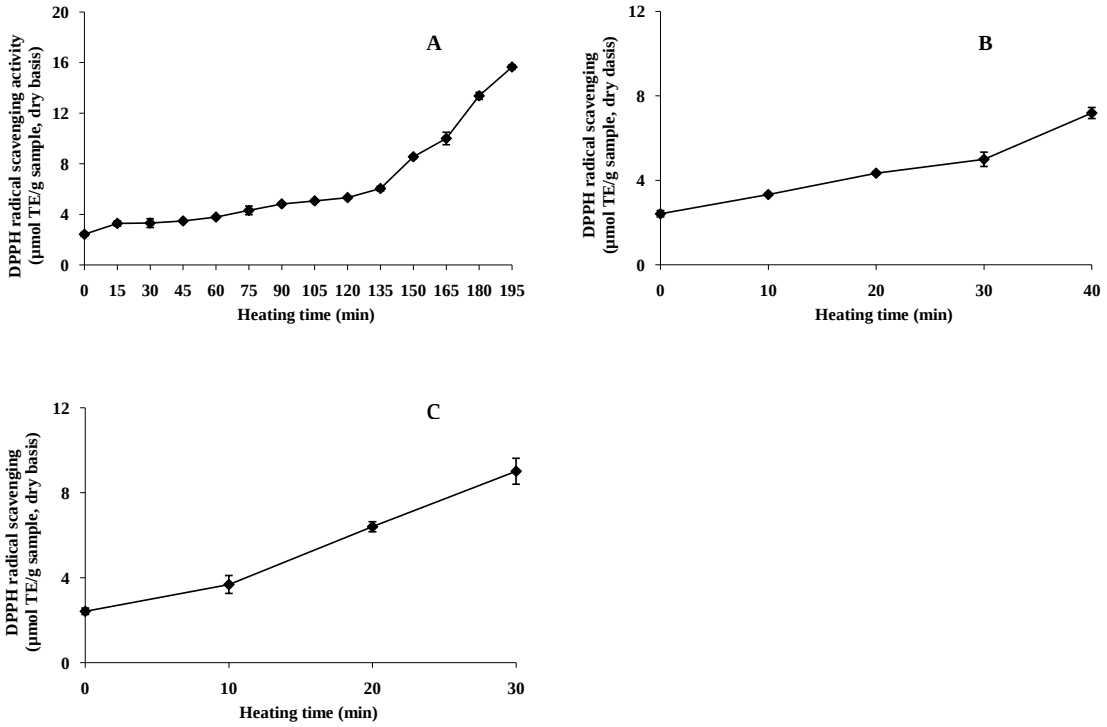


Figure 30. Changes in DPPH radical scavenging during the production of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70^{o}C (B) and 80^{o}C (C)

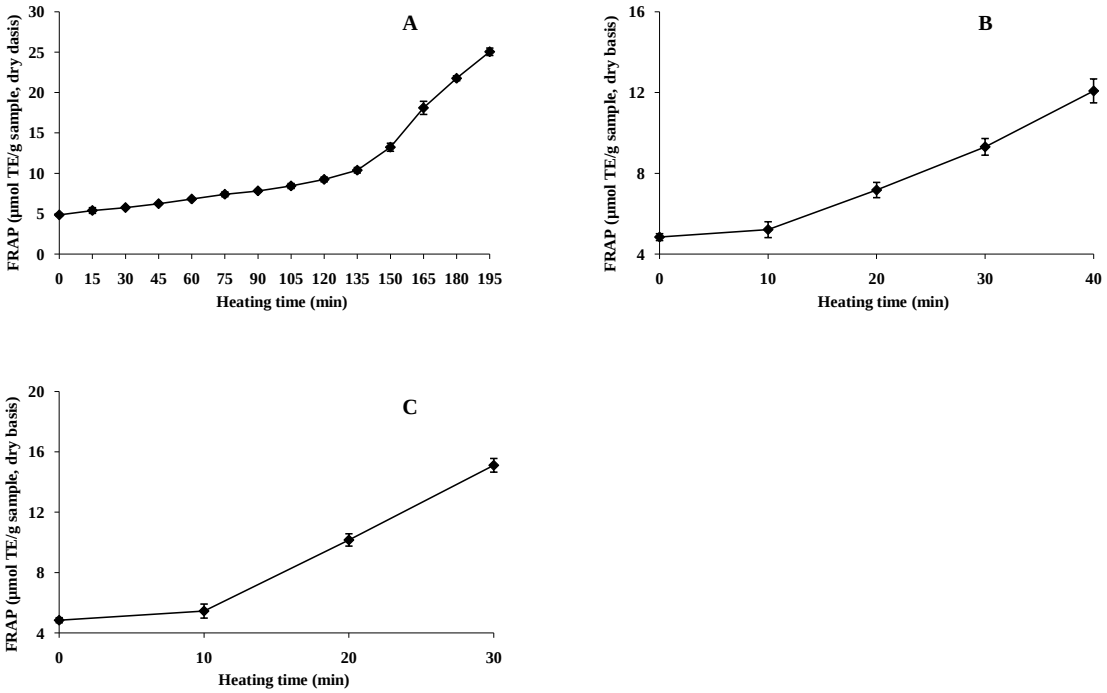


Figure 31. Changes in FRAP during the production of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70^{o}C (B) and 80^{o}C (C)

Changes in reducing power during heating

Reducing power is another method to determine antioxidant activity. In this study, palm sap with and without Kiam wood added were determined for reducing power. It was found that reducing power in palm sap were 0.25 and 0.15 for palm sap with and without Kiam wood added, respectively. Reducing power in palm sap added Kiam wood was higher than that no Kiam wood added. The changes in reducing power during the production of palm sugar syrup that produced by an open pan and a vacuum evaporation were shown in Figure 32. A continuous increase in reducing power was found in all samples with heating time. Initally, there was slowly increase in reducing power in the first 135 of heating for palm sugar syrup that produced by an open pan.

Thereafter, a rapid increase in reducing power was observed until the end of process. While a continuous increase in reducing power was found in samples produced by a vacuum evaporator at 70^{o}C and 80^{o}C. Yoshimura *et al.* (1997) found that the reducing power increased with increasing heating time of glucose-glycone mixture. MRPs from xylose-lysine (Yen and Hsieh, 1995) and glucose-lysine (Yoshimura *et al.*, 1997) model systems also possessed reducing power. Moreover, CPs also showed reducing power activity (Pongkanpai, 2005). Lee and Lee (1997) reported the CPs from sucrose prepared at pH 4 and heated at 200^{o}C for 90 min had a reducing power and reducing power of CPs increased after solution was heated for 60 min. The reducing power was used to indicate the hydrogen-donating ability (Benjakul *et al.*, 2005). Thus, the results revealed that MRPs and CPs of heated palm sap had hydrogen-donating activity. In addition, higher reducing power was found in sample that produced by an open pan compared to a vacuum evaporator due to heating by an open pan process had high rate of nonenzymatic browning reactions, resulting in high MRPs and CPs were formed.

DPPH radical scavenging activity, FRAP and reducing power of heated palm sap and palm sugar syrup was correlated to the changes in IBP, BI and HMF contents. This indicated that brown pigments and colourless intermediate compounds had the hydrogen-donating properties, which varied with processing methods.

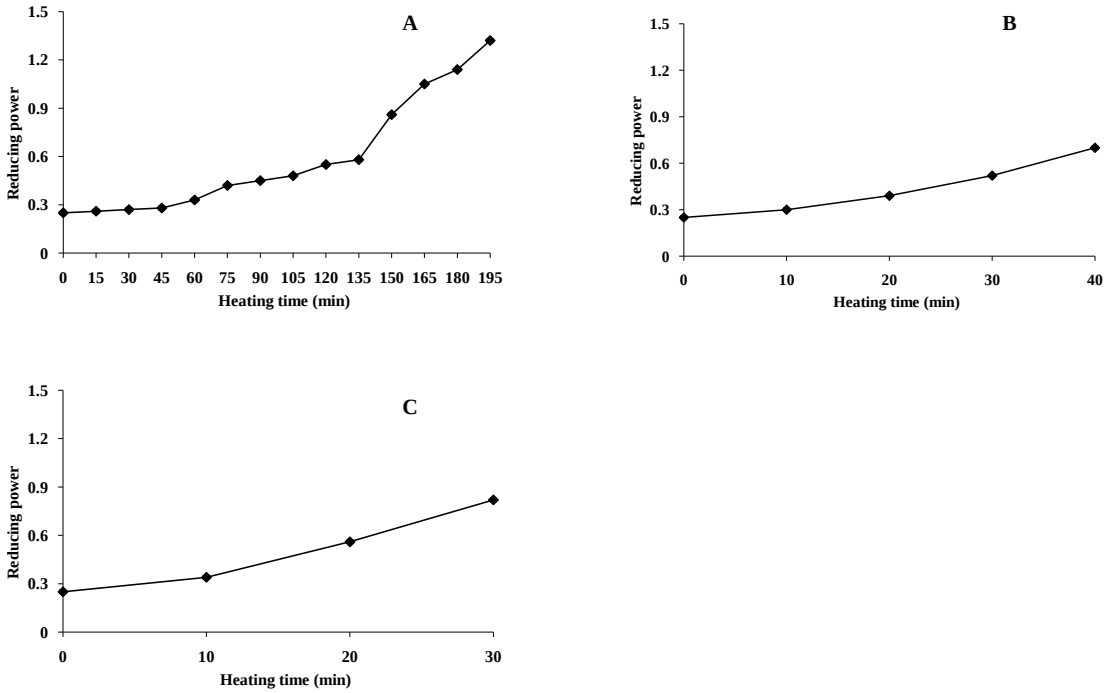


Figure 32. Changes in reducing power during the production of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70^{o}C (B) and 80^{o}C (C)

Changes in physical properties during storage of palm sugar syrup produced by an open pan and a vacuum evaporator

Normally, syrup with concentration approximately 70^{o}Brix is stable to store under room temperature for one year (Potter and Hotchkiss, 1995). The most common chemical reactions that influence on the property of syrup during storage are inversion reaction and Maillard reaction (Akochi-K *et al.*, 1997; Apriyantono *et al.*, 2002; Ho *et al.*, 2007; Perkin and Van den Berg, 2009). In this study, the effect of storage temperature (4^{o}C and 30^{o}C) and storage time (0-12 months) on the changes in property of palm sugar syrup that produced by an open pan and a vacuum evaporator was

investigated. Appendix Figure 10 shows the samples of palm sugar syrup produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C during storage under 4^{o}C and 30^{o}C for 12 months.

Changes in colour values (L*, a* and b*) of palm sugar syrup during storage

The colour change of palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C during storage under two temperatures (4^{o}C and 30^{o}C) for 12 months was observed. The L* values of palm sugar syrup was monitored during storage as depicted in Figure 33. Temperature and time during storage had a significant effect on L*, a* and b* values ($P<0.05$). The L* values of all samples were significantly decreased with increasing storage temperature ($P<0.05$). Additionally, the L* values of all samples in each processing method were also significantly decreased with increasing storage time and temperature ($P<0.05$). The L* value was initially found to be 55.33, 58.64 and 57.78 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. The L* values decreased to 39.44, 48.40 and 47.59 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C and stored under 4^{o}C until the end of storage, respectively. Additionally, L* values were also reduced to 12.28, 23.81 and 18.30 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C and stored under 30^{o}C until the end of storage, respectively. Decreasing of these values indicate the colour of palm sugar syrup changed to brown and dark, especially at higher temperature and longer time during storage.

The a* values can be used to evaluate browning of palm sugar syrup. Changes in a* values of palm sugar syrup during storage are shown in Figure 34. The a* values of all samples in each processing method were significantly increased with increasing storage temperature ($P<0.05$). Additionally, the a* values of all samples in each processing method were also significantly increased with increasing storage time and temperature ($P<0.05$). The a* value increased during storage from an initial value of

24.35, 9.96 and 13.88 to 34.31, 14.07 and 18.73 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, and stored under 4^{o}C until the end of storage, respectively. Moreover, the a* values increased to 25.72 and 28.31 for palm sugar syrup that produced by a vacuum evaporator at 70^{o}C and 80^{o}C and stored under 30^{o}C until the end of storage, respectively.

The b* values indicate the variation between yellow and blue colour. Changes in b* value of palm sugar syrup during storage are presented in Figure 34. The b* values of all samples in each processing method were significantly decreased with increasing storage temperature ($P<0.05$). Additionally, the b* values of all samples in each processing method were also significantly decreased with increasing storage time and temperature ($P<0.05$). Initial the b* values were found to 86.14, 49.19 and 56.99 and these values decreased to 67.17, 35.45 and 40.46 at the end of storage for palm sugar syrup that produced by an open pan and a vacuum evaporator 70^{o}C and 80^{o}C and stored under 4^{o}C. The b* values reduced to 20.84, 35.45 and 30.53 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C and stored under 30^{o}C until the end of storage, respectively. The greatest decrease in b* value during storage occurred in palm sugar syrup stored under 30^{o}C, followed by those stored under 4^{o}C.

Generally, L* and b* value decrease while a* value increases during browning reaction occur. It indicated that the colour of palm sugar syrup became darker with more red component and less yellow component (Krapfenbauer *et al.,* 2006). Changes in L*, a* and b* values are responsible for dark colour and they may contribute to the nonenzymatic browning reactions (Maskan, 2006; Ibarz, 1999; Rattanathanalerk *et al.,* 2005; Damasceno *et al.,* 2008). During storage under 4^{o}C and 30^{o}C, only Maillard reaction took place and caused the browning colour in palm sugar syrup. The Maillard reaction rate increased with increasing temperature and time (Martins *et al.,* 2001). Temperature affects the activities of the reducing sugars. The active form of sugar is considered to be opened chain, which is formed markedly with increasing temperature (Van Boekel and Martins, 2002). Thus, higher opened chain of sugar greatly took place

in samples stored under 30°C when compared to those stored under 4°C causing darkening in a product.

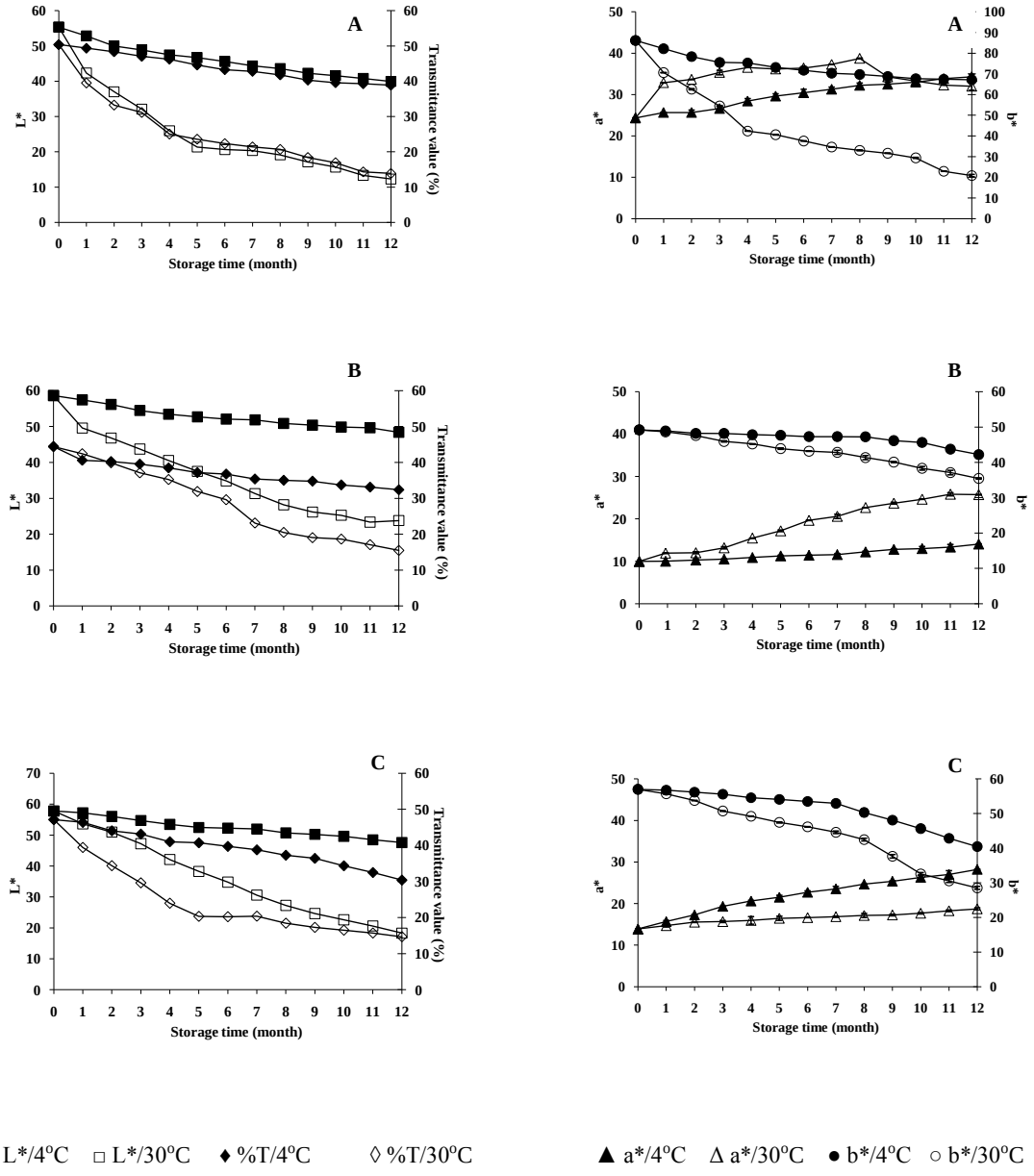


Figure 33. Changes in L*, a*, b* and transmittance values during storage of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70°C (B) and 80°C (C)

Changes in clarity of palm sugar syrup during storage

Clarity was determined by measuring the percentage of light transmittance at 650 nm. This value can be used to evaluate the clarity in fruit juice. High transmittance value shows the juice more clarified than low transmittance value. Changes in transmittance of palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C during storage for 12 month are shown in Figure 33. Temperature and time during storage had a significant effect on the transmittance values ($P<0.05$). The transmittance values of all samples in each processing method were significantly decreased with increasing storage temperature ($P<0.05$). Additionally, transmittance values of all samples in each processing method were also significantly decreased with increasing storage time and temperature ($P<0.05$). Initial transmittance values of palm sugar syrup were 50.39% (for an open pan), 44.42% (for a vacuum evaporator at 70^{o}C) and 47.16% (for a vacuum evaporator at 80^{o}C). At the end of storage, the transmittance value declined to 38.86%, 32.37% and 30.40% for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C and stored under 4^{o}C, respectively. On the other hand, palm sugar syrup that stored under 30^{o}C showed lower transmittance values than those that stored under 4^{o}C, which were 13.86%, 15.49% and 14.68% for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. The clarity of palm sugar syrup depends greatly on its protein concentration and the polyphenol compounds, which is dissolved from Kiam wood and as presented by nature in palm sap itself. The complex between protein and polyphenol can be induced and therefore, a large colloid size or haze can be developed (Kermasha *et al.,* 1995, Siebert *et al.,* 2006). The protein-phenol haze forms via hydrogen and/or hydrophobic interaction. The hydrogen bonds occur between the hydroxyl groups of polyphenols and the carbonyl oxygen in the peptide backbone, whereas the hydrophobic interactions are generated via attraction between the aromatic structure of polyphenols and the nonpolar moiety in proteins (Katrine *et al.,* 2006). The transmittance decreased slightly under lower storage temperature (4^{o}C), and more dramatically under 30^{o}C for all

palm sugar syrup samples. At higher temperature, molecular mobility is higher thus, allowing more interactions to take place polyphenol and protein interaction (Calderon *et al.*, 1986; Lee *et al.*, 2007). Moreover, the Maillard reaction taking place during storage might affect the decrease in clarity of palm sugar syrup due to the formation of the colloid particles (brown polymer), resulting higher light scattering (Takano, 2005).

Changes in intermediate browning and browning intensity of palm sugar syrup during storage

As Maillard reaction was found to be a main reaction caused of colour change in palm sugar syrup during storage. The changes in absorbance values at 280 nm for intermediate browning product (IBP) and 420 nm for browning intensity (BI) of palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C after storage were monitored as shown in Figure 34. Temperature and time during storage had a significant effect on IBP and BI ($P<0.05$). The IBP and BI of all samples in each processing method were significantly increased with increasing storage temperature and time ($P<0.05$). At the beginning, the IBP of palm sugar syrup was 0.40 (for an open pan), 0.21 (for a vacuum evaporator at 70^{o}C) and 0.26 (for a vacuum evaporator at 80^{o}C). At the end of storage and at under 4^{o}C, the IBP increased to 0.88, 0.48 and 0.55 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. Likewise, an increase of the IBP was found in all samples that stored under 30^{o}C, which were 1.03, 0.66 and 0.70 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. The increase in IBP was in accordance with an absorbance value at 420 nm (BI).

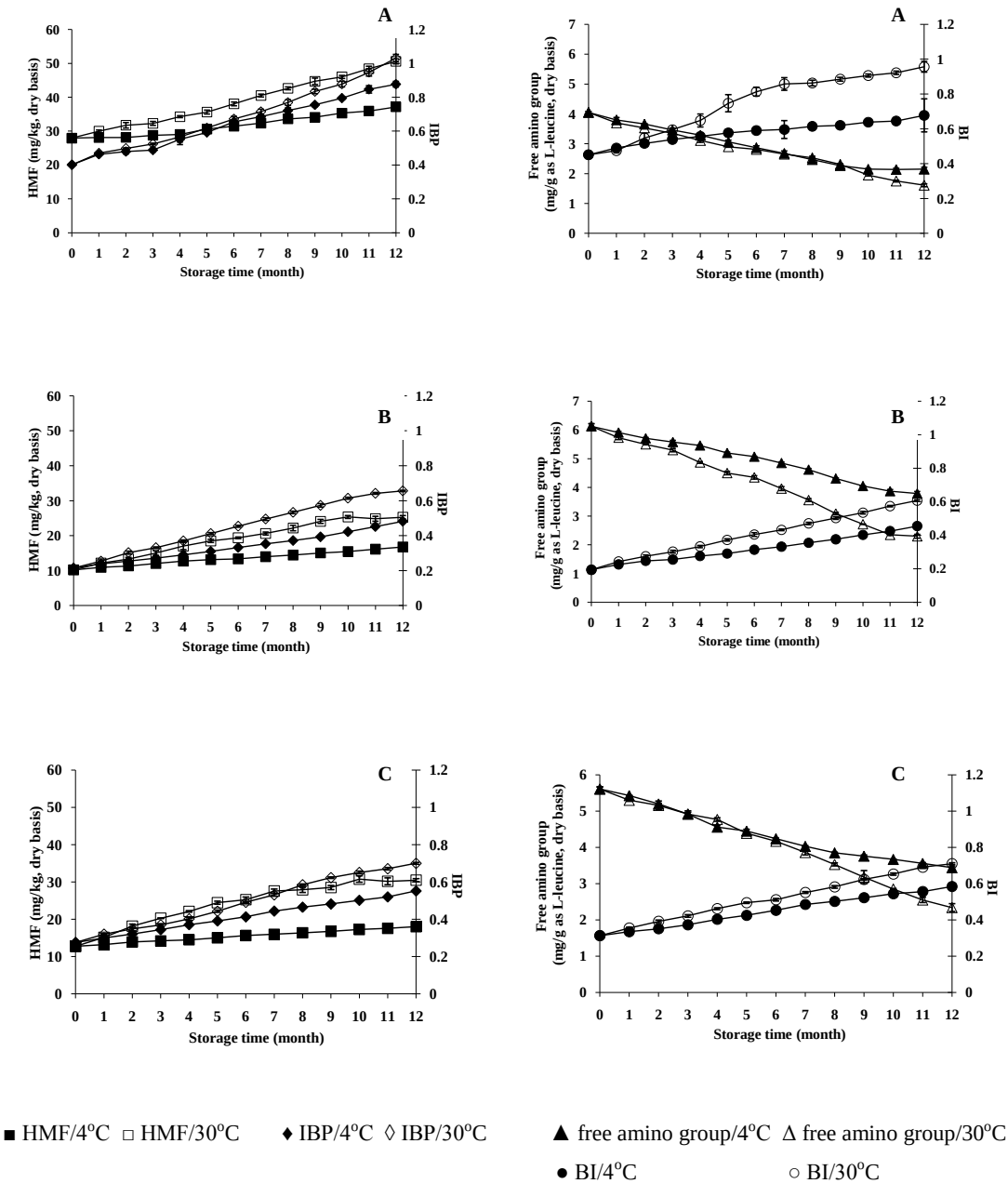


Figure 34. Changes in HMF, IBP, free amino group and BI during storage of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70°C (B) and 80°C (C)

The initial BI of palm sugar syrup was 0.45 (for an open pan), 0.19 (for a vacuum evaporator at 70^{o}C) and 0.31 (for a vacuum evaporator at 80^{o}C). These were then increased to 0.68, 0.46 and 0.58 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C at the end of storage under 4^{o}C, respectively. At the end of storage, samples that stored under 30^{o}C presented higher BI than those that stored under 4^{o}C, which were 0.96, 0.61 and 0.71 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively.

The changes in IBP and BI during storage were affected by Maillard reaction. The browning developments during storage are greatly enhanced by storing palm sugar syrup under 30^{o}C and were suppressed under 4^{o}C due to the Maillard reaction that can be promoted by high storage temperature (Eskin, 1990; Martins *et al.,* 2001). In addition, a similar relationship between the increase in IBP and BI suggested that some intermediate products might undergo conversion to the final brown compounds, while some intermediates are still being generated during storage.

Changes in chemical properties during storage of palm sugar syrup produced by an open pan and a vacuum evaporator

Changes in total soluble solid of palm sugar syrup during storage

Initial TSS of all palm sugar syrup samples was approximately 70^{o}Brix. The TSS of all palm sugar syrup samples remained constant during 12 months of storage either stored under 4^{o}C or 30^{o}C. Temperature and time during storage had not a significant effect on TSS ($P \geq 0.05$). Thai Industrial Standards Institute Ministry of Industry (2003) stated that the standard of TSS in palm sugar syrup shall not be less than 65^{o}Brix in order to prevent the microorganisms growth during storage under room temperature. From the result, all palm sugar syrup samples met this standard.

Changes in moisture content and water activity of palm sugar syrup during storage

Moisture content (MC) and (Aw) can be used as an indicator to evaluate the microbiological properties of sugar based product. Both of MC and Aw are highly important for extension the shelf life stability of palm sugar syrup during storage (De Rodriguez *et al.*, 2004). An average of MC and Aw during storage for 12 months either stored under 4^{o}C or 30^{o}C was approximately 25% and 0.80, respectively. Storage temperature and storage time had not a significant effect on MC and Aw ($P \geq 0.05$).

Changes in pH and total acidity of palm sugar syrup during storage

Changes in pH and total acidity were observed during 12 months of storage as shown in Figure 35. Temperature and time during storage had a significant effect on pH and total acidity ($P<0.05$). The pH of all samples in each processing method were significantly decreased with increasing storage temperature and time ($P<0.05$). Initial the pH values were found to be 5.16, 5.33 and 5.39. These decreased to 5.00, 5.15 and 5.21 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C and stored under 4^{o}C until the end of storage, respectively. On the other hand, the pH values were reduced to 4.91, 5.04 and 5.10 for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C and stored under 30^{o}C until the end of storage, respectively. The total acidity of all samples were significantly ($P<0.05$) increased with increasing storage temperature in each processing method. Additionally, the total acidity of all samples were also significantly ($P<0.05$) increased with increasing storage time in each processing method. The total acidity increased during storage. This rose from an initial value of 0.25%, 0.23% and 0.23% to 0.38%, 0.35% and 0.35% for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C and stored under 4^{o}C until the end of storage, respectively. Moreover, total acidity increased to 0.47%, 0.43% and 0.43% for palm

sugar syrup that produced by an open pan and a vacuum evaporator at 70°C and 80°C and stored under 30°C until the end of storage, respectively. Results suggest that the decrease in pH value and increase in total acidity were probably due to chemical reaction and the growth of microorganisms. The reduction in pH value and increase in total acidity occurring in Maillard reaction was due to the formation of organic acids such as formic acid and acetic acid (Ames, 1998; Brands and Van Boekel, 2002; Lertittikul *et al.*, 2007).

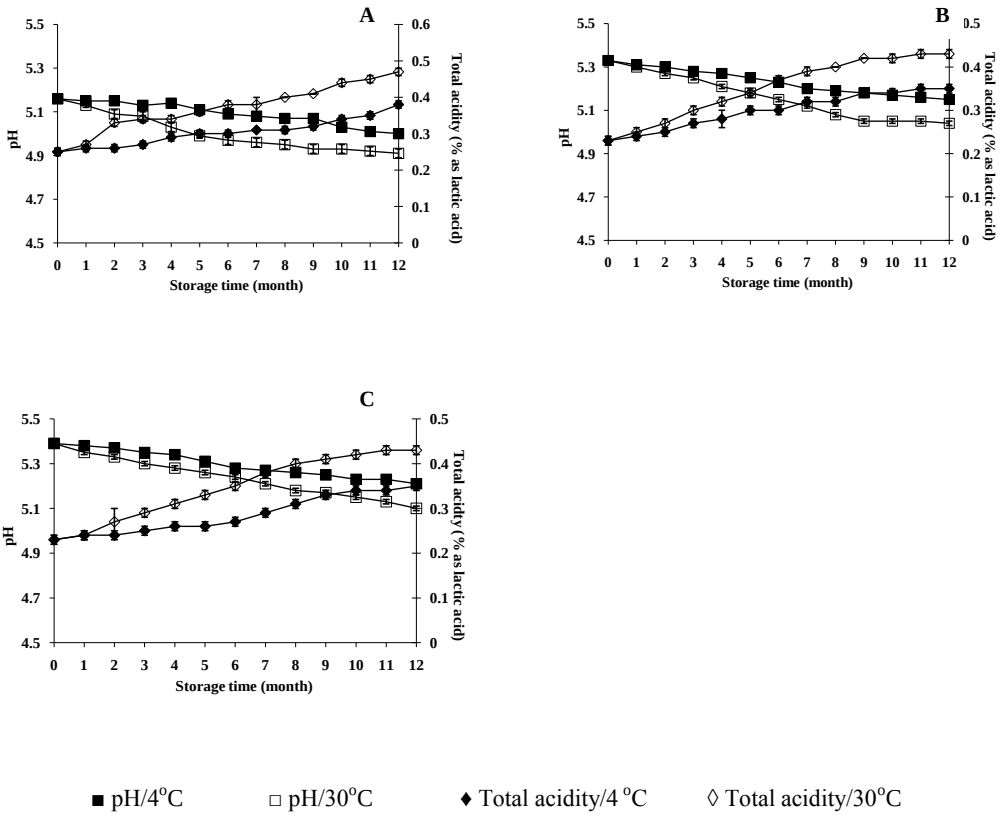


Figure 35. Changes in pH and total acidity during storage of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70°C (B) and 80°C (C)

Changes in HMF content of palm sugar syrup during storage

Temperature and time during storage can induce the increase in HMF content of palm sugar syrup. Changes in HMF content during storage of all palm sugar syrup samples were presented in Figure 34. Storage temperature and time had a significant effect on HMF content ($P<0.05$). HMF content of all samples in each processing method was significantly increased with increasing storage temperature and time ($P<0.05$). Initally, HMF content of palm sugar syrup was 26.05 mg/kg (for an open pan), 10.22 mg/kg (for a vacuum evaporator at 70^{o}C) and 12.76 mg/kg (for a vacuum evaporator at 80^{o}C). At the end of storage for 12 months under 4^{o}C, the HMF content increased to 37.15 mg/kg, 16.74 mg/kg and 18.01 mg/kg for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C. Meanwhile, palm sugar syrup samples that stored under 30^{o}C showed a higher HMF content than those that stored under 4^{o}C, which were 50.58 mg/kg, 25.27 mg/kg and 30.48 mg/kg for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C, respectively. An increase in HMF was coincidental with an increase in IBP. Only the sample that produced by an open pan and stored at 30^{o}C contained HMF content (50.58 mg/kg) higher than the permitted maximum limit (40 mg/kg as recommend by the Codex Alimentarious) (Turhan *et al.*, 2008). Thus, low temperature during storage (4^{o}C) can be used to retard the increase in HMF content in palm sugar syrup, especially by using a combination of a vacuum evaporator during production.

Changes in free amino group content of palm sugar syrup during storage

At early stage of Maillard reaction, terminal α-amino groups of peptides and ε-NH_2 groups of lysine react with the carbonyl function of reducing sugars present in the reaction medium. Thus, the loss of available primary amino groups can be used to evaluate Maillard reaction in palm sugar syrup during storage. The free amino group content in palm sugar syrup was monitored during storage as shown in Figure 34.

Temperature and time during storage had a significant effect on free amino group content ($P<0.05$). Free amino group content of all samples in each processing method were significantly decreased with increasing storage temperature and time ($P<0.05$). Initial free amino group content was found to be 4.02 mg/g, 6.13 mg/g and 5.61 mg/g and this then decreased to 2.15 mg/g, 3.79 mg/g and 3.44 mg/g in the palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C and stored under 4^{o}C at the end of storage for 12 months. Under 30^{o}C during storage, the free amino group content declined to 1.61 mg/g, 2.30 mg/g and 2.34 mg/g for palm sugar syrup that produced by an open pan and a vacuum evaporator at 70^{o}C and 80^{o}C at the end of storage for 12 months. The free amino group content tended to decrease during storage since it declined during Maillard reaction especially at high temperature.

Changes in sugar of palm sugar syrup during storage

Sugar is a major component in palm sap and palm sugar syrup. Figure 36 showed the changes in total sugar and reducing sugar contents of palm sugar syrup during storage. Changes in fructose, glucose and sucrose contents of palm sugar syrup are shown in Figure 37. Temperature and time during storage had a significant effect ($P<0.05$) on total sugar and reducing sugar as well as fructose and glucose and sucrose contents.

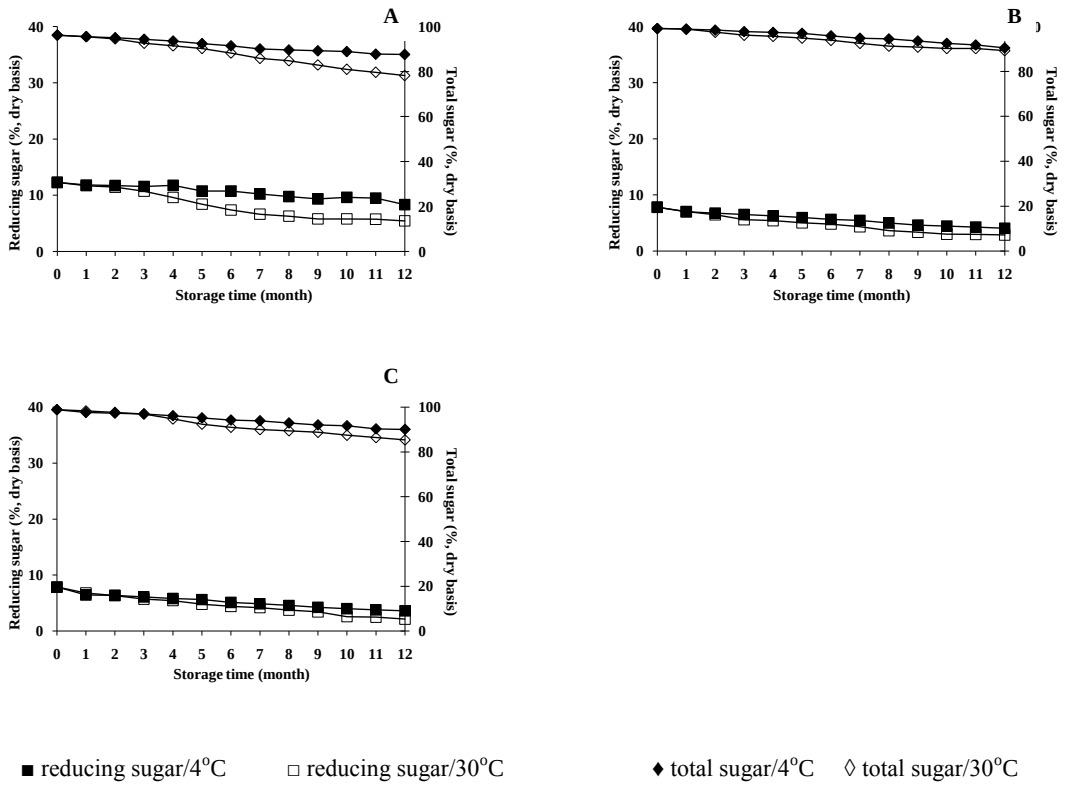


Figure 36. Changes in reducing sugar and total sugar during storage of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70°C (B) and 80°C (C)

Fructose, glucose and sucrose contents of all samples in each processing method were significantly decreased with increasing storage temperature and time ($P<0.05$). Initally, fructose, glucose and sucrose contents were found to be 6.36%, 6.43%, 83.03% for an open pan, 3.54%, 3.59%, 88.92% for a vacuum evaporator at 70°C and 3.59%, 3.63%, 88.00% for a vacuum evaporator at 80°C. At the end of storage, the fructose, glucose and sucrose of all samples that stored under 4°C decreased to 4.04%, 4.52%, 75.17% for an open pan, 2.00%, 2.30%, 85.31% for a vacuum evaporator at 70°C and 1.85%, 1.98%, 82.51% for a vacuum evaporator at 80°C. In addition, the fructose, glucose and sucrose of all samples that stored under 30°C were also reduced to 2.72%, 3.01%, 68.80% for an open pan, 1.02%, 1.43%, 78.54% for a vacuum evaporator at 70°C

and 0.85%, 1.30%, 76.04% for a vacuum evaporator at 80^{o}C. Results suggested that, the decrease in fructose and glucose contents during storage were due to they acted as a substrate of Maillard reaction. Additionally, a decrease in sucrose can be used to confirm the inversion of sucrose contents occurred during storage. The greatest decrease in sucrose during storage occurred in palm sugar syrup that stored under 30^{o}C, followed by those that stored under 4^{o}C for all palm sugar syrup samples.

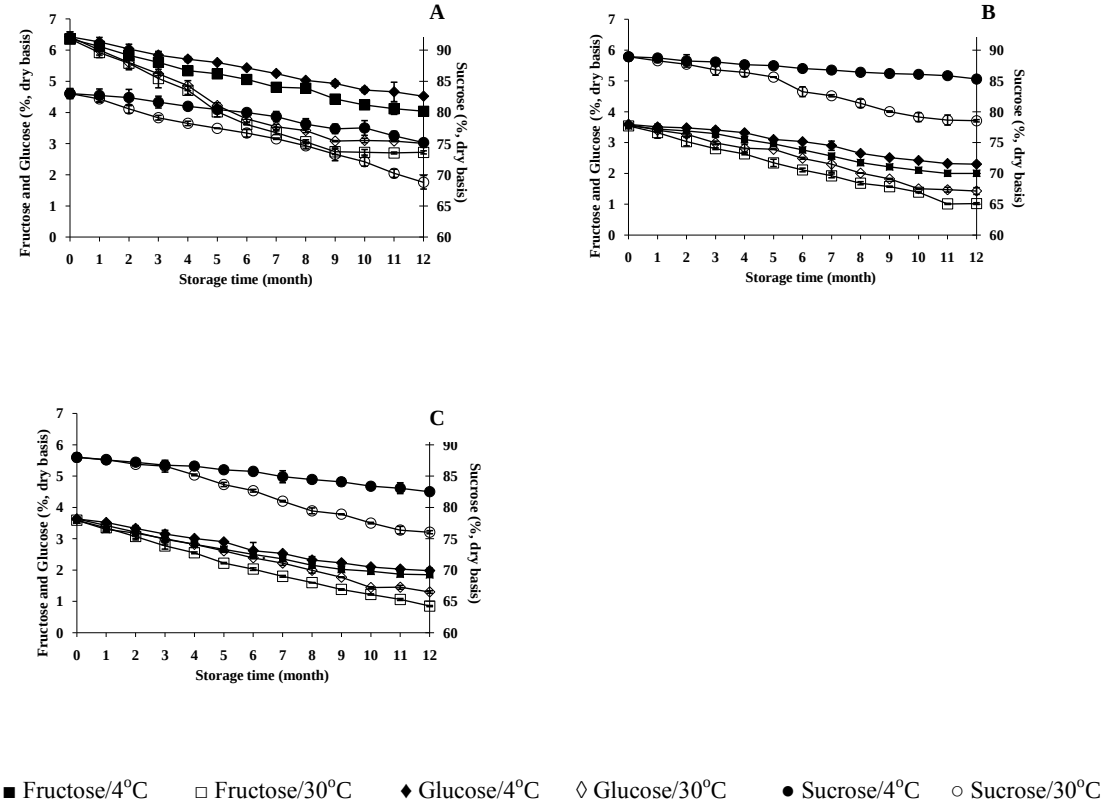


Figure 37. Changes in fructose, glucose and sucrose during storage of palm sugar syrup produced by an open pan (A) and a vacuum evaporator at 70^{o}C (B) and 80^{o}C (C)

From the results, the decrease in the free amino group, fructose and glucose content were in accordance with the increase in IBP, BI and HMF. This indicated that the interaction between free amino group and sugar through the glycation process

took place during the storage of palm sugar syrup. Thus, intermediate products were formed and further converted to brown pigment, as observed by the increase in BI.

Changes in volatile flavour compounds of palm sugar syrup during storage

Changes in volatile flavour compounds of palm sugar syrup produced by an open pan and a vacuum evaporator during storage were analysed using Headspace Soild Phase Microextraction technique (HS-SPME) coupled with Gas chromatography-mass spectrometry (GC-MS) as shown in Table 24, 25 and 26, respectively. Similar profiles of volatile flavour compounds from all palm sugar syrup samples were noticed. However, low amount of volatile flavour compounds were observed in samples produced by a vacuum evaporator.

Volatile flavour compouds were commonly found in a sample produced by an open pan, consisting of 2 alcohols, 1 ester, 1 ketone, 1 acid, 1 pyrrole, 8 furans and 4 pyrazines. For samples produced by a vacuum evaporator either 70^{o}C or 80^{o}C, 2 alcohols, 1 ester, 1 ketone, 1 acid, 4 furans and 2 pyrazines were detected. Less typical volatile flavour compounds were found in samples that produced by a vacuum evaporator. This data can explain as low nonenzymatic browning reaction occurred during the production. This was due to a vacuum evaporator required low temperature under vacuum during production. Therefore, a short processing time was required.

Volatile flavour compounds from Maillard reaction and Caramelisation were found in palm sugar syrup including furans, pyrazines and pyrroles. These compounds have been reported as a typical flavour in syrup and found in various plant syrup such as maple syrup, coconut syrup and birch syrup (Kallio, 1989; Akochi *et al.*, 1994; Akochi *et al.*, 1997; Purnomo, 2007). Furan derivatives such as 2-furanmethanol, 2-furancarboxaldehyde, 3-furancarboxaldehyde, 2-acetylfuran, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2-methyl dihydro-3(2H)-furanone, 2-methyl dihydro-2(3H)-furanone and 2,3-dihydro-3,5-dihydroxy-6methyl-pyran-4-one were detected in samples produced by an open pan. Only 4 furan derivatives such as 2-furanmethanol, 2-

furancarboxaldehyde, 3-furancarboxaldehyde and 2,3-dihydro-3,5-dihydroxy-6methyl-pyran-4-one were found in samples that produced by a vacuum evaporator. All detected furans contribute to sweet, caramel, cook sugar or burnt sugar (Ho *et al.,* 2007). High temperature during storage (30^{o}C) had more effect on the formation of furan derivatives than low temperature during storage (4^{o}C). Generally, the formation of furan derivatives can be formed through two pathways either via lipid peroxidation or the degradation of carbohydrate involved Maillard reaction. In this study, furan is known to be formed by the degradation of carbohydrate. Furfural is formed from the Amadori intermediate of the Maillard reaction by 1,2-enolization followed by a deamination and dehydration and can be formed via Caramelisation (Nursten, 1980; Meynier and Mottram, 1995).

Pyrazine derivatives were detected in samples produced by an open pan including 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, methyl pyrazine, 2-methoxy-6-methylpyrazine and 2-ethyl-3,5-dimethylpyrazine. However, only 2 pyrazines such as methyl pyrazine and 2,6-dimethylpyrazine were detected in samples produced by a vacuum evaporator. All pyrazines are correlated with sensory attributes, such as nutty, sweet and roasty odour which consequently contribute to the typical aroma notes of palm sugar syrup (Ho *et al.,* 2007). The presence of pyrazines in palm sugar syrup indicated that not only Caramelisation, but also the Maillard reaction took place during preparation of palm sugar syrup (Apriyantono *et al.,* 2002). Normally, alkyl pyrazine are commonly found in heated food. High temperature during storage (30^{o}C) had more effect on the formation of pyrazine derivatives than low temperature during storage (4^{o}C). One of the main routes of pyrazine formation is via the Strecker degradation of amino acids in the presence of dicarbonyl compounds. In addition to the Strecker aldehyde, this reaction yields an aminocarbonyl compounds, and the condensation of two of these molecules results in an alkyl pyrazine (Amrani-Hemaimi *et al.,* 1995; Hwang *et al.,* 1995; Meynier and Mottram, 1995). One pyrrole (acetylpyrrol) was identified in palm sugar syrup. This pyrrole tends to contribute to sweet or burnt sugar notes and it has been reported in various heated foods. There are two pathways to form pyrrole. First pathway is the interaction between an amino acid and a 3-

dexoyhezosone through the Strecker degradation followed by dehydration and ring closure. The second pathway is the reaction of furans with amines or amino acids. (Rizzie, 1974; Hwang *et al.*, 1995).

The 3-hydroxy-2-butanone was reported to be responsible for sweet odour (Cheetham, 2002). It is the main volatile flavour compound in palm sap as reported by Taipaiboon (2004). A decrease in 3-hydroxy-2-butanone was found during storage in all samples. Phenethyl alcohol or benzene ethanol had been reported to possess a sweet or rose aroma. It is derived from L-phenylalanine through metabolic reaction of yeast (Soufleros *et al.,* 2004). Phenethyl alcohol increased with increasing storage temperature and time. Acetic acid increased with increasing storage temperature and time. There are two possible pathways for acetic acid formation including osmophilic yeast metabolism and Maillard reaction. Acetic acid may be derived from isoamyl alcohol and acetyl coenzyme A by alcohol acetyl transferase in yeasts (Inoue *et al.,* 1994). In addition, the α-dicarbonyl compounds are unstable and undergo a cleavage reaction (at the C-C bond), resulting in acetic acid during Maillard reaction (Martins and Van Boekel, 2005).

Changes in microbiological properties during storage of palm sugar syrup produced by an open pan and a vacuum evaporator

In general, microorganisms reported to be found in sugar based product are yeasts and molds. Yeasts can grow under acid conditions and cannot inhibite by sucrose. Osmophilic or sugar tolerant yeasts are a problem in syrup industry, because they can grow even at the limited level of water (Aw ranged from 0.65 to 0.80), high sucrose content and acid condition. Heat from the heating process can destroy the microorganism presented in palm sap. However, osmophilic yeasts may be found to survive, and to grow after heating process due to sugar can protect their spores (Dumont *et al.,* 1993; Snowdon and Cliver, 1996; Phaichamnan, 2009). According to The Thai Industrial Standards Institute Ministry of Industry (2003) indicates that the total microbial and the yeast and mold counts in palm sugar syrup shall not be more than 5×10^2 cfu/g and

100 cfu/g, respectively. Total microbial count and yeast and mold count of all samples were lower than the maximum limit of permission that allowed by The Thai Industrial Standards Institute Ministry of Industry (Table 27-29). Low temperature during storage (4^{o}C) retarded the growth of osmophilic yeasts and, thus reduced a risk of acid formation and a product spoilage. The growth of osmophilic yeasts is corresponding to the decrease of pH value and increase in total acidity of syrup during storage.

Table 24. Changes in volatile flavour compounds (ppb) of palm sugar syrup produced by an open pan during storage under 4°C and 30°C for 12 months

RT[A]	RI[B]	Volatile flavour compounds	Attributes[C]	Storage temperature (°C)/Storage time (month)								
				0	3 months		6 months		9 months		12 months	
				month	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C
		Pyrazines										
5.81	1292	methyl pyrazine	nutty, roasty	644	657	687	637	748	627	847	600	899
6.84	1314	2,3-dimethylpyrazine	roasted nut, sweet	995	975	1025	947	1189	927	1287	887	1369
6.50	1309	2,5-dimethylpyrazine	sweet, roasty	884	856	912	875	936	841	998	800	1005
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	3657	3625	3698	3611	3874	3587	3784	3566	3996
7.82	1470	2-ethyl-3,5-dimethylpyrazine	roasty	1044	1002	1125	987	1254	956	1369	944	1440
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	1501	1489	1589	1456	1621	1247	1785	1204	1897
		Furans										
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	508	489	548	471	602	447	689	402	784
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	234	224	296	204	341	185	411	154	487
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	332	321	397	300	456	266	587	244	661
8.35	1537	2-acetylfuran	sweet, caramel	112	100	159	86	187	88	204	68	300
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	98	84	152	77	127	87	205	66	298
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	728	700	789	675	854	645	952	603	1002
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	2534	2501	2596	2475	268	2423	2774	2369	2896
11.72	2577	2,3-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one	caramel, sweet, burnt sugar	357	325	425	300	418	284	487	257	523
		Pyrrole										
10.62	2023	Acetylpyrrole	sweet, burnt sugar	220	211	256	189	304	167	389	144	447
		Alcohols										
10.36	1951	Phenethyl alcohol	flora	172	178	189	196	217	200	228	215	269
8.81	1587	2,3-butanediol	fruity	2393	2347	2441	2456	2499	2489	2554	2541	2966
		Acids										
7.84	1473	Acetic acid	sour	23569	24478	25631	25876	26471	26874	27846	26991	28996
		Ketone										
6.00	1307	3-hydroxy-2-butanone	sweet	2425	2400	2584	2347	2574	234	2411	2301	2311
		Ester										2311
1.86	997	Ethyl acetate	fruity, sweet	8084	8076	7956	8002	7968	7984	7844	7900	7800

Note: [A] RT refers to retention time (min). [B] RI refers to retention index that were based on a series of alkane (C_{10}-C_{24}).

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

Table 25. Changes in volatile flavour compounds (ppb) of palm sugar syrup produced by a vacuum evaporator at 70°C during storage under 4°C and 30°C for 12 months

RT[A]	RI[B]	Volatile flavour compounds	Attributes[C]	Storage temperature (°C)/Storage time (month)								
				0	3 months		6 months		9 months		12 months	
				month	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C
		Pyrazines										
5.81	1292	methyl pyrazine	nutty, roasty	263	265	286	245	301	240	322	233	347
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	585	568	601	578	654	544	689	500	712
		Furans										
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	233	224	256	214	287	210	301	204	326
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	151	150	174	134	196	124	218	107	254
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	121	110	148	98	184	77	210	74	241
11.72	2577	2,3-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one	caramel, sweet, burnt sugar	139	128	156	114	184	103	213	92	248
		Alcohols										
10.36	1951	phenethyl alcohol	flora	525	530	542	547	559	550	568	564	584
8.71	1587	2,3-butanediol	fruity	3208	3201	3246	3224	3287	3251	3311	3262	3382
		Acid										
7.84	1473	acetic acid	sour	18898	18994	19045	19004	19187	19124	19346	19248	19564
		Ketone										
6.00	1307	3-hydroxy-2-butanone	sweet	3790	3756	3684	3700	3600	3667	3584	3604	3451
		Ester										
1.86	997	ethyl acetate	fruity, sweet	14254	14047	13247	13985	12654	13756	12056	13660	11234

Note: [A] RT refers to retention time (min). [B] RI refers to retention index that were based on a series of alkane (C_{10}-C_{24}).

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

Table 26. Changes in volatile flavour compounds (ppb) of palm sugar syrup produced by a vacuum evaporator at 80°C during storage under 4°C and 30°C for 12 months

RT[A]	RI[B]	Volatile flavour compounds	Attributes[C]	Storage temperature (°C)/Storage time (month)								
				0	3 months		6 months		9 months		12 months	
				month	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C
		Pyrazines										
5.81	1292	methyl pyrazine	nutty, roasty	301	286	315	297	336	264	374	255	389
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	774	765	796	754	824	721	856	705	896
		Furans										
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	285	275	301	245	335	256	386	221	412
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	194	185	215	165	236	142	275	124	310
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	157	142	185	125	215	101	257	105	300
11.72	2577	2,3-dihydro-3,5-dihydroxy-6-methyl-pyran-4-one	caramel, sweet, burnt sugar	180	174	213	154	246	167	287	141	324
		Alcohols										
10.36	1951	phenethyl alcohol	flora	660	684	701	697	756	711	778	725	812
8.71	1587	2,3-butanediol	fruity	2857	2874	2901	2899	2956	2924	3001	2956	3158
		Acid										
7.84	1473	acetic acid	sour	18005	18234	18400	18369	18679	18561	18842	18742	19741
		Ketone										
6.00	1307	3-hydroxy-2-butanone	sweet	3540	3530	3475	3514	3369	3487	3245	3457	3201
		Ester										
1.86	997	ethyl acetate	fruity, sweet	12869	12745	12648	12674	12369	12487	12114	12244	11988

Note: [A] RT refers to retention time (min). [B] RI refers to retention index that were based on a series of alkane (C_{10}-C_{24}).

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

Table 27. Changes in total microbial count (cfu/g) of palm sugar syrup produced by an open pan and a vacuum evaporator during storage under 4°C and 30°C for 12 months

Storage time (month)	Processing method/Storage temperature (°C)					
	Open pan		Vacuum evaporator at 70°C		Vacuum evaporator at 80°C	
	4°C	30°C	4°C	30°C	4°C	30°C
0	<10	<10	<10	<10	<10	<10
6	<10	<10	<10	<10	<10	<10
12	<10	<10	<10	<10	<10	<10

Table 28. Changes in yeast and mold (cfu/g) of palm sugar syrup produced by an open pan and a vacuum evaporator during storage under 4°C and 30°C for 12 months

Storage time (month)	Processing method/Storage temperature (°C)					
	Open pan		Vacuum evaporator at 70°C		Vacuum evaporator at 80°C	
	4°C	30°C	4°C	30°C	4°C	30°C
0	<10	<10	<10	<10	<10	<10
6	<10	<10	<10	<10	<10	<10
12	<10	<10	<10	<10	<10	<10

Table 29. Changes in osmophilic yeast (cfu/g) of of palm sugar syrup produced by an open pan and a vacuum evaporator during storage under 4°C and 30°C for 12 months

Storage time (month)	Processing method/Storage temperature (°C)					
	Open pan		Vacuum evaporator at 70°C		Vacuum evaporator at 80°C	
	4°C	30°C	4°C	30°C	4°C	30°C
0	<10	<10	<10	<10	<10	<10
6	30	40	40	50	30	50
12	40	70	50	80	40	70

4.5 Conclusion

Heating temperature and time showed a pronounced effect on the browning development and antioxidant activity of palm sap and its changes during the production of palm sugar syrup either by an open pan or a vacuum evaporator. During

each heating process, the browning development due to MRPs and CPs formation was found as indicated from an increase in IBP, BI and HMF content and a decrease in free amino group content. The main nonenzymatic browning reaction taking place during the production of palm sugar syrup is the Maillard reaction. Reducing sugar content as well as glucose and fructose contents increased during heating process due to an inversion reaction of sucrose took place. The browning development during each heating process was coincidental with an increase in antioxidant activity. Thus, MRPs and CPs of heated palm sap exhibited antioxidant activity via electron donating and radical scavenging. Moreover, the browning development and antioxidant activity of palm sugar syrup that produced by an open pan were higher than those produced by a vacuum evaporator. Therefore, palm sugar syrup can be used as a new source of antioxidant ingredient in food products afterward.

Temperature and time during storage showed a pronounced effect on the properties changed of palm sugar syrup that produced by an open pan and a vacuum evaporator. Low temperature during storage (4^{o}C) can be used to reduce the browning development of palm sugar syrup as indicated by lower IBP, BI and HMF content when compared with high storage temperature (30^{o}C) ($P<0.05$). Storage time also affected the browning development of palm sugar syrup as evidenced by the increase in IBP, BI and HMF content as storage time increased ($P<0.05$). Processing method influenced the browning development of palm sugar syrup during storage. High heating temperature and long time during the production can promote the dark colour formation of palm sugar syrup during storage. The Maillard reaction is a major reaction responsible for the dark colour formation during storage. Thus, the heating process by a vacuum evaporator and the storage condition under low temperature can be used as an alternative way to produce less browning development of palm sugar syrup during storage.

CHAPTER 5

EFFECT OF SUCROSE AND GLUCOSE SYRUP ADDITION ON PROPERTIES OF PALM SUGAR CAKE AND ITS CHANGES DURING STORAGE

5.1 Abstract

The aim of this study was to determine the effect of sucrose and glucose syrup addition on properties of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator and the effect of storage temperature (4^{o}C and 30^{o}C) and storage time (12 months and 4 weeks for sample stored under 11% and 75% of RH, respectively) on properties of palm sugar cake that produced from palm sugar syrup wuth either using an open pan or a vacuum evaporator during storage.

Two different processing methods either an open pan or a vacuum evaporator were used to produce palm sugar syrup. That later on, it was used as a raw material to produce palm sugar cake. The different concentrations of sucrose (30%, 40%, and 50%) and glucose syrup (10% and 20%) were added to palm sugar syrup. All ingredients were heated by an open pan until the total soluble solid reached 80^{o}Brix (at temperature approximately 120^{o}C). After that, it was removed from heat and stirred until its became crystalline and poured in a mold. Palm sugar cake that produced from 100% palm sugar syrup was used as a control. It was found that an increase in hardness and crystallinity was found in all samples with increasing sucrose content. An increase in Tg and a decrease in IBP, BI and HMF content was found with increasing sucrose and glucose syrup content. However, there was a decrease in hardness and crystallinity with increasing glucose syrup content. The highest dark colour formation and lowest hardness was observed in samples that produced from 100% palm sugar syrup with either using an

open pan or a vacuum evaporator as indicated by the lowest L* and hardness and the highest a*, IBP, BI and HMF content. Moreover, all samples that produced from palm sugar syrup with using an open pan presented higher BI and HMF content and lower hardness, crystallinity and Tg than those that produced from palm sugar syrup with using an vacuum evaporator. Furthermore, the highest overall acceptability score was observed in samples that produced from 50% palm sugar syrup, 40% sucrose and 10% glucose syrup. Therefore, this formulation was selected to study the effect of storage temperature and time afterward.

Storage temperature affected the browning development of all samples in both RHs. During storage, Maillard reaction took place in samples that stored under 4^{o}C lower than those that stored under 30^{o}C in both RHs. This result was shown by lower a* value, BI and higher L* value in sample that stored under 4^{o}C than 30^{o}C at the end of storage ($P<0.05$). Storage temperature did not affect on hardness and crystallinity of all samples stored in both RHs. Storage time influenced the increase in a* and BI and the decrease in L* in all samples that stored in both RHs ($P<0.05$). The hardness and crystallinity of all samples increased within the first month ($P<0.05$), and then no changes was found until 12 months of storage under 11% of RH ($P\geq0.05$). While, continuous decrease in hardness and crystallinity was found in all samples that stored under 75% of RH when storage time increased ($P<0.05$). Thus, storage condition under 4^{o}C and 11% of RH is suitable to store palm sugar cake when compared to under 30^{o}C and 75% of RH due to these conditions can reduce the changes of texture and dark colour of palm sugar cake.

5.2 Introduction

Sugar cake is made from various materials such as palm sap, coconut sap and maple sap. In southern part of Thailand particularly in Songkhla province, palm sugar cake was originally made from the sugary sap of the Palmyra palm (*Borassus flabellifer* Linn). The sap is boiled to obtain palm sugar syrup that it can be used as a raw

material for palm sugar cake production. During heating process for a sugar cake production, water is removed until its becomes concentrate (the total soluble solid is more than 80^{o}Brix). As soon as the temperature of this heated palm syrup approximately reaches 120^{o}C, it is removed from the heat and stirred. The stirring process is continued until the solution begins to crystallise and stiffen. At this stage, it can be poured into a mould.

There are many factors affecting the properties of palm sugar cake including quality of raw material and its formulation. Nowadays, palm sugar syrup produced by an open pan was used as a raw material for the production of palm sugar syrup. High temperature during processing could be affected the properties of palm sugar syrup due to high inversion reaction rate. This reaction can promote the increment of reducing sugar content in palm sugar syrup. The palm sugar syrup contained high reducing sugar content was hard to obtain crystallise sugar cake. Therefore, processing method such as a vacuum evaporation under low temperature can be used to produce palm sugar syrup as an alternative way to reduce inversion reaction during heating. Furthermore, some ingredients added could be affected the properties of palm sugar cake. Sucrose was used as an ingredient to induce crystallisation of sugar. In addition, glucose syrup was usually added to increase the stability of product.

In addition, palm sugar cake is hygroscopic. It rapidly absorbs moisture from an environment and easily melts. Chemical reaction such as nonenzymatic browning reactions is accelerated when moisture content in sugar based product increased (Eskin, 1990). Nonenzymatic browning reactions may cause unacceptable nutritional and sensory effects in sugar based food products and may be a limiting factor in the shelf life of a product. Since quality is supremely important in food, therefore thermal deterioration has to be controlled during storage. Some factors such as storage temperature, storage time and relative humidity generally affected the properties of palm sugar cake. However, scientific supports on palm sugar cake in Thailand have rarely been yet investigated. Therefore the aim of this work was to study the effect of sucrose and glucose syrup addition on properties of palm sugar cake produced by palm sugar syrup

obtained from an open pan and a vacuum evaporator. Additionally, the effect of storage temperature and storage time on properties of palm sugar cake during storage under different relative humidity was also investigated.

5.3 Materials and Methods

Chemicals

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, methanol and phosphoric acid were obtained from Merck (Darmstadt, Germany). Bovine serum albumin (BSA) and Coomassie brilliant blue were obtained from Fluka (Buchs, Switzerland). Potassium sodium tartrate, lead acetate and potassium oxalate were purchased from Riedel-deHaen (Seelze, Germany). Glucose syrup (DE 40) was purchased from Taweekit, LTD, Thailand.

Raw material

Palm sap was collected from a contact farm in Songkhla province. Natural palm sap was tapped and harvested after 12 h of collecting in an open container. During tapping, natural wood called Kiam (3 g/L) was added into a container since the beginning state of tapping. Palm sap was kept in an icebox (4^{o}C) during transportation (30 min). The sample was filtrated using cloth sheet at room temperature and immediately used to produce palm sugar syrup.

Production of palm sugar syrup

Two different processing methods either using an open pan or a vacuum evaporator were used to produce palm sugar syrup. Palm sap (15 liters) was concentrated by an open pan (at temperature approximately 110^{o}C) and a vacuum evaporator (at temperature 80^{o}C) until its total soluble solids reached 70^{o}Brix to obtain palm sugar syrup.

Production of palm sugar cake

Palm sugar cake was prepared from palm sugar syrup which was concentrated by two methods either an open pan or a vacuum evaporator. A sample of palm sugar syrup from each processing method was mixed with sucrose and glucose syrup with different concentrations and four formulations were obtained (Table 30). The ingredients from each formulation were boiled using an open pan until the total soluble solid reached 80^{o}Brix (at temperature approximately 120^{o}C). After that, it was removed from the heat and stirred by using wood spatula until its became crystalline and then the sample was poured in thin (0.5 cm) slabs and allowed to cool at ambient temperature. Immediately after production, the physical, chemical properties and sensory evaluation of palm sugar cake were determined.

Table 30. Formulations of palm sugar cake

Formulation	Palm sugar syrup (%w/w)	Sucrose[a] (%w/w)	Glucose syrup[a] (%w/w)
1	50	0	0
2	50	50	0
3	50	40	10
4	50	30	20

Note: [a]Commercial grade of sucrose and glucose syrup (DE 40, containing 4% glucose and 6% maltose) was used

Measurement of colour

The colour measurements of the samples were carried out using a Hunter Lab Colourflex colourimeter. A colourimeter was adjusted for reflectance, illuminant D 65, and angle of 10^{o}. Instrumental colour data was provided in accord with the CIE system in terms of L* (lightness), a* (redness and greenness) and b* (yellowness and blueness).

Measurement of intermediate browning product and browning intensity

Intermediate browning product (IBP) and browning intensity (BI) of palm palm sugar cake were determined by monitoring the absorbance at 280 and 420 nm, respectively. The absorbance was measured by using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan), as described by Takano (2005) and Kawai *et al.* (2005). Appropriate dilution (20-fold for IBP and 10-fold for BI) was made using distilled water to obtain a reliable absorbance reading.

Measurement of hardness and stickiness

The hardness and stickiness of palm sugar cake was measured by using a TA-XT2i Texture Analyzer (Stable Micro System, UK), calibrated by using a 5 kg stainless steel weight. Hardness was measured as the force (Newton) required for a 6 mm diameter cylinder probe (P/6) to penetrate (0.1 mm/s) the surface to a depth of 30% of the height sample. For the measurement of stickiness, a cylinder probe (P/6) penetrated the surface to a depth of 30% of the height sample, and was held there for 3 sec. Stickiness was measured as the force (Newton) required to remove the probe (0.1 mm/sec) from the sample. Twenty samples were used for each testing (Nowakowski and Hartel, 2002).

Measurement of solubility

Solubility of palm sugar cake was determined according to Cano-Chauca *et al.* (2005). A thin slab of sample (approximately 2 g) and distilled water (50 ml) were transferred into a beaker. The mixture was agitated with a magnetic stirrer, using a stirring bar with a size of 2×7 mm at 30^{o}C for 5 min. After that the solution was filtrated through pre-weighed filter paper (Whatman, No. 1) using a vacuum aspirator and immediately dried in vacuum oven at 60^{o}C for 5 h. Then the percentage of solubility value was calculated by weight difference.

Measurement of glass transition temperature

The glass transition temperature (Tg) was determined by the differential scanning calorimetry model DSC7 (Perkin Elmer, U.S.A.). Palm sugar cake (5 mg) was powdered using a mortar and weighted in an aluminum pan, hermetically sealed and then taken for DSC analysis. First a sample was cooled from 25^{o}C to -40^{o}C at the rate of 10^{o}C/min. After that a sample was heated at the rate of 10^{o}C/min from -40^{o}C to 200^{o}C followed by cooling to -40^{o}C at the rate of 120^{o}C/min. A repeat run was then performed.

The reference was an empty pan. The mid-point of Tg was considered as the characteristic temperature of the transition. Only palm sugar cake was measured for Tg.

Measurement of crystallinity

The crystallinity of palm sugar cake was identified by the X-ray diffraction method. The powder sample was placed in a sample holder for the powder X-ray diffraction and the surface was smoothed with a glass slide. The measure was carried out with the powder X-ray diffraction meter using Cu radiation, under operational conditions of 40 kV of potency and 30 mA. Each sample was scanned with the 2θ being between 5^{o} and 40^{o}. Data acquisition was accomplished using a step size of 0.05° and a step time of 1 sec (Bhandari and Hartel, 2002). The crystallinity was calculated by intregrating total peak area.

Microstructure of palm sugar cake

Microstructure of palm sugar cake was determined by Scanning electron microscope (SEM) (FEI Quanta 400). Palm sugar cake was broken off and mounted on SEM stubs using a double backed cellophane tape. The samples were coated with gold and then examined using a SEM.

Determination of pH

The pH value was measured at ambient temperature with a pH meter (Satorious, USA) which calibrated at pH 4.0 and 7.0.

Determination of total acidity

The sample was diluted with distilled water and titrated with 0.01 N NaOH using a few drops of 1% phenolphthalein solution as an indicator. The sample was transferred as a measured quantity (1-3 g) into approximately 10 ml of distilled water. The result was calculated as a percentage of acetic acid for palm sugar cake (Uttraporn, 2006).

Determination of moisture content

The moisture content of palm sugar syrup and palm sugar cake was measured using a vacuum oven. About 2-5 g of the sample was placed in a pre-dried aluminum dish and dried in a vacuum oven at 60^{o}C (pressure<70mmHg) for 6 h. The dried sample was placed in a desiccator and cooled for 0.5 h to room temperature. The weight was recorded, and the percentage moisture based on the initial wet weight was calculated.

Determination of water activity

Water activity of palm sugar cake was measured at room temperature using a water activity meter (Novasina, Thermostanter). The sample was inserted into a sample cup and the measurement was made immediately to restrict moisture transfer from the air to the sample.

Determination of total sugar and reducing sugar

The total sugar and reducing sugar content were quantified by the Lane and Eynon Volumetric method using titration with Fehling's reagents. The results were expressed as gram of glucose per 100 g of sample (Rangana, 1986).

Determination of type and concentration of sugars

The type and concentration of sugar was determined using HPLC (Shimadzu, CR6A Chromatopac) with a Hypersil NH_2 column and refractive index detector. The mobile phase was the solution of acetonitrile and water (80:20), pumped at a flow rate of 1.5 ml/min and an injection volume of 20 µl. The samples were prepared by making appropriate dilutions with distilled water. All sample solutions were passed through a 0.45 µm nylon syringe filter to remove particulates prior to HPLC analysis. D-glucose, D-fructose and sucrose were used as the external standards. The calibration curve of each sugar was plotted between peak areas and concentrations (Stuckel and Low, 1996).

Determination of free amino group content

Free amino group content was determined according to the method of Benjakul and Morrissey (1997). First, the sample was diluted through appropriate dilution. Then 125 µl of the sample was mixed with 2.0 ml of 0.21 M phosphate buffer, pH 8.2, and 1.0 ml of 0.01% TNBS solution was then added. The solutions were mixed thoroughly and placed in a temperature-controlled water bath (Memmert, Bavaris, Germany) at 50^{o}C for 30 min in dark. The reaction was terminated by adding 2.0 ml of 0.1 M sodium sulfite. The mixtures were cooled at room temperature for 15 min. The blank was prepared in the same manner as the samples except that distilled water was used instead of 0.01% TNBS. The absorbance was measured at 420 nm. The free amino group content was expressed in terms of L-leucine.

Determination of 5-Hydroxymethylfurfural content

The palm sugar cake (5-10 g) was dissolved in deionized water and made up to 50 ml with deionized water. After that it was centrifuged at 5,000 rpm for 15 min.

The supernatant was used to measure 5-hydroxymethylfurfural (HMF) content. To determine the HMF content, 2 ml of supernatant was introduced into the tube. Two ml of 12% trichloroacetic acid and 2 ml of 0.025 M thiobabituric acid were subsequently added and mixed thoroughly. The tube with the sample was then placed in a water bath at 40^{o}C. After incubating for 50 min, the tube was cooled immediately, using water, and the absorbance was measured at 443 nm. A calibration curve of HMF was utilised to quantify the HMF concentration (Rattanathanalerk *et al.,* 2005). Only palm sugar syrup and palm sugar cake were measured for HMF content.

Determination of volatile flavour compounds

Volatile flavour compounds were analysed using the HS-SPME-GC-MS technique. A 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber was used (Supelco, Bellefonte, PA, USA). Each sample was powdered and injected (2 g) in a 12 ml headspace vial and then internal standard 2-methyl-3-heptanone was spiked into a sample. After that, the vial was sealed tightly with a crimp cap and a PTFE/silicone septum and equilibrated at 50^{o}C for 30 min in a water bath. A manual SPME holder containing fiber was inserted into the vial and exposed to the sample headspace at 50^{o}C for 15 min. The fiber was then transferred directly into the injector port of the GC-MS system. Thermal desorption of analytes from the fiber in the GC injector port was carried out with an SPME inlet liner (0.75 mm i.d., Supelco) in the splitless mode at a desorption temperature of 240^{o}C. The SPME fiber was conditioned at 250^{o}C for 10 min before starting the first measurement. It was then left in the injection port for re-conditioning during the whole GC run before taking the next sample. GC-MS analysis was conducted using the Agilent 6890 Plus GC/HP 5973 MSD, equipped with an HP-FFAP column (25 m×0.32 mm i.d.×25 µm film thickness). The injector temperature was 240^{o}C. The GC oven temperature was programmed from 40 to 230^{o}C at the rate of 8^{o}C/min and held at 230^{o}C for 10 min. The carrier gas was ultra high purity helium at a constant flow of 1.5 ml/min. The mass spectrometer condition was as follows: MSD

capillary direct-interface temperature was 280^{o}C; the Ionization energy was 70 eV; and the mass range was between 20-450 a.m.u. Positive identification of a component was performed by comparison of the mass spectrum. Tentatively identified compounds were specifically identified in the basis of the mass spectra from the Wiley 275.L mass spectra database (Hewlett-Packard Co.). The integration of peaks was done on HP chemstation software (Hewlett-Packard Co.). The minimum peak area for detection was 10,000 counts.

In this study, the given concentration values were noted as equivalents to the internal standard. The relative concentrations of the investigated compounds (IC) were calculated by relating the areas of the internal standard (IS) to the areas of the compounds of interest.

$$\text{Relative concentration} = \frac{\text{(Peak area of IC)}}{\text{(Peak area of IS)}} \times \text{Concentration of IS}$$

Sensory evaluation

Sensory evaluation was done based on nine-point hedonic scale (1: extremely poor; 3: poor; 5: acceptable; 7: good; 9: excellent). Four attributes were evaluated including colour, texture, flavour and overall acceptable. Colour was evaluated by visual observation. Texture was evaluated by breaking off the sample. Flavour was evaluated by smell and taste the sample. Sixty untrained panelists were used.

Statistical analysis

All analysis and measurements were performed in triplicates. Four formulations of palm sugar cake in each processing method were run using completely randomized design (CRD). The effect of storage temperature (4^{o}C and 30^{o}C) on a given data within each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator and storage time was run using paired test of the

difference. The effect of storage time (0-12 months) on a given data within each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator and storage temperature was run using CRD. Data was subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test (Steel and Torrie, 1980). Analysis was performed using a SPSS package (SPSS 6.0 for windows, SPSS Inc, Chicago, IL).

5.4 Results and Discussion

Effect of sucrose and glucose syrup addition on the physical properties of palm sugar cake

Colour

Colour is an important attribute because it is usually the first property the consumer observes (Saenz *et al.*, 1993). The samples of palm sugar cake that produced from palm sugar syrup with either using an open pan (120^{o}C) or a vacuum evaporator (80^{o}C) were presented in Appendix Figure 11. Colour of all palm sugar cake samples was shown in Table 31 and 32. All samples appeared in red-brown to brown colour shades. This observation was reflected by a low L* value and a high a* value. The L*, a* and b* values of palm sugar cake were significantly affected by the addition of sucrose and glucose syrup ($P<0.05$). Higher L* and b* and lower a* values were found in samples that sucrose and glucose syrup added when compared to samples with no sucrose and glucose syrup added. The highest L* and b* and lowest a* values were found in formulation No. 4 for both of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator (Table 38 and 39). Nonenzymatic browning reactions including Maillard reaction and Caramelisation are responsible for brown colour of palm sugar cake. Many factors affected the colour of palm sugar cake that caused by nonenzymatic browning reactions including processing temperature,

processing time and formulation of palm sugar cake. Processing temperature and time was controlled in all treatments in this study. Hence, the different properties of each sample may depend on its formulation. Samples that produced from 100% palm sugar syrup (formulation No. 1) with either using an open pan or a vacuum evaporator took place the highest nonenzymatic browning reactions. This probably due to this sample contained the highest reducing sugar content. Moreover, the addition of sucrose and glucose syrup in its formulation can reduce nonenzymatic browning reactions during the production of palm sugar cake. When sucrose was added, nonreducing sugar, can not precipitate with protein via Maillard reaction. Glucose syrup molecules might impede nonenzymatic browning reactions by preventing the reaction between amino groups and reducing sugars. In addition, all palm sugar cake samples that produced from palm sugar syrup with using an open pan presented higher a* and lower L* and b* value than those that produced from palm sugar syrup with using a vacuum evaporator. This probably due to palm sugar syrup that produced by a vacuum evaporator contained lower reducing sugar content than that produced by an open pan.

Intermediate browning product and browning intensity

Intermediate browning product (IBP) and browning intensity (BI) of palm sugar cake that produced by palm sugar syrup obtained from an open pan and a vacuum evaporator were showed in Table 31 and 32. The IBP and BI of palm sugar cake were significantly affected by the addition of sucrose and glucose syrup ($P<0.05$). Lower IBP and BI were found in a sample that sucrose and glucose syrup added when compared to samples with no sucrose and glucose syrup added. The highest IBP and BI were found in formulation No. 1 in both of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. These data could be explained as high reducing sugar content in formulation No. 1. Thus, the addition of sucrose and glucose syrup can reduce IBP and BI of palm sugar cake. Furthermore, samples was added only sucrose presented higher IBP and BI than those of samples that contained glucose syrup

and sucrose added. These phenomena could be attributed to sucrose added that may be hydrolysed to invert sugar (glucose and fructose) and caused in an increase in substrate of nonenzymatic browning reactions during heating. In addition, all palm sugar cake samples that produced from palm sugar syrup with using an open pan presented higher IBP and BI than those that produced from palm sugar syrup with using a vacuum evaporator. This probably due to palm sugar syrup that produced by a vacuum evaporator contained lower reducing sugar content than that produced by an open pan.

During the production of palm sugar cake, Caramelisation had more effect on browning development than the Maillard reaction. This probably due to Caramelisation greatly progresses at temperature approximately 120^{o}C or above (Kroh, 1994; Eskin, 1990) as well as the processing temperature used during palm sugar cake production.

Table 31. Physical properties of palm sugar cake produced from palm sugar syrup with using an open pan

Formulation	Physical properties				
	L*	a*	b*	IBP[a]	BI[b]
1	25.01 ± 0.91^{d}	9.73 ± 0.43^{a}	12.35 ± 0.28^{c}	0.75 ± 0.02^{a}	1.47 ± 0.01^{a}
2	31.26 ± 0.42^{c}	9.45 ± 0.44^{ab}	14.83 ± 0.29^{b}	0.45 ± 0.01^{b}	1.29 ± 0.03^{b}
3	33.60 ± 0.38^{b}	8.82 ± 0.51^{b}	15.44 ± 0.37^{b}	0.39 ± 0.01^{c}	1.06 ± 0.03^{c}
4	40.85 ± 0.92^{a}	7.60 ± 0.26^{c}	18.46 ± 0.41^{a}	0.32 ± 0.01^{d}	0.73 ± 0.01^{d}

Note: Each value is the mean ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

[a] The IBP was measured by diluting the sample with distilled water in the ratio of sample:water = 1:8.

[b] The BI was measured by diluting the sample with distilled water in the ratio of sample:water = 1:4.

Table 32. Physical properties of palm sugar cake produced from palm sugar syrup with using a vacuum evaporator

Formulation	Physical properties				
	L*	a*	b*	IBP[a]	BI[b]
1	36.53 ± 0.32[d]	7.87 ± 0.20[a]	16.61 ± 0.22[d]	0.44 ± 0.01[a]	1.06 ± 0.02[a]
2	49.65 ± 0.66[c]	7.08 ± 0.38[b]	17.07 ± 0.19[c]	0.35 ± 0.03[b]	0.72 ± 0.01[b]
3	43.94 ± 1.03[b]	6.30 ± 0.18[c]	18.46 ± 0.26[b]	0.32 ± 0.01[c]	0.51 ± 0.01[c]
4	49.65 ± 0.66[a]	4.78 ± 0.39[d]	20.28 ± 0.16[a]	0.26 ± 0.01[d]	0.47 ± 0.01[d]

Note: Each value is the mean ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

[a] The IBP was measured by diluting the sample with distilled water in the ratio of sample:water = 1:8.

[b] The BI was measured by diluting the sample with distilled water in the ratio of sample:water = 1:4.

Hardness and stickiness

Hardness is the property of a material that enables it to resist deformation, usually by penetration or compression. It is generally characterised by strong intermolecular bonds such as in a structure of a sample. During the production of palm sugar cake, syrup was concentrated until crystalline obtained. Thus, degree of crystallisation of sugar affected the hardness of palm sugar cake. Texture of palm sugar cake was measured in terms of hardness and stickiness as presented in Table 33 and 34. Hardness of palm sugar cake was significantly affected by the addition of sucrose and glucose syrup ($P<0.05$). Hardness increased with increasing sucrose content. On the other hand, the increase in glucose syrup caused a decrease in hardness of palm sugar cake. The highest hardness was found in formulation No. 2 in both an open pan and a vacuum evaporator methods. This could be explained by the lowest reducing sugar content contained in formulation No. 2. The lowest hardness was found in a sample that produced from 100% palm sugar syrup with either using an open pan or a vacuum evaporator (formulation No. 1). This result is directly related to this formulation that contained the highest reducing sugar content. Moreover, palm sugar cake added glucose syrup

(formulation No. 3 and No. 4) presented lower hardness than those added only sucrose (formulation No. 2). This probably due to the presence of reducing sugar contents as well as fructose and glucose or polysaccharide contents such as glucose syrup. These ingredients could be retarded sugar crystallisation and caused low hardness of a product (Faria *et al.,* 2003; Aider *et al.,* 2006; Abdel-Rahman *et al.,* 2008).

Some food products exhibit a marked tendency to adhere to a contact surface, which is generally known as stickiness. The stickiness in food products greatly depends on water content, storage temperature and time. The interaction of water with solids is the prime cause of stickiness in low moisture food. Water is a suitable catalyst for stickiness (Adhikari *et al.,* 2001). Stickiness of palm sugar cake was significant affected by the addition of sucrose and glucose syrup ($P<0.05$). Stickiness decreased with increasing sucrose content. The lowest stickiness was found in formulation No. 2 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. On the other hand, the highest stickiness of samples that produced from palm sugar syrup with either using an open pan or a vacuum evaporator was found in formulation No. 1. This probably due to this formulation contained the highest reducing sugar content. Reducing sugars such as fructose and glucose are very hygroscopic. Palm sugar cake that contained high reducing sugar can easily absorbed water, resulting in high stickiness. Additionally, an increase in glucose syrup caused an increase in stickiness of palm sugar cake. Samples added glucose syrup (formulation No. 3 and No. 4) presented higher stickiness than those only sucrose added (formulation No. 2). Glucose syrup is a polydextrose obtained from starch hydrolysis. There are many -OH group in a structure of glucose syrup that can interact with water molecules via Hydrogen bond (Hartel, 2001). Moreover, glucose syrup acts as a humectant that has a property of absorbing water (Branen *et al.,* 1990). Hence, glucose syrup can increase in the stickiness and reduce the hard texture of palm sugar cake.

Solubility

Solubility is a hydration process. The interaction between water and solute molecules are called hydration. In the presence of polar molecules such as sugars and water, these interactions are dominated by hydrogen bonding (Joupplia, 2006). The addition of sucrose and glucose syrup had a significant effect on the solubility of palm sugar cake ($P<0.05$). The addition of sucrose caused a decrease in solubility of palm sugar cake. The lowest solubility was found in formulation No. 2 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This probably due to this formulation presented the highest crystallinity or rigid structure. Thus, water molecule is more difficult to penetrate into this crystallised structure than amorphous structure (Whistler and James, 1996). On the other hand, the highest solubility was found in formulation No. 1 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This probably due to this formulation contained the highest reducing sugar content. Reducing sugar is hygroscopic and rapidly absorbed water, results in the increase in solubility of a product. In addition, glucose syrup increased the solubility of palm sugar cake. This probably due to glucose syrup function by interfering with the orderly intermolecular forces, mainly hydrogen bonds, that from between sugar molecules. The sugar hydroxyl groups then become more available to precipitate in hydrogen bonding to ambient water (Whistler and James, 1996).

Crystallinity

Crystallinity of all palm sugar cake samples was showed in Table 33 and 34. XRD patterns of all palm sugar cake samples were shown in Appendix Figure 12 and 13. The addition of sucrose and glucose syrup had a significantly effect on crystallinity ($P<0.05$). Sucrose increased crystallinity of palm sugar cake as indicated by the highest

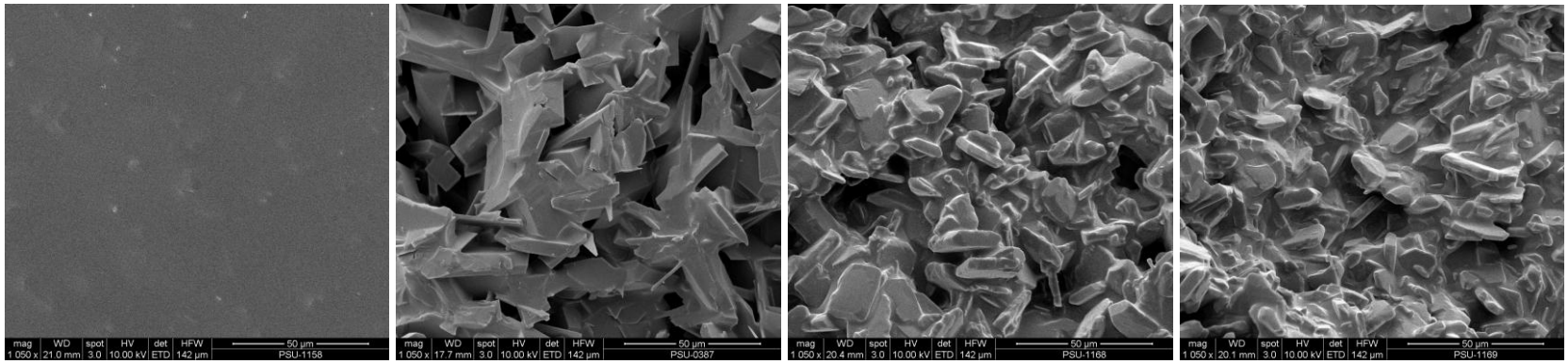
crystallinity was found in formulation No. 2 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. However, the lowest crystallinity was found in formulation No. 1 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This probably due to this formulation contained the highest reducing sugar content. Most sugars, especially sucrose can be induced a process of crystallisation. However, it is difficult to obtain certain reducing sugars in crystalline form because the presence of anomers and ring isomers in solution make the reducing sugars intrinsically "impure". Thus, crystallisation of non reducing sugar could be retarded by the presence of reducing sugar (Shallenberger and Birch, 1989; Hartel, 2001). Glucose syrup decreased crystallinity of palm sugar cake as indicated by the reduction of crystallinity. This was found in palm sugar cake when increasing of glucose syrup content. This probably due to glucose syrup molecules interfere the crystallisation of sucrose and reduce the solubility of sucrose (Gabarra and Hartel, 1998; Howell and Hartel, 2001). Moreover, crystallinity of all palm sugar cake that produced from palm sugar syrup with using an open pan was lower than those that produced from palm sugar syrup with using a vacuum evaporator. This could be explained by palm sugar syrup that produced by an open pan contained higher reducing sugar than that produced by a vacuum evaporator.

Microstructure

Microstructure structure of all palm sugar cake samples was shown in Figure 38 and 39. The addition of sucrose and glucose syrup influenced the microstructure of palm sugar cake. It was found an agglomerated sugar crystal was observed in all samples, except only sample produced from 100% palm sugar syrup with using an open pan (formulation No. 1) that presented liqufied. This result was in accordance with the lowest crystallinity was found in formulation No. 1. This probably due to palm sugar syrup produced by an open pan contained the highest reducing sugar

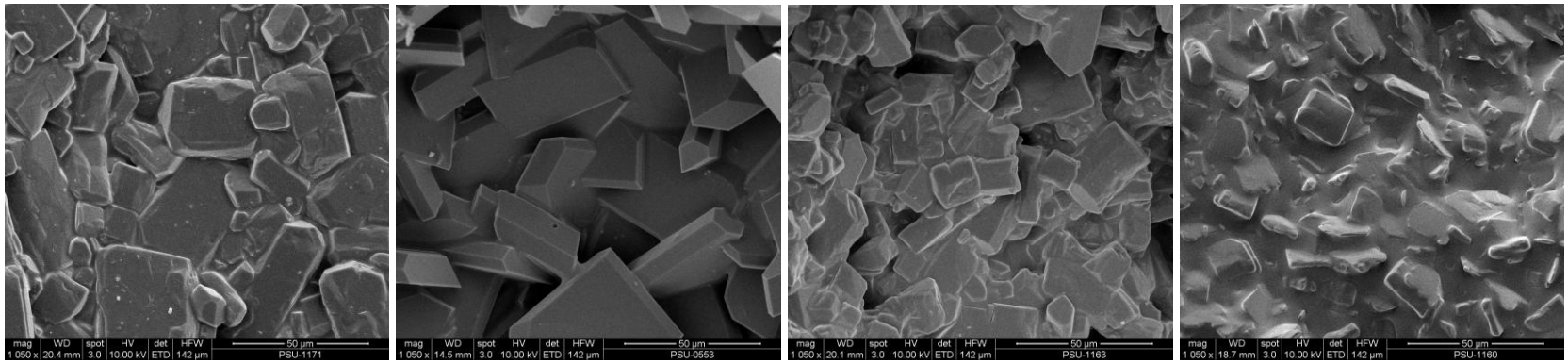
content. Reducing sugar generally can act as a barrier for crystallisation in palm sugar cake (Hartel, 2001).

On the other hand, palm sugar cake that produced from 100% palm sugar syrup with using a vacuum evaporator presented a clear crystal. This probably due to palm sugar syrup produced by a vacuum evaporator contained higher sucrose content than that produced by an open pan, resulting in lower amorphous fraction. The clearest and largest crystal was found in formulation No. 2 of palm sugar cake that produced from palm sugar syrup with using a vacuum evaporator, followed by formulation No. 2 of palm sugar cake that produced from palm sugar syrup with using an open pan. This result was in agreement with the highest crystallinity was found in formulation No. 2 of each palm sugar cake that produced from palm sugar syrup with either using a vacuum evaporator or an open pan. This indicated that the addition of sucrose can promote sugar crystallisation. The shape of sugar crystal is monoclinic form that referred to sucrose crystal (Hartel, 2001). Lower crystal size was found in formulation No. 3 and formulation No. 4 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. Additionally, samples added glucose syrup (formulation No. 3 and No. 4) presented a film of glucose syrup coating the sugar crystals. High molecular weight compound such as glucose syrup can inhibit sugar crystallisation, resulting in higher amorphous fraction was found in samples that added glucose syrup compared with samples that added only sucrose. Similar size of sucrose crystal between formulation No. 3 and No. 4 was found. While the hardness and crystallinity between formulation No. 3 and No. 4 presented significantly different ($P<0.05$). This probably due to the effect of glucose syrup added. Formulation No. 4 contained higher amount of glucose syrup than formulation No. 3 as shown by a film of glucose syrup coating among sugar crystals. This phenomenon may cause a lower hardness and crystallinity in formulation No. 4 when compared to formulation No. 3, although the crystal size was not different.



Formulation 1 Formulation 2 Formulation 3 Formulation 4

Figure 38. Microstructure of palm sugar cake produced from palm sugar syrup with using an open pan



Formulation 1 Formulation 2 Formulation 3 Formulation 4

Figure 39. Microstructure of palm sugar cake produced from palm sugar syrup with using a vacuum evaporator

Glass transition temperature

Glass transition temperature (Tg) of all palm sugar syrup samples was shown in Table 33 and 34. Tg is the temperature at which the amorphous phase is converted between rubbery and glassy states. In food systems, Tg is mainly affected by the water content and the average molecular weight (Joupplia, 2006; Sablani *et al.*, 2007). The addition of sucrose and glucose syrup had a significantly effect on Tg ($P<0.05$). Sucrose or glucose syrup added can increase Tg of palm sugar cake. The lowest Tg was observed in formulation No. 1 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This probably due to these samples contained the highest reducing sugar content. Monosaccharide such as fructose and glucose act as plasticizers for higher molecular weight carbohydrates (sucrose). Tg of fructose and glucose generally are approximately $5^{o}C$ and $31^{o}C$, respectively (Roos, 1995). Thus, the presence of fructose and glucose can decrease Tg of palm sugar cake. On the other hand, the highest Tg was found in formulation No. 4 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. Tg of sucrose and glucose syrup are approximately $67^{o}C$ and $90^{o}C$, respectively (Roos, 1995). Thus, the addition of sucrose and glucose syrup can increase Tg of product. Moreover, Tg of all palm sugar cake that produced from palm sugar syrup with using an open pan was lower than those that produced from palm sugar syrup with using a vacuum evaporator. This could be explained by lower inversion of sucrose took place in palm sugar syrup produced by a vacuum evaporator due to this process used low heating temperature and short heating time when compared with an open pan process.

Table 33. Physical properties of palm sugar cake produced from palm sugar syrup with using an open pan

Formulation	Physical properties				
	Hardness (N)	Stickiness (N)	Solubility (%)	Tg (^{o}C)	Crystallinity (%)
1	16.02 ± 3.78^{d}	0.44 ± 0.08^{a}	98.35 ± 0.21^{a}	-3.72 ± 0.25^{d}	63.29 ± 0.14^{d}
2	48.00 ± 5.88^{a}	0.20 ± 0.05^{c}	96.34 ± 0.19^{c}	22.72 ± 0.23^{c}	78.07 ± 0.04^{a}
3	31.53 ± 5.00^{b}	0.31 ± 0.13^{b}	97.51 ± 0.34^{b}	24.41 ± 0.05^{b}	75.51 ± 0.05^{b}
4	26.32 ± 6.35^{c}	0.31 ± 0.10^{d}	97.43 ± 0.41^{b}	26.43 ± 0.08^{a}	73.20 ± 0.10^{c}

Note: Each value is the mean ± standard deviation.

The different superscripts in the same column denote the significant different (P<0.05).

Table 34. Physical properties of palm sugar cake produced from palm sugar syrup with using an vacuum evaporator

Formulation	Physical properties				
	Hardness (N)	Stickiness (N)	Solubility (%)	Tg (^{o}C)	Crystallinity (%)
1	24.14 ± 1.91^{d}	0.20 ± 0.05^{a}	98.15 ± 0.08^{a}	7.31 ± 0.05^{d}	70.14 ± 0.04^{d}
2	70.92 ± 5.74^{a}	0.13 ± 0.14^{b}	95.51 ± 0.31^{c}	28.52 ± 0.04^{c}	82.25 ± 0.04^{a}
3	45.63 ± 6.42^{b}	0.20 ± 0.09^{a}	97.16 ± 0.13^{b}	30.28 ± 0.03^{b}	80.52 ± 0.07^{b}
4	34.43 ± 5.85^{c}	0.15 ± 0.07^{a}	97.15 ± 0.11^{b}	33.66 ± 0.04^{a}	78.13 ± 0.09^{c}

Note: Each value is the mean ± standard deviation.

The different superscripts in the same column denote the significant different (P<0.05).

Effect of sucrose and glucose syrup addition on the chemical properties of palm sugar cake

pH and total acidity

Table 35 and 36 showed chemical properties of palm sugar cake samples. The addition of sucrose and glucose syrup affected the pH and total acidity of palm sugar cake. The lowest pH and the highest total acidity were observed in formulation No. 1 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This probably due to this formulation contained the highest

reducing sugar content. High reducing sugar content promotes the nonenzymatic browning reactions during heating. The reduction in pH values and increment of total acidity occurring in Maillard reaction was due to the formation of organic acids such as formic acid and acetic acid (Ames, 1998; Brands and Van Boekel, 2002; Lertittikul *et al.*, 2007). Acetic acid and formic acid were formed via the Maillard reaction (Martins and Van Boekel, 2003). The pH of all palm sugar cake samples that added sucrose and glucose syrup was higher than those no sucrose and glucose syrup added. On the other hand, the total acidity was lower. This probably due to samples that added sucrose and glucose syrup had lower nonenzymatic browning reactions than those no sucrose and glucose syrup added.

Moisture content and water activity

Moisture content (MC) and water activity (Aw) of palm sugar cake were showed in Table 35 and 36. Aw is the intrinsic product characteristic that most influences the microbial ecology of sugar-rich product. It defined as free moisture content in product (de Rodriguez, 2004). Both of moisture content and water activity are highly important for the shelf life of palm sugar cake during storage. The addition of sucrose and glucose syrup had a significantly effect on MC ($P<0.05$). On the other hand, there was no significant effect of sucrose and glucose syrup addition on Aw ($P\geq0.05$). MC decreased with increasing sucrose content as evidenced by the lowest MC was found in a formulation No. 2 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. The addition of sucrose added can reduce the ratio of invert sugar (hygroscopic) during palm sugar cake production. In addition, sucrose can promote crystalline structure, resulting in lower water holding in a sample (Bell and Labuza, 2000). Higher MC was observed in a sample that produced from 100% palm sugar syrup (formulation No. 1) and samples added glucose syrup (formulation No. 3 and No. 4) when compared to a sample that added only sucrose. A sample that produced from 100% palm sugar syrup (formulation No. 1) contained the

highest reducing sugar content. As the amount of glucose or fructose increased, the moisture level also increased, indicating the presence of increased amount of amorphous fraction in sample (Saleki-Gerhardt *et al.,* 1994; Bhandari and Hartel, 2002). Additionally, moisture content of a sample that added glucose syrup and sucrose were higher than those added only sucrose. As mentioned previously, glucose syrup acts as a humectant that absorbed water to its molecule in term of bound form, thus water activity was reduced while a sample still kept moist (Branen *et al.,* 1990).

Total sugar, reducing sugar, type and concentration of sugar

Type and concentration of sugar in palm sugar cake were presented in Table 37 and 38. Type and concentration of sugar in palm sugar cake were significant affected by the addition of sucrose and glucose syrup ($P<0.05$). It was found that all palm sugar cake samples consisted of mainly three sugars including sucrose, fructose and glucose. However, maltose was detected in some formulations e.g. formulation No. 3 and 4). The addition of sucrose and glucose syrup can reduce an increase of reducing sugar content in palm sugar cake. The highest fructose and glucose contents were observed in formulation No. 1 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. The presence of fructose and glucose is responsible for inversion reaction during heating (Whalen and Morselli, 1985). This indicated that formulation No. 1 took place the highest inversion reaction. The lowest fructose and glucose content were observed in formulation No. 2 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. During heating, sucrose in palm sugar syrup and sucrose added were hydrolyzed to glucose and fructose. This reaction is accelerated by thermal process and acid condition (Wiene and Shallenberger, 1988). Low pH in formulation may responsible for the highest inversion reaction that obtained in formulation No. 1 as mentioned previously. Glucose syrup is the most viable substitute for sugar. It is an aqueous solution of several compounds principally polydextrose and trace glucose and maltose.

From the results, maltose can be detected in a sample that added glucose syrup (formulation No. 3 and No. 4). This amount of maltose can be increased with increasing glucose syrup content.

HMF content

HMF is an indicator of heat stress to foods during processing and storage because of its toxicological status (Kukurova *et al.,* 2005). HMF resulted from the dehydration of hexoses under acidic condition. It was formed as an intermediate in Maillard reaction and Caramelisation. These reactions can be produced when the materials containing fructose and glucose were subjected to heat. HMF content in palm sugar cake was significant affected by the addition of sucrose and glucose syrup ($P<0.05$). The addition of sucrose and glucose syrup can reduce HMF formation in palm sugar cake. The highest HMF content was observed in formulation No. 1 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This result indicated that this formulation took place the highest nonenzymatic browning reactions. HMF content of samples that added sucrose and glucose syrup was lower than those without the addition of sucrose and glucose syrup. This could be attributed to the decrease of reducing sugar content during palm sugar cake production.

HMF content of all palm sugar cake samples was found to be varied from 66.14 to 319.62 mg/kg as shown in Table 35 and 36. It can be noticed that HMF content of all samples was higher than the allowed maximum limit of 40 mg/kg as recommended by Codex Alimentarius (Turhan *et al.,* 2008). HMF content of all palm sugar cake sampkes that produced from palm sugar syrup with using an open pan was ranged from 136.81 to 319.62 mg/kg. HMF content of all palm sugar cake samples that produced from palm sugar syrup with using a vacuum evaporator was ranged from 66.14 to 107.06 mg/kg. All palm sugar cake samples that produced from palm sugar syrup with using a vacuum evaporator had HMF content lower than those that produced from palm sugar syrup with using an open pan.

Free amino group content

At the early stage of Maillard reaction, terminal α-amino groups of peptides and ε-NH_2 groups of lysine react with the carbonyl functions of reducing sugars present in the reaction medium. Thus the loss of available primary amino groups is another indicator used to compare sugar reactivity in Maillard reaction (Laroque *et al.*, 2008). Therefore, the free amino group content can be used to evaluate Maillard reaction in palm sugar cake. Free amino group content in palm sugar cake was significant affected by the addition of sucrose and glucose syrup ($P<0.05$). The addition of sucrose and glucose syrup can reduce the loss of free amino group content in palm sugar cake. The lowest free amino group content remaining was observed in formulation No. 1 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This result can be confirmed that these formulations had the highest Maillard reaction during heating. The α- or ε-NH_2 groups of amino acids or proteins, covalently attached to a sugar to form glycated proteins to a greater extent when reducing sugar increased as found in formulation No. 1. The first glycation product, or Schiff base, rearranges to a more stable ketoamine or Amadori product. The Amadori products can then form cross-links between adjacent proteins or with other amino groups, resulting in polymeric aggregates called advanced glycation end-products (Friedman, 1996). The highest free amino group content remaining was observed in formulation No. 4 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This probably due to this formulation contained low reducing sugar content. Moreover, glucose syrup molecules might impeded nonenzymatic browning reactions by functioning as the bulky molecules preventing the reaction between amino group and reducing sugar.

Table 35. Chemical properties of palm sugar cake produced from palm sugar syrup with using an open pan

Formulation	Chemical properties					
	pH	Total acidity (%)	Moisture content (%)	Aw	HMF (mg/kg)	Free amino group (mg/g)
1	4.67 ± 0.02^{d}	0.26 ± 0.01^{a}	6.43 ± 0.15^{a}	0.68 ± 0.02^{ns}	319.62 ± 1.15^{a}	0.85 ± 0.03^{d}
2	4.88 ± 0.01^{c}	0.17 ± 0.04^{b}	5.11 ± 0.35^{b}	0.68 ± 0.01^{ns}	237.74 ± 0.92^{b}	1.09 ± 0.01^{c}
3	4.93 ± 0.01^{b}	0.16 ± 0.01^{c}	6.47 ± 0.45^{a}	0.68 ± 0.01^{ns}	202.12 ± 0.57^{c}	1.39 ± 0.01^{b}
4	4.96 ± 0.01^{a}	0.14 ± 0.01^{c}	6.47 ± 0.07^{a}	0.68 ± 0.01^{ns}	136.81 ± 0.47^{d}	1.52 ± 0.02^{a}

Note: Each value is the mean ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

Table 36. Chemical properties of palm sugar cake produced from palm sugar syrup with using a vacuum evaporator

Formulation	Chemical properties					
	pH	Total acidity (%)	Moisture content (%)	Aw	HMF (mg/kg)	Free amino group (mg/g)
1	5.11 ± 0.01^{d}	0.12 ± 0.01^{a}	6.48 ± 0.28^{a}	0.68 ± 0.02^{ns}	107.06 ± 1.12^{a}	1.87 ± 0.02^{d}
2	5.22 ± 0.01^{c}	0.13 ± 0.01^{b}	4.77 ± 0.13^{b}	0.68 ± 0.01^{ns}	94.41 ± 0.84^{b}	2.04 ± 0.01^{c}
3	5.28 ± 0.01^{b}	0.10 ± 0.01^{c}	6.22 ± 0.17^{a}	0.68 ± 0.01^{ns}	74.14 ± 0.39^{c}	2.35 ± 0.03^{b}
4	5.32 ± 0.01^{a}	0.09 ± 0.01^{d}	6.45 ± 0.29^{a}	0.68 ± 0.01^{ns}	66.14 ± 0.70^{d}	2.50 ± 0.01^{a}

Note: Each value is the mean ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

Table 37. Types of sugar and their concentrations of palm sugar cake produced by palm sugar syrup with using an open pan

Formulation	Type of sugar					
	Reducing sugar (%)	Total sugar (%)	Fructose (%)	Glucose (%)	Maltose (%)	Sucrose (%)
1	20.58 ± 0.30^{a}	90.76 ± 0.44^{a}	6.18 ± 0.12^{a}	12.58 ± 0.08^{a}	0.00 ± 0.00^{c}	70.51 ± 0.57^{b}
2	10.51 ± 0.20^{d}	90.02 ± 0.43^{a}	3.18 ± 0.23^{c}	6.08 ± 0.12^{d}	0.00 ± 0.00^{c}	78.19 ± 0.05^{a}
3	12.10 ± 0.13^{c}	82.07 ± 0.73^{b}	4.28 ± 0.32^{b}	7.26 ± 0.14^{c}	1.07 ± 0.06^{b}	69.85 ± 0.25^{b}
4	13.61 ± 0.17^{b}	72.27 ± 0.77^{c}	4.24 ± 0.05^{b}	7.64 ± 0.21^{b}	2.16 ± 0.12^{a}	58.83 ± 0.34^{c}

Note: Each value is the mean ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

Table 38. Types of sugar and their concentrations of palm sugar cake produced from palm sugar syrup with using a vacuum evaporator

Formulation	Type of sugar					
	Reducing sugar (%)	Total sugar (%)	Fructose (%)	Glucose (%)	Maltose (%)	Sucrose (%)
1	10.09 ± 0.13^{a}	89.55 ± 0.93^{a}	3.13 ± 0.08^{a}	6.34 ± 0.28^{a}	0.00 ± 0.00^{c}	78.98 ± 0.06^{b}
2	3.55 ± 0.02^{d}	90.19 ± 0.45^{a}	1.05 ± 0.11^{c}	2.07 ± 0.04^{d}	0.00 ± 0.00^{b}	89.97 ± 0.90^{a}
3	5.61 ± 0.04^{c}	82.31 ± 0.36^{b}	1.43 ± 0.00^{bc}	4.22 ± 0.09^{b}	1.10 ± 0.12^{b}	76.02 ± 0.32^{c}
4	7.73 ± 0.13^{b}	72.37 ± 0.50^{c}	1.75 ± 0.40^{b}	3.03 ± 0.13^{c}	2.17 ± 0.02^{a}	64.68 ± 0.74^{d}

Note: Each value is the mean ± standard deviation.

The different superscripts in the same column denote the significant different ($P<0.05$).

Volatile flavour compounds

Volatile flavour compounds in palm sugar cake samples were investigated using Headspace Soild Phase Microextraction technique (HS-SPME) coupled with Gas chromatography-mass spectrometry (GC-MS). Similar profiles of volatile flavour compounds from all palm sugar cake samples were noticed. However, the different of volatile components identified in palm sugar cake samples is possibly due to the different of property of palm sugar syrup as influenced by processing method and ingredients added. Volatile flavour compouds were commonly found in all samples, consisting of 2 alcohols, 1 ketone, 1 acid, 1 pyrrole, 10 furans and 6 pyrazines. Table 39 and 40 showed the composition of volatile flavour compounds in palm sugar cake samples.

The formation of volatile flavour compounds from Maillard reaction and Caramelisation were found in palm sugar cake including furan, pyrazine and pyrrole. These compounds have been reported in various plant syrup such as maple syrup, coconut syrup and birch syrup (Kallio, 1989; Akochi *et al.,* 1994; Akochi *et al.,* 1997; Purnomo, 2007). These volatile flavour compounds are typical flavours in palm sugar cake. Furan derivatives such as 2-furanmethanol, 2-furancarboxaldehyde, 2-furancarboxaldehyde, 2-acetylfuran, 5-methylfurfural, 5-hydroxymethylfurfural, 2-furan methane thiol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2-methyl dihydro-3(2H)-furanone, 2-methyl dihydro-2(3H)-furanone and 2,3-dihydro-3,5-dihydroxy-6M-pyran-4-one were found in all samples. These furans contribute to the sweet, caramel, cook sugar or burnt sugar. Furans account for the caramel-like odour of heated carbohydrates (Ho *et al.*, 2007). The formation of furans can take place through two pathways such as lipid peroxidation and degradation of carbohydrates involved Maillard reaction. In this study, furan is known to be formed by the degradation of carbohydrates. Furfural is formed from the Amadori intermediate of the Maillard reaction by 1,2-enolization followed by a deamination and dehydration and can be formed via Caramelisation (Nursten, 1980; Meynier and Mottram, 1995). Pyrazine derivatives were detected in all palm sugar cake samples including to 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine,

methyl pyrazine, 2-methoxy-6-methylpyrazine and 2-ethyl-3 5-dimethylpyrazine. All these pyrazines are correlated with sensory attributes, such as nutty, sweet and roasty odour which consequently contribute to typical flavour of palm sugar cake (Ho *et al.*, 2007). The presence of pyrazines in palm sugar cake indicated that not only Caramelisation, but also the Maillard reaction took place during the production of palm sugar cake (Apriyantono *et al.*, 2002). Normally, alkyl pyrazine are commonly found in heated food. One of the main routes of pyrazines formation is through the Strecker degradation of amino acids in the presence of dicarbonyl compounds. In addition to the Strecker aldehyde, this reaction yields an aminocarbonyl compounds, and the condensation of these two molecules results in an alkyl pyrazine (Amrani-Hemaimi *et al.*, 1995; Hwang *et al.*, 1995; Meynier and Mottram, 1995). One pyrrole (acetylpyrrol) was identified in palm sugar cake. This pyrrole tends to contribute to sweet or burnt sugar attributes. There are two pathways to form a pyrrole. First pathway is the interaction between an amino acid and a 3-dexoyhezosone through the Strecker degradation followed by dehydration and ring closure. The second pathway is the reaction of a furan with an amine or an amino acids (Rizzie, 1974; Hwang *et al.*, 1995). From the results, the highest pyrazines, furans and pyrrole were obtained in formulation 1 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This probably due to these formulation contained the highest reducing sugar the acts as a substrate of nonenzymatic browning reactions during the production of palm sugar cake by heating process.

Phenethyl alcohol or benzene ethanol had been reported to possess a sweet or rose aroma. It is derived from L-phenylalanine through metabolic reaction of yeast (Soufleros *et al.*, 2004). The 3-hydroxy-2-butanone was reported to be responsible for sweet odour (Cheetham, 2002). This compound is the main volatile flavour compounds in palm sap as reported by Taiapaiboon (2004). The highest remaining of 3-hydroxy-2-butanone was found in formulation 1 of each palm sugar cake produced from palm sugar syrup with either using an open pan or a vacuum evaporator. Acetic acid can be found in palm sugar cake. This probably due to the α-dicarbonyl compounds are unstable and

undergo a cleavage reaction (at the C-C bond), resulting in acetic acid during Maillard reaction (Martins and Van Boekel, 2005). The highest acetic acid was obtained in formulation 1 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This probably due to these samples took place the highest nonenzymatic browning reactions.

Table 39. Volatile flavour compounds (ppb) of palm sugar cake produced from palm sugar syrup with using an open pan

RT[A]	RI[B]	Volatile flavour compounds	Attribute[C]	Formulation			
				1	2	3	4
		Furans					
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	6175	4195	3284	1912
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	4846	4288	3375	2715
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	656	489	316	191
12.88	2344	5-hydroxymethylfurfural	caramel	5058	3226	2767	1080
8.81	1608	5-methylfurfural	sweet, caramel, nutty	9584	8116	7135	6259
8.35	1537	2-acetylfuran	sweet, caramel	1477	1314	1084	1089
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	1569	1239	917	738
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	197	190	157	141
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	1217	955	786	613
11.72	2577	2,3-dihydro-3,5-dihydroxy-6M-pyran-4-one	caramel, sweet, burnt sugar	1473	1377	1178	1161
		Pyrazines					
5.81	1292	methyl pyrazine	nutty, roasty	3391	3112	2275	2205
6.84	1314	2,3-dimethylpyrazine	roasted nut, sweet	687	516	428	296
6.50	1309	2,5-dimethylpyrazine	sweet, roasty	669	557	457	331
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	10140	9756	6729	5001
7.82	1470	2-ethyl-3,5-dimethylpyrazine	roasty	2675	2237	2056	1839
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	1021	839	652	606
		Alcohols					
10.36	1951	phenethyl alcohol	sweet	282	197	198	149
8.81	1587	2,3-butanediol	fruity	553	366	343	399
		Acid					
7.84	1473	acetic acid	sour	8112	7662	4048	4019
		Ketones					
6.00	1307	3-hydroxy-2-butanone	sweet	892	504	454	522
		Pyrrole					
10.62	2023	acetylpyrrole	sweet, burnt sugar	1021	839	652	606

Note: [A] RT refers to retention time (min); [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24})

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

Table 40. Volatile flavour compounds (ppb) of palm sugar cake produced from palm sugar syrup with using a vacuum evaporator

RT[A]	RI[B]	Volatile flavour compounds	Attribute[C]	Formulation			
				1	2	3	4
		Furans					
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	4306	2178	1920	1571
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	2425	2180	1668	1060
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	440	407	295	220
12.88	2344	5-hydroxymethylfurfural	caramel	3570	2503	1287	971
8.81	1608	5-methylfurfural	sweet, caramel, nutty	7410	4827	3467	2733
8.35	1537	2-acetylfuran	sweet, caramel	1053	758	442	432
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	1033	846	476	393
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	125	109	107	108
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	833	695	440	396
11.72	2577	2,3-dihydro-3,5-dihydroxy-6M-pyran-4-one	caramel, sweet, burnt sugar	1211	1012	886	788
		Pyrazines					
5.81	1292	methyl pyrazine	nutty, roasty	2647	1964	1504	1342
6.84	1314	2,3-dimethylpyrazine	roasted nut, sweet	452	350	223	192
6.50	1309	2,5-dimethylpyrazine	sweet, roasty	44	323	204	123
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	7672	4751	4073	2542
7.82	1470	2-ethyl-3,5-dimethylpyrazine	roasty	2146	1828	1647	1497
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	678	557	323	262
		Alcohols					
10.36	1951	phenethyl alcohol	sweet	151	115	101	106
8.81	1587	2,3-butanediol	fruity	433	221	202.	202
		Acid					
7.84	1473	acetic acid	sour	6476	4402	3442	3311
		Ketone					
6.00	1307	3-hydroxy-2-butanone	sweet	1219	772	779	709
		Pyrrole					
10.62	2023	acetylpyrrole	sweet, burnt sugar	678	557	323	262

Note: [A] RT refers to retention time (min); [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24})

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

Effect of sucrose and glucose syrup addition on the sensory evaluation of palm sugar cake

The sensory evaluation of palm sugar cake was shown in Table 41 and 42. It can be seen that the addition of sucrose and glucose syrup affected the colour, texture, flavour and overall acceptability of palm sugar cake. The lowest mean score of colour was found in formulation No. 1 and 4 for palm sugar cake that produced from palm sugar syrup with using an open pan. This probably due to formulation No. 1 had dark colour while formulation No. 4 presented pale colour, resulting in unacceptability of a product. A higher mean score of colour was observed in formulation No. 2 and 3. This result indicated that the addition of sucrose and glucose syrup in a suitable ratio can improve the colour of a product. Another process of palm sugar cake that produced from palm sugar syrup with using a vacuum evaporator, the lowest mean colour score was found in formulation No. 4. This probably due to this sample presented pale colour. The lowest mean score of texture was found in formulation No. 1, either palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This probably due to this formulation presented soft texture. On the other hand, the highest mean score of texture was found in formulation No. 3 either palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This probably due to low hardness or less crystallisation of a product from glucose syrup added. The sensory evaluation is in agreement with the hardness and crystallinity of palm sugar cake. The hardness and crystallinity of palm sugar cake depend on the amount of glucose syrup added. High amount of glucose syrup caused a soft texture in palm sugar cake as presented in formulation No. 4. The amount of glucose syrup had more effect than the crystal size as can be seen in SEM result (Figure 38 and 39). The lowest mean score of flavour was found in formulation No. 1 and No. 4 either palm sugar cake that produced from palm sugar syrup obtained from an open pan or a vacuum evaporator. This probably due to formulation No. 1 had the highest nonenzymatic browning reactions and caused high amount of burnt flavour as reported in Table 9 and 10. The lowest mean

score of flavour was also found in formulation No. 4 either palm sugar cake that produced by palm sugar syrup obtained from an open pan or a vacuum evaporator. This probably due to formulation No. 4 had the lowest of nonenzymatic browning reactions and caused in less typical flavour. The highest mean score of flavour was observed in formulation No. 3. This result indicated that the addition of sucrose and glucose syrup in suitable ratio can improve the flavour of a product. However, the addition in high concentration of glucose syrup may reduce the typical colour, caused in unacceptability of a product. Furthermore, the highest overall acceptability score was observed in formulation No. 3 of each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. This result can be concluded that the addition of sucrose and glucose syrup in suitable ratio can improve the quality and acceptability of palm sugar cake.

Table 41. Sensory evaluation of palm sugar cake produced from palm sugar syrup with using an open pan

Formulation	Sensory evaluation			
	Colour	Texture	Flavour	Overall
1	6.50^{b}	6.68^{b}	6.47^{b}	6.65^{c}
2	7.23^{a}	7.08^{ab}	7.15^{a}	7.11^{b}
3	7.25^{a}	7.48^{a}	7.37^{a}	7.57^{a}
4	6.78^{b}	6.62^{b}	6.58^{b}	6.85^{bc}

Note: The different superscripts in the same column denote the significant different ($P<0.05$).

Table 42. Sensory evaluation of palm sugar cake produced from palm sugar syrup with using a vacuum evaporator

Formulation	Sensory evaluation			
	Colour	Texture	Flavour	Overall
1	6.98^{a}	6.48^{b}	6.95^{c}	6.81^{b}
2	7.03^{a}	6.58^{b}	6.98^{b}	7.00^{b}
3	7.38^{a}	7.12^{a}	7.82^{a}	7.88^{a}
4	6.08^{b}	6.18^{b}	6.15^{c}	6.37^{c}

Note: The different superscripts in the same column denote the significant different ($P<0.05$).

Effect of storage temperature and storage time on the physical properties of palm sugar cake during storage in different relative humidities (RHs)

Since, the highest overall acceptability score of palm sugar cake was observed in a sample that contained 50% palm sugar syrup, 40% sucrose and 10% glucose syrup. Thus, this formulation was selected for further study. Two palm sugar cake samples that produced from palm sugar syrup with either using an open pan or a vacuum evaporator were stored under a temperature (4^{o}C and 30^{o}C) and two relative humidities (11% and 75%). The physical and chemical properties during storage temperature and storage time were monitored. During storage under 11% of RH in both temperatures, all palm sugar cake samples were monitored at one month interval for twelve months. However, under 75% of RH during storage in both temperatures, all palm sugar cake samples became watery within one month, thus the properties of palm sugar cake were monitored at one week interval for a month. Appendix Figure 14-17 presents the samples of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum vaporator and stored under a temperature (4^{o}C and 30^{o}C) and two relative humidities (11% and 75%).

Changes in colour values (L*, a* and b*) of palm sugar cake during storage

The change in the browning of palm sugar cake was observed by using the CIE colour system (L*, a*, b*). The L*, a* and b* values of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator were monitored during storage. The results are shown in Figure 40 and 41. The effects of storage temperature and time in each processing method on L*,a* and b* values were investigated. It was found that temperature and time during storage had a significant effect on L*, a* and b* values ($P<0.05$).

The L* value was significantly decreased by increasing the storage temperature and time in all samples that stored under either 11% or 75% of RH ($P<0.05$).

The decrease in L* value in all samples that stored under 30^{o}C was higher than those stored under 4^{o}C in both RHs ($P<0.05$). Additionally, the reduction in L* value was found in all samples with increasing storage time under both RHs ($P<0.05$). A slow decrease in L* value was found in all samples that stored under 11% of RH under both temperatures. The rapid decrease in L* was found in all samples that stored under 75% of RH and 30^{o}C. Initial L* values were 33.60 and 43.94 and then decreased to 20.16 and 34.47 for samples that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, respectively and stored under 30^{o}C at the end of storage for 4 weeks. The a* values can be used to evaluate the browning of palm sugar cake. The a* value was significantly increased by increasing storage temperature and time in all samples stored either under 11% or 75% of RH ($P<0.05$). The increase in a* value in all samples that stored under 30^{o}C was higher than those that stored under 4^{o}C in both RHs during storage ($P<0.05$). At 12 months of storage, the highest a* value was was found in a sample produced from palm sugar syrup with either using an open pan ($a^*=14.46$) or a vacuum evaporator ($a^*=10.54$), and stored under 30^{o}C and 75% of RH. The b* value indicates the variation between yellow and blue colour. The b* value was significantly decreased by increasing storage temperature and time in all samples stored either under 11% or 75% of RH ($P<0.05$). The decrease in b* value in all samples that stored under 30^{o}C was higher than those that stored under 4^{o}C in both RHs during storage ($P<0.05$). A slow decrease in b* value was found in all samples that stored under 11% of RH in both temperatures. The rapid decrease in b* was found in all samples that stored under 75% of RH and 30^{o}C. Initial b* values were 15.44 and 18.46 and then decreased to 8.71 and 11.72 for samples that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, respectively and stored under 30^{o}C at the end of storage for 4 weeks.

Generally, the L* and b* values decrease while the a* value increases during browning. This indicated that the colour of the palm sugar cake became darker with more red component and less yellow components. The changes in browning may

contribute to the Maillard reaction during storage. More browning colour in palm sugar cake during storage under 30^{o}C was found higher that stored under 4^{o}C.

From the result, the colour of samples that stored at 30^{o}C was higher change to dark colour than those that stored at 4^{o}C. This indicated that storage temperature affected the colour of palm sugar cake. The rate of Maillard reaction increased with increasing temperature and time. Temperature affects the activities of the reducing sugars. The active form of sugar is considered to be opened chain, which is formed markedly with increasing temperature (Van Boekel and Martins, 2002). Thus, more opened chain of sugar greatly took place in samples that stored under 30^{o}C than 4^{o}C.

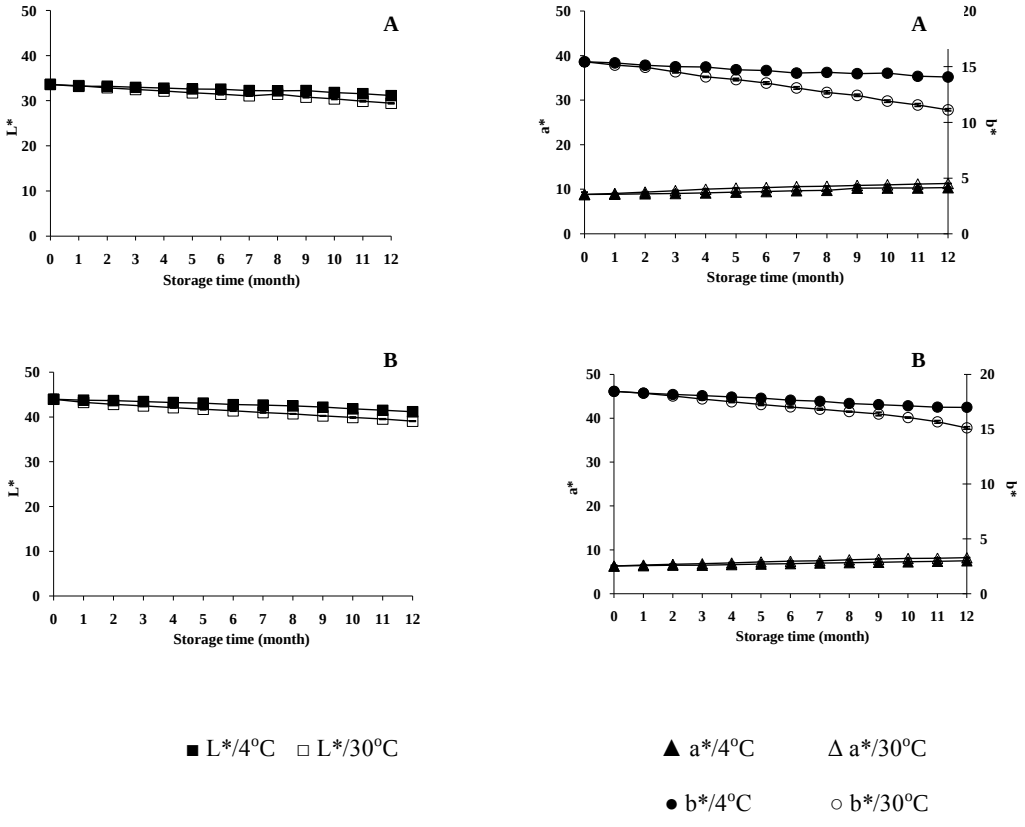


Figure 40. Changes in L*, a* and b* values during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 11% of RH

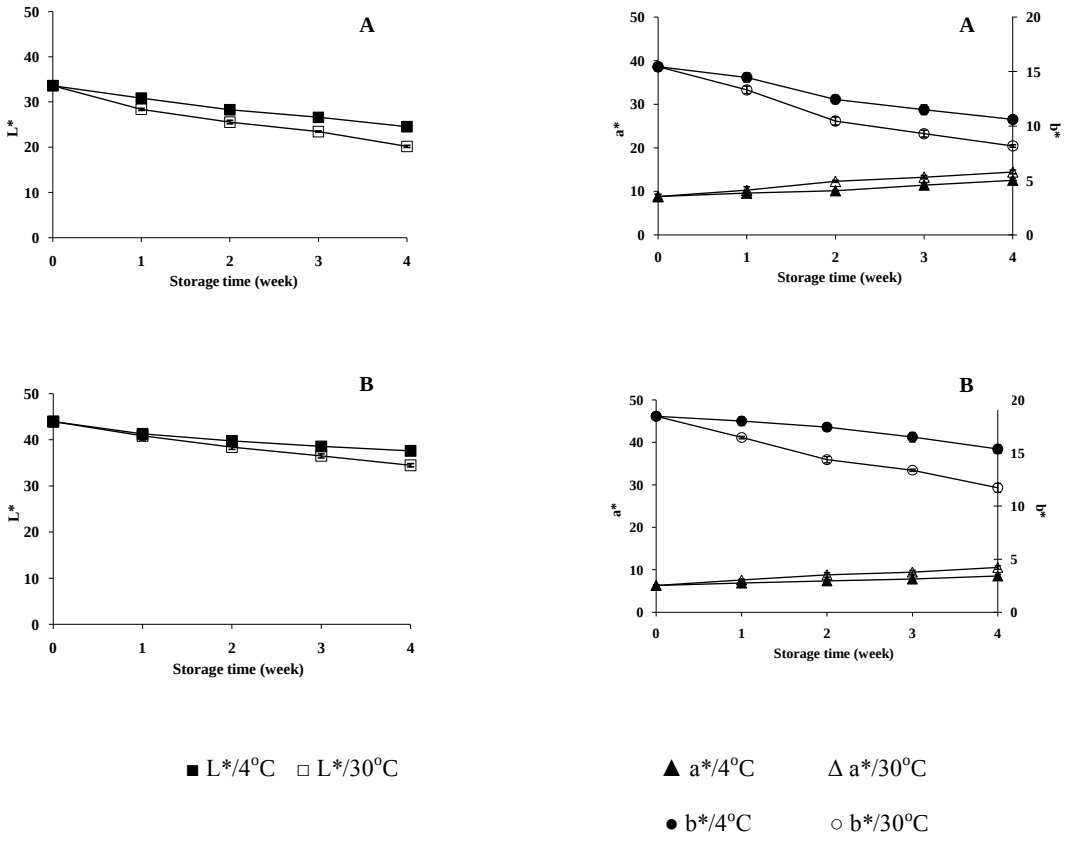


Figure 41. Changes in L*, a* and b* values during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 75% of RH

Moreover, RH is one of the parameters affecting the colour of palm sugar cake during storage. High RH (75%) can accelerate the Maillard reaction in palm sugar cake as evidenced by a rapid decrease in L* and increase in a* values during storage. When palm sugar cake stored under high RH, it rapidly absorbed moisture, resulting in an increase in moisture content. As moisture is absorbed into the dry system, reactants begin to dissolve and mobilise. With greater moisture adsorption, more reactants dissolve and their mobilities increase, resulting in faster reaction rate (Bell, 2007). On ther other hand,

the samples stored under 11% of RH had a slow decrease in L* and increase in a* values. This was probably due to moisture loss from the sample.

Changes in intermediate browning product and browning intensity of palm sugar cake during storage

The Maillard reaction caused colour change in the palm sugar cake during storage. The IBP and BI of palm sugar cake that produced from palm sugar syrup with either using an open pan and a vacuum evaporator were monitored during storage under two temperatures (4^{o}C and 12^{o}C) and two relative humidities (11% and 75%) as shown in Figure 42 and 43. The effects of storage temperature and time on IBP and BI were investigated. It was found that storage temperature and time had a significant effect on IBP and BI ($P<0.05$).

At the beginning, the IBP of palm sugar cake was 0.39 and 0.32 in samples that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, respectively. At the end of storage for 12 months, under 4^{o}C, the IBP of all samples stored under 11% of RH still constant ($P\geq0.05$) while a slowly increase when storage time increased was found in all samples that stored under 30^{o}C which were 0.45 and 0.43 in samples that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, respectively at 12 months of storage ($P<0.05$). A sharp increase in IBP of all samples that stored under 75% of RH was observed with storage temperature and time ($P<0.05$).

The BI was significantly increased with storage temperature and time in all samples either stored under 11% or 75% of RH ($P<0.05$). Additionally, an increase in BI was found in all samples with storage time in both RHs ($P<0.05$). The BI was initially found to be 1.06 and 0.51 in samples that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, respectively. A slowly increase in BI was found in all samples that stored under 11% of RH and in both temperature. The BI sharply increased in all samples that stored under 75% of RH and 30^{o}C. The BI value was

1.87 and 1.04 in a sample that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, respectively, at the end of storage for 4 weeks.

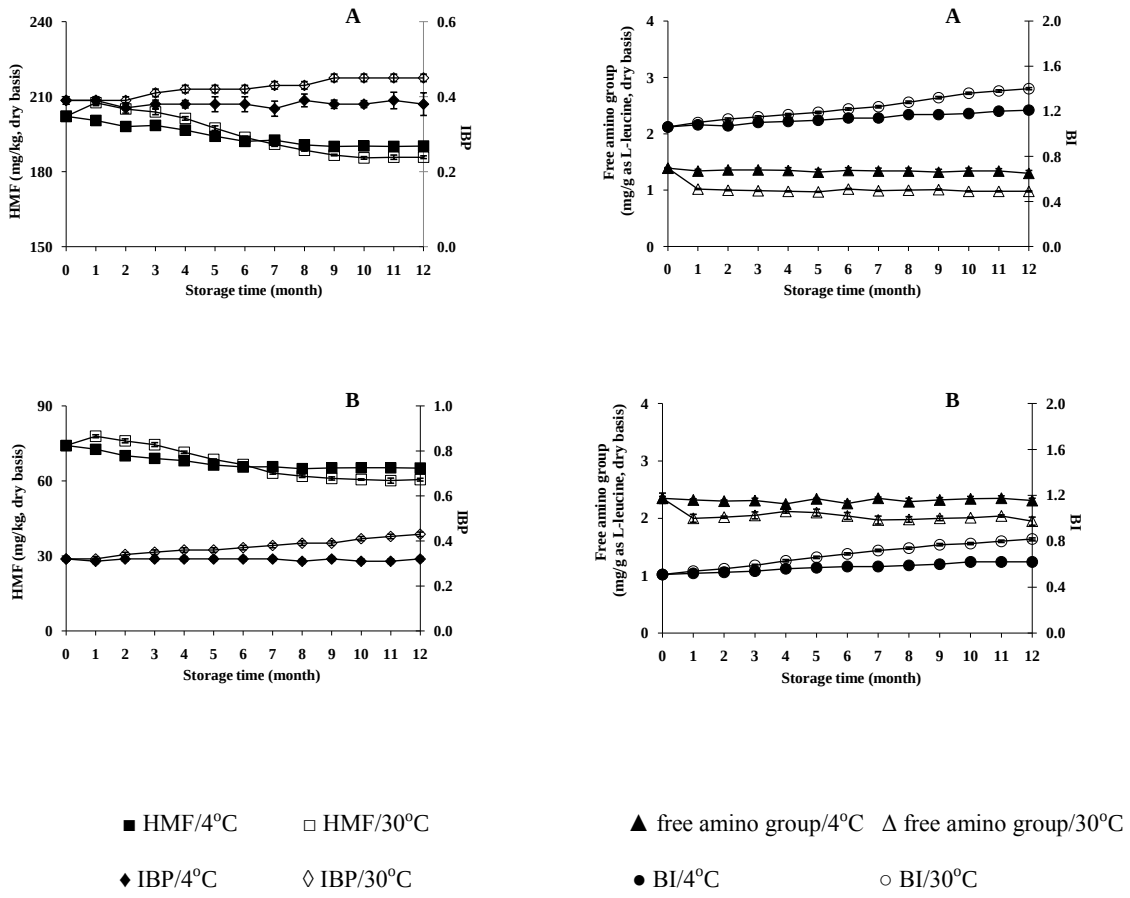


Figure 42. Changes in HMF, IBP, free amino group and BI during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 11% of RH

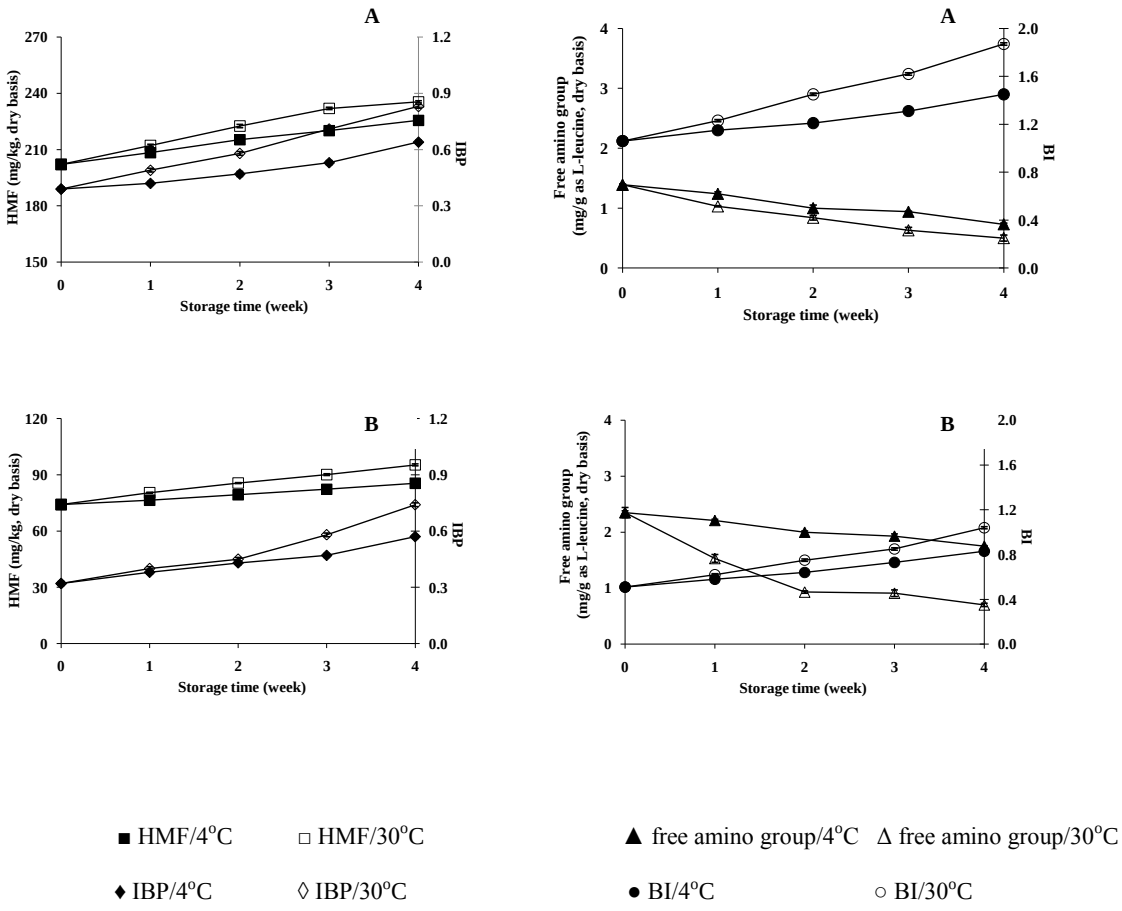


Figure 43. Changes in HMF, IBP, free amino group and BI during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 75% of RH

The results suggest that, the Maillard reaction during storage is greatly enhanced by temperature. In addition, a positive correlation was found between an increase in IBP and BI. This suggested that an intermediate product as indicated by IBP was converted to a brown pigment as indicated by BI while partial intermediates products are still being generated during storage. However, no change in IBP in a sample that stored under 4°C and 11% of RH was detected. This is in agreement with Lertittikul *et al.* (2007) who suggested that some intermediate products might undergo polymerisation to

form the brown pigment and thus only a small amount of intermediate products was generated (Lertittikul *et al.*, 2007). Additionally, the IBP and BI was found higher in samples that stored under 75% of RH than those that stored under 11% of RH. This probably available water under this condition can promote the Maillard reaction as it greatly taking place in RH approximately 60-70% (Bell, 2007).

Changes in hardness and stickiness of palm sugar cake during storage

The hardness and stickiness of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator were monitored during storage under two temperatures (4^{o}C and 12^{o}C) and two relative humidities (11% and 75%) as shown in Figure 44 and 45.

The hardness of all samples increased markedly from their initial values within the first month during storage under 11% of RH and in both temperatures ($P<0.05$). After one month, no change in hardness was found in all samples during storage ($P\geq0.05$). Storage temperature did not affect hardness ($P\geq0.05$). The increment of hardness within one month was due to the loss of sorbed water in a product, resulting in a rigid structure was formed. A continuous decrease in hardness was observed in all samples that stored under 75% of RH when storage time increased ($P<0.05$). There are no significant different of hardness between samples that stored under 4^{o}C and 30^{o}C ($P\geq0.05$). The reduction of hardness of palm sugar cake that stored under 75% of RH was due to an increase in moisture content in a product, resulting in a decrease in crystallinity of samples. After that, a sample became watery and partial liquefied. This result is in agreement with the work of Nowakowski and Hartel (2002). They found a decrease in hardness of sugar cake when a sample absorbed moisture from an environment during storage under high RH. Moreover, Uttraporn (2006) reported that hardness of coconut cake also decreased during storage in high RH.

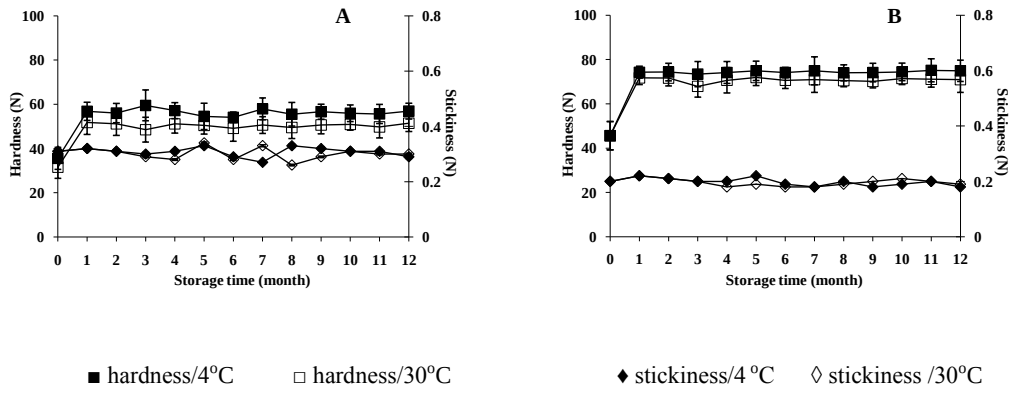


Figure 44. Changes in hardness and stickiness during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 11% of RH

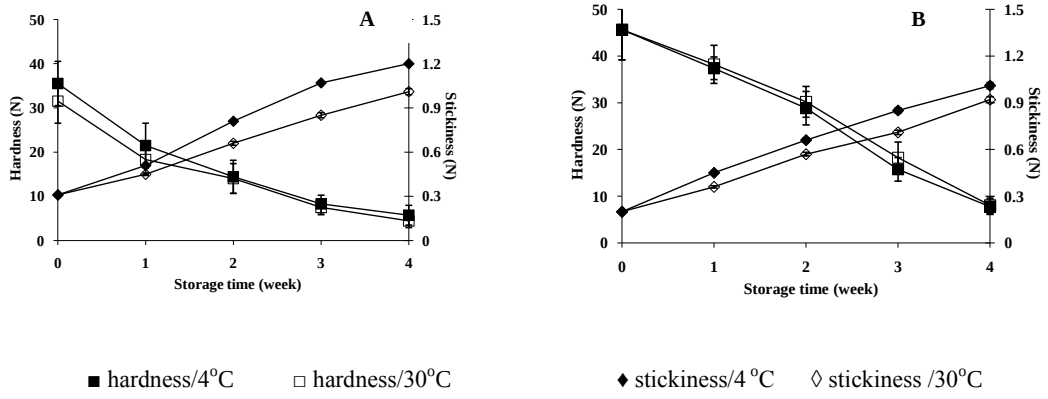


Figure 45. Changes in hardness and stickiness during storage of palm sugar cake produced from palm sugar syrup with using either an open pan (A) or a vacuum evaporator (B) and stored under 75% of RH

The stickiness in food greatly depends on water, temperature and food ingredient. The interaction of water with solids is the prime cause of stickiness in low moisture food. Water is a suitable catalyst for stickiness (Adhikari *et al.,* 2001). No

change in stickiness of all samples that stored under 11% of RH and in both temperatures was found during 12 months of storage ($P \geq 0.05$). Storage temperature did not affect stickiness ($P \geq 0.05$). A continuous increase in stickiness was observed in all samples that stored under 75% of RH and in both temperatures during storage ($P < 0.05$). Nowakowski and Hartel (2002) reported that stickiness increased as a function of moisture content. There are no significant different in stickiness between samples stored under 4^{o}C and 30^{o}C ($P \geq 0.05$). The increase in stickiness was probably due to sample absorbed more moisture.

Changes in solubility of palm sugar cake during storage

The solubility of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator were monitored during two storage temperatures (4^{o}C and 12^{o}C) and two relative humidities (11% and 75%) as shown in Figure 46 and 47. The effects of storage temperature and storage time on hardness and stickiness were investigated. The solubility of all samples decreased markedly from their initial value within first month during storage under 11% of RH and in both temperatures ($P < 0.05$). Thereafter, no change in solubility was found in all samples ($P \geq 0.05$) and storage temperature did not affect solubility ($P \geq 0.05$). The reduction of solubility within first month was due to the loss of sorbed water in the product, resulting in a rigid structure was formed (Nowakowski and Hartel, 2002). Thus, water is difficult to penetrate into the crystal structure. A continuous increase of solubility was observed in all samples that stored under 75% of RH and in both temperatures when storage time increased ($P < 0.05$). There are no significant different of solubility between samples stored under 4^{o}C and 30^{o}C ($P \geq 0.05$).

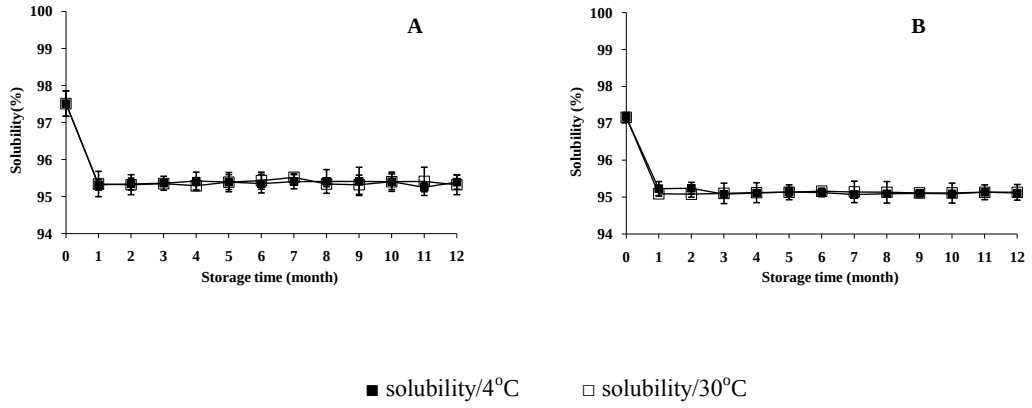


Figure 47. Changes in solubility during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 11% of RH

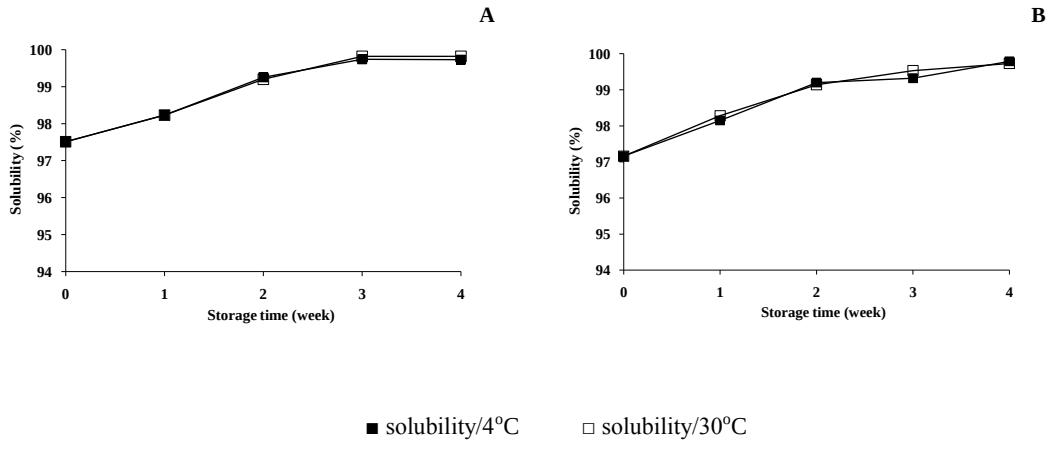


Figure 48. Changes in solubility during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 75% of RH

Sugar is generally very soluble in water at room temperature. Therefore, a sample stored under high RH rapidly absorbed moisture from anenvironment. Water dissolved a crystal structure, caused in the decrease in crystallinity of sugar. This

phenomenon caused an increase in amorphous fraction of a sample, resulting in water easily to penetrate and dissolve.

Changes in crystallinity of palm sugar cake during storage

The crystallinity of palm sugar cake that produced from palm sugar syrup either using an open pan or a vacuum evaporator were monitored during storage under two temperatures (4^oC and 12^oC) and two relative humidities (11% and 75%) as shown in Figure 48 and 49. XRD patterns of all samples during storage were shown in Appendix Figure 18-21. The effects of storage temperature and time on crystallinity were investigated. The crystallinity of all samples increased from their initial values within the first 6 month during storage under 11% of RH and in both temperatures ($P<0.05$). Thereafter, no change in crystallinity was found in all samples ($P\geq0.05$). Storage temperature did not affect crystallinity ($P\geq0.05$). The increment of crystallinity was due to the loss of sorbed water in the product, resulting in a rigid structure was formed. A continuous decrease in crystallinity was observed in all samples that stored under 75% of RH and in both temperatures when storage time increased ($P\geq0.05$). There are no significant different of crystallinity between samples that stored under 4^oC and 30^oC ($P\geq0.05$). The reduction of crystallinity during storage of palm sugar cake that stored under 75% of RH was due to the increase in moisture content in product, resulting in the decrease in crystallinity of samples and sample became watery and partial liquefied.

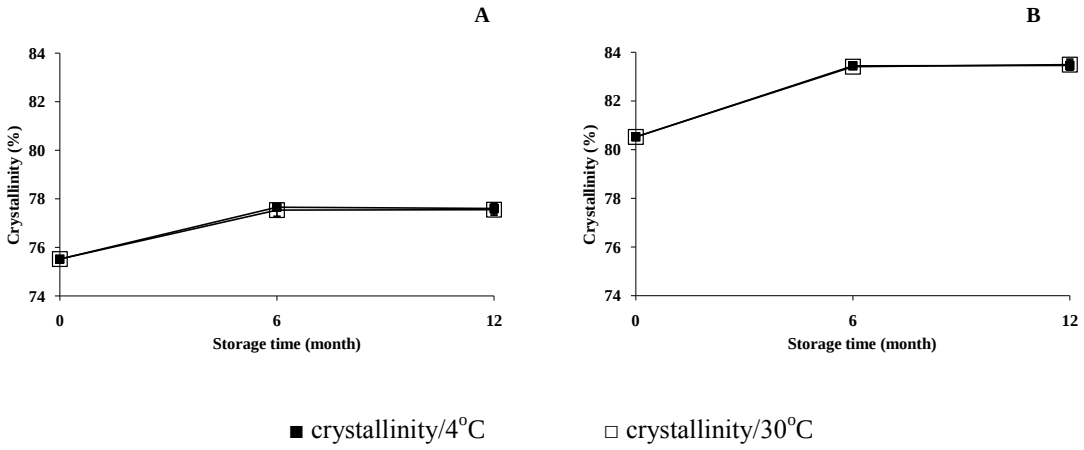


Figure 48. Changes in crystallinity during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 11% of RH

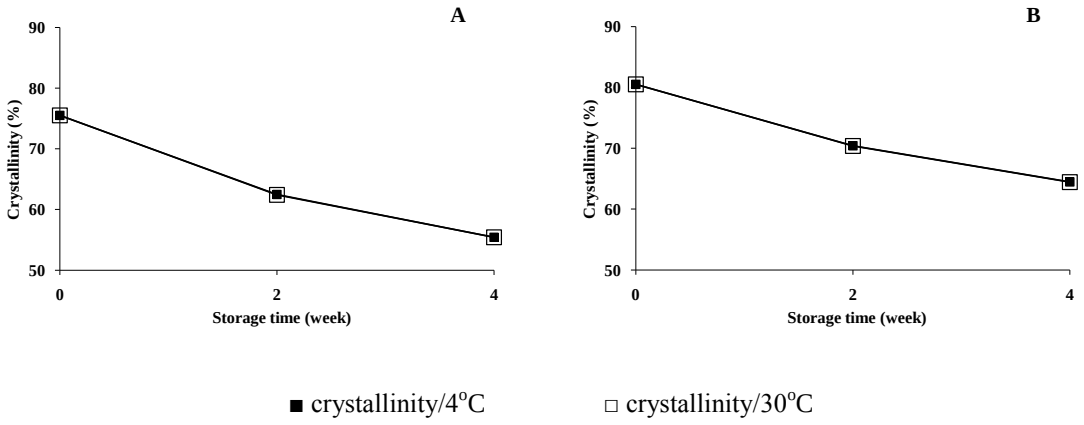


Figure 49. Changes in crystallinity during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 75% of RH

Changes in glass transition temperature of palm sugar cake during storage

The glass transition temperature (Tg) is a temperature at which the amorphous phase is converted from glassy to rubbery states. In food systems, the Tg is mainly affected by the water content and molecular weight of compound (Joupplia, 2006; Sablani *et al.,* 2007). The Tg of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator were monitored during storage under two temperatures (4^{o}C and 12^{o}C) and two relative humidities (11% and 75%). The effects of storage temperature and time on Tg in palm sugar cake were investigated. Initially, Tg of sample was 24.42^{o}C and 30.27^{o}C in sample that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, respectively. Storage temperature did not affect on Tg ($P \geq 0.05$). An increase in Tg was found in all samples that stored under 11% of RH and in both temperatures when storage time increased ($P < 0.05$). The Tg increased to 27.21^{o}C and 33.15^{o}C in samples that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, and stored under 4^{o}C at the end of storage for 12 months, respectively. The Tg also increased to 27.02^{o}C and 33.04^{o}C in samples that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, and stored under 30^{o}C at the end of storage for 12 months, respectively. The increment of Tg when sample stored under low RH was due to the loss of sorbed water in the product. On the other hand, a decrease in Tg was observed in all samples that stored under 75% of RH and in both temperatures when storage time increased ($P < 0.05$). There are no significant different of Tg between samples that stored under 4^{o}C and 30^{o}C ($P \geq 0.05$). At the end of storage for 4 weeks, Tg of palm sugar cake declined to -3.15^{o}C and 12.17^{o}C in samples that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, stored under 4^{o}C, respectively. Tg of samples that stored under 30^{o}C decreased to -3.57^{o}C and 11.23^{o}C in samples that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, at the end of storage for 4 weeks, respectively. The reduction of Tg during storage of palm sugar cake that stored under 75% of RH was due to the increase in moisture content

in product. Normally, water can act as a plasticizer in a sugar based product, resulting in lower Tg (Joupplia, 2006). Moreover, the inversion reaction was taken place in samples that stored under 75% of RH, resulting in the decrease of Tg. Generally, Tg of a sugar mixture likewise in palm sugar cake product depends on the proportion of sugar types in this product. Lower molecular weight sugar has been shown to plasticize higher than high molecular weight sugar (Roos and Karel, 1991). Monosaccharide such as fructose and glucose can act as plasticizers for higher molecular weight carbohydrates (sucrose).

Effect of storage temperature and time on the chemical properties of palm sugar cake during storage in different relative humidities (RHs)

Changes in pH and total acidity of palm sugar cake during storage

The pH value and total acidity of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator were monitored during storage under two temperatures (4^{o}C and 12^{o}C) and two relative humidities (11% and 75%). The effects of storage temperature and time on pH value and total acidity were investigated.

Changes in pH value and total acidity were observed during storage as shown in Figure 50 and 51. No changes in the pH value and total acidity were observed in a sample that produced from palm sugar syrup with either using an open pan or a vacuum evaporator during storage under 11% of RH and in both temperatures ($P \geq 0.05$). In contrast, a decrease in pH value and increase in total acidity was found in a sample that stored under 75% of RH and in both temperatures ($P < 0.05$). In addition, there are no significant different in pH values and total acidity among samples that stored under 4^{o}C and 30^{o}C and 75% of RH ($P \geq 0.05$). The reduction in pH values occurring in Maillard reaction was due to the formation of organic acids such as formic acid and acetic acid (Ames, 1998; Brands and Van Boekel, 2002; Lertittikul *et al.*, 2007).

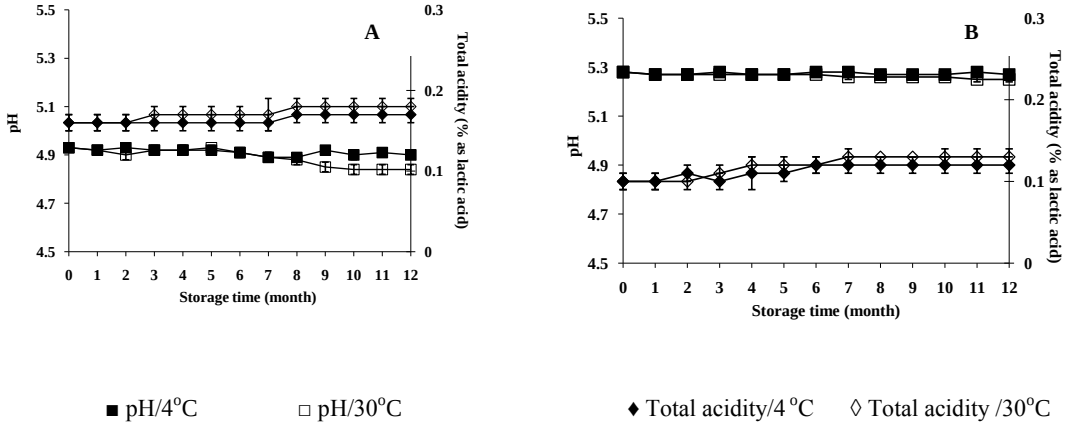


Figure 51. Changes in pH value and total acidity during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 11% of RH

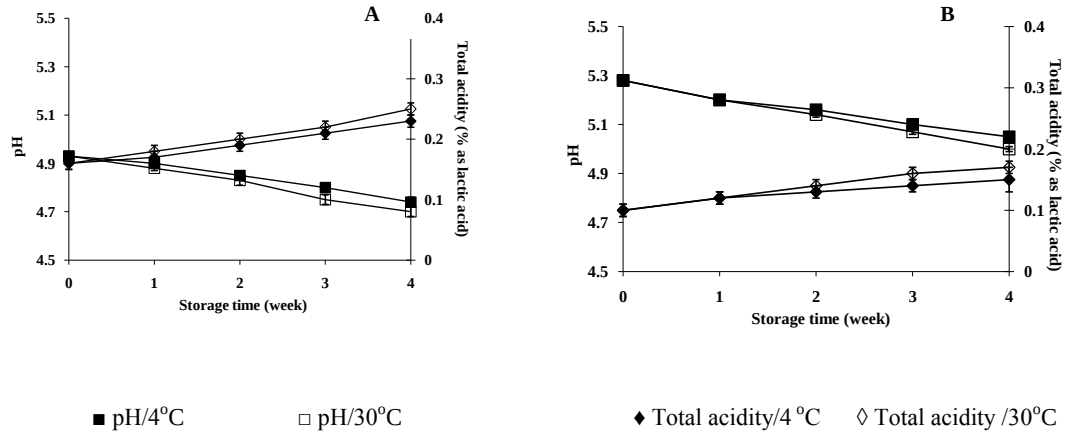


Figure 52. Changes in pH value and total acidity during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 75% of RH

Changes in moisture content and water activity of palm sugar cake during storage

The moisture content (MC) and water activity (Aw) of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator were monitored during storage under two temperatures (4^{o}C and 12^{o}C) and two relative humidities (11% and 75%). The effects of storage temperature and time on MC and Aw were investigated. Changes in moisture content (MC) and water activity (Aw) of palm sugar syrup are shown in Figure 52 and 53, respectively.

The MC and Aw of all samples decreased within the first month during storage under 11% of RH and in both temperatures ($P<0.05$). Thereafter, no change in MC and Aw was found in all samples ($P\geq0.05$). Storage temperature did not affect MC and Aw ($P\geq0.05$). The reduction of MC and Aw in samples that stored under 11% of RH within the fitst month was due to the loss of sorbed water in their products. After that, the MC and Aw of all samples that stored under 11% of RH were remained constant until the end of storage ($P\geq0.05$). This probably due to MC of samples reached equilibrium. Conversely, the MC and Aw was increased in all samples that stored under 75% of RH and in both temperatures with storage time ($P<0.05$). There are no significant different in MC and Aw among samples that stored under 4^{o}C and 30^{o}C ($P\geq0.05$). Since palm sugar cake is hygroscopic, it rapidly absorbed moisture during storage under high RH, resulting in the increase in MC and Aw. This result is similar to Uttraporn (2006) who reported that MC of coconut cake also increased during storage under high RH.

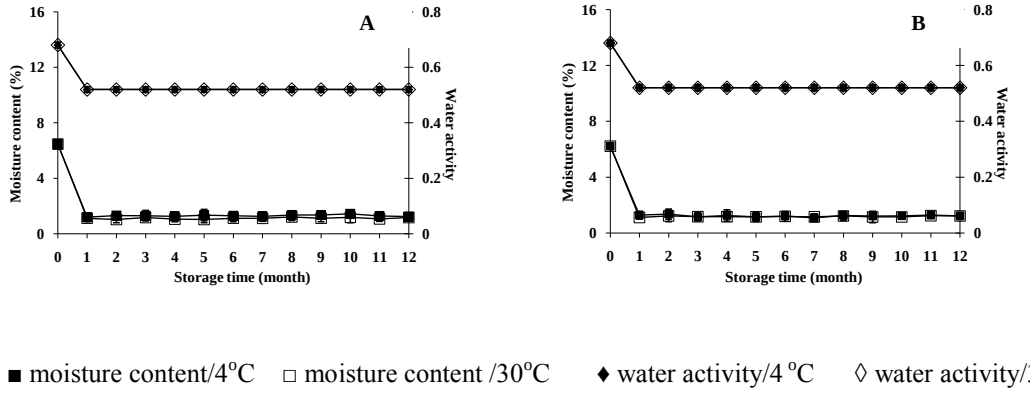


Figure 52. Changes in moisture content and water activity during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 11% of RH.

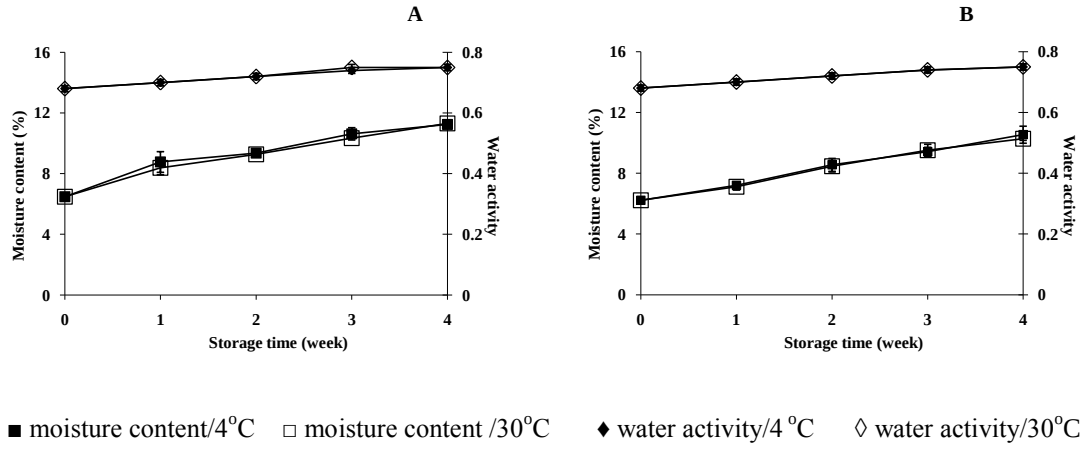


Figure 53. Changes in moisture content and water activity during storage of palm sugar cake produced by palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 75% of RH.

Changes in type and concentration of sugar of palm sugar cake during storage

The total sugar, reducing sugar including glucose, fructose and maltose, and sucrose of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator were monitored during storage under two temperatures (4^{o}C and 12^{o}C) and two relative humidities (11% and 75%). The effects of storage temperature and time on total sugar, reducing sugar including glucose, fructose and maltose and sucrose were investigated. Changes in total sugar, reducing sugar, fructose, glucose, maltose and sucrose were shown in Figure 54-57.

The total sugar, sucrose and reducing sugar as well as fructose and glucose was remained constant in all samples during storage under 11% of RH and 4^{o}C during 12 months of storage ($P \geq 0.05$). While, total sugar, sucrose and reducing sugar as well as fructose and glucose of all samples that stored under 30^{o}C decreased within the first month ($P < 0.05$). Thereafter, the total sugar, sucrose and reducing sugar as well as fructose and glucose were remained constant in all samples until the end of storage ($P \geq 0.05$). Maltose content of all samples was also constant during storage for 12 months ($P \geq 0.05$). The reduction of reducing sugars within the first month was due to Maillard reaction. After that, it was remained constant. This was probably due to the Maillard reaction rate is relatively slow in low RH (Bell, 2007).

The total sugar, sucrose and reducing sugar as well as fructose, glucose and maltose was found to decrease in all samples that stored under 75% of RH and in both temperatures with storage time ($P < 0.05$). The greatest decrease in total sugar, sucrose and reducing sugar as well as fructose, glucose and maltose during storage occurred in palm sugar cake samples that stored under 30^{o}C ($P < 0.05$). A decrease in fructose and glucose may contribute to the Maillard reaction over times. A decrease in sucrose was referred to the inversion of sucrose that took place during storage. Normally, the Maillard reaction is expected to show a maximum value in RH varied from 60% to

80% RH. This probably due to these ranges of RH contained sufficient water to promote the Maillard reaction (Eichner and Karel, 1972; Bell, 2007).

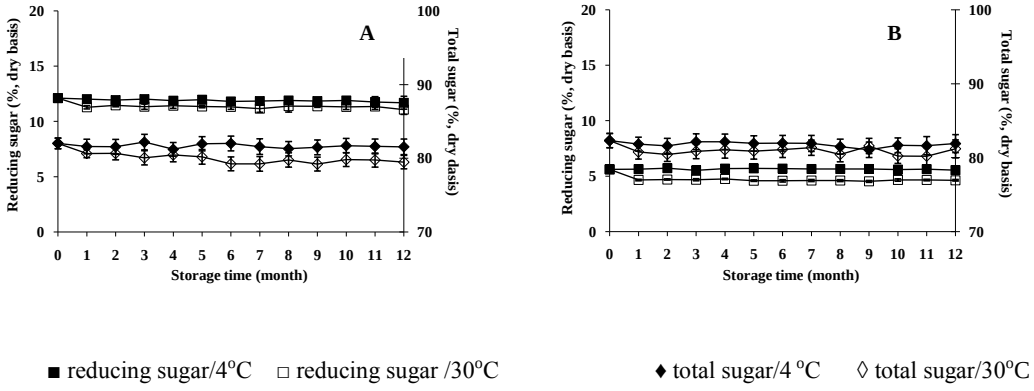


Figure 55. Changes in reducing sugar and total sugar during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 11% of RH

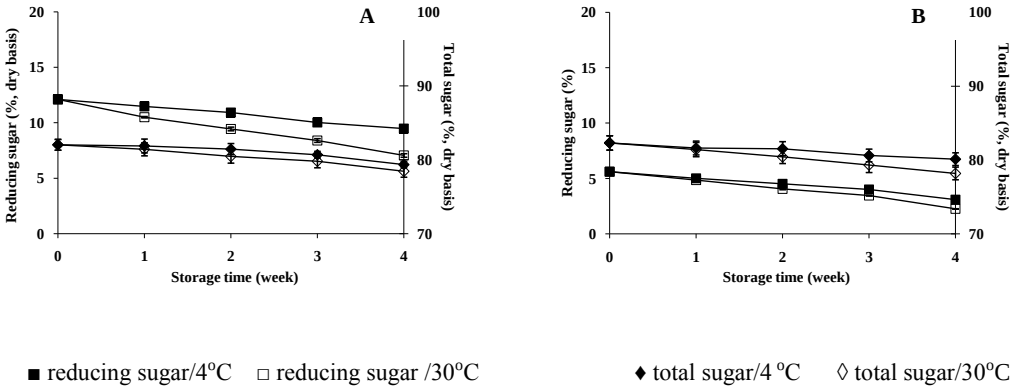


Figure 56. Changes in reducing sugar and total sugar during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 75% of RH

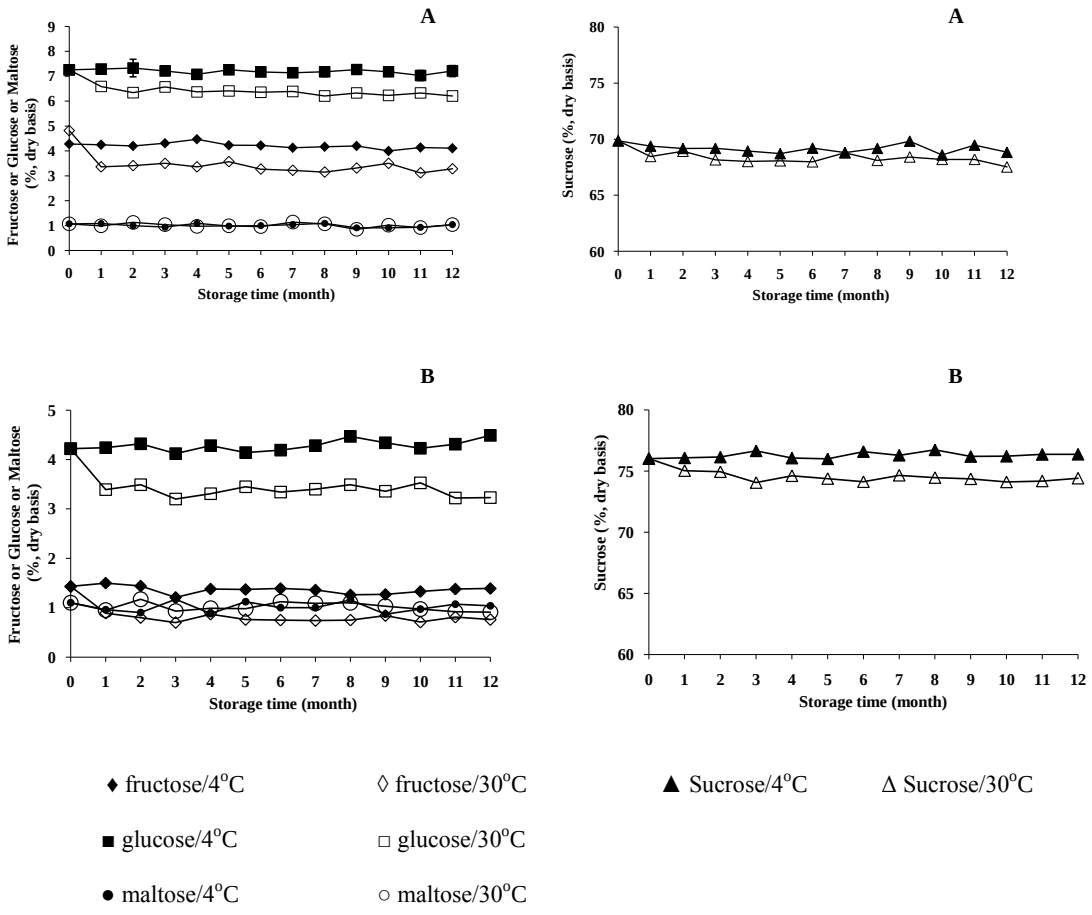


Figure 57. Changes in fructose, glucose, maltose and sucrose during storage of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) and stored under 11% of RH

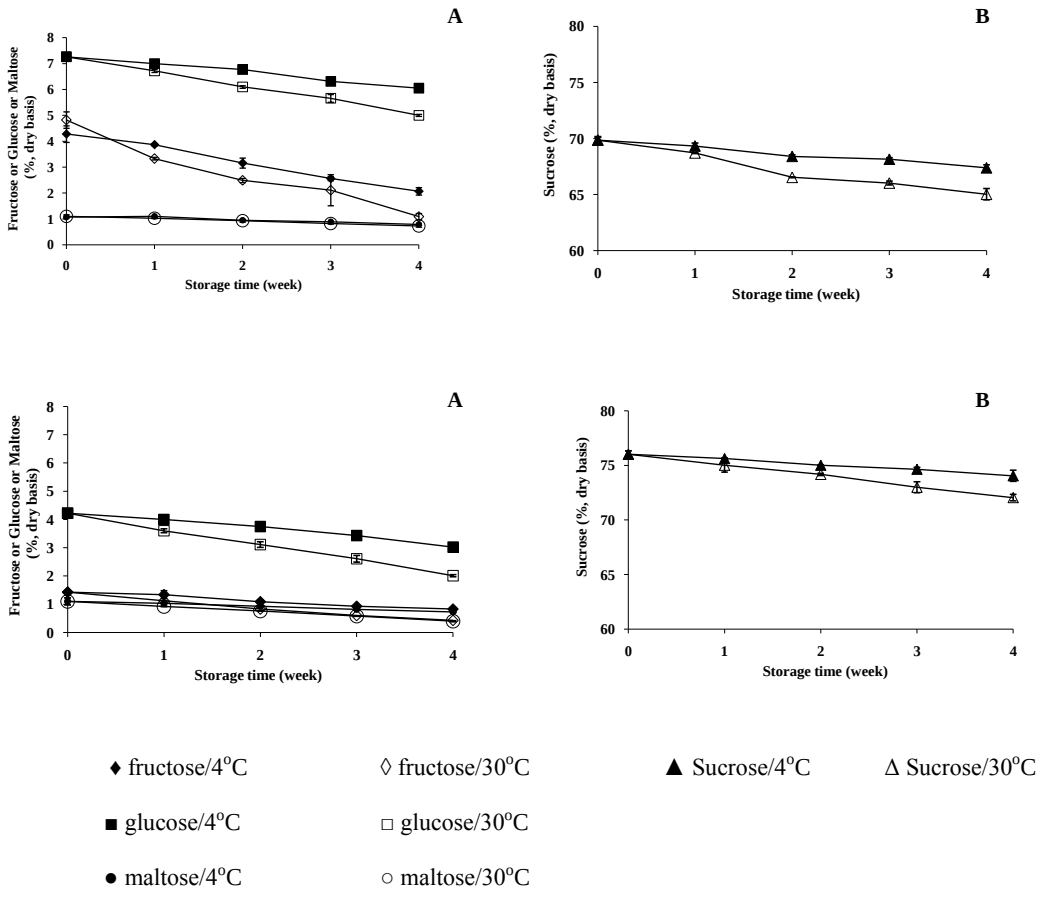


Figure 58. Changes in fructose, glucose, maltose and sucrose during storage of palm sugar cake produced by palm sugar syrup obtained from an open pan (A) and a vacuum evaporator (B) and stored under 75% of RH

Changes in HMF content of palm sugar cake during storage

The HMF content of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator were monitored during storage under two temperatures (4^{o}C and 12^{o}C) and two relative humidities (11% and 75%). The effects of storage temperature and time on HMF content were investigated. Changes in HMF content of palm sugar cake during storage were presented in Figure 42 and 43.

The HMF content was found to decrease in all samples stored under 11% of RH and in both temperatures with storage time ($P<0.05$). The greatest decrease in HMF content during storage occurred in the samples that stored under 30^{o}C ($P<0.05$). Maillard reaction is associated with the development of these intermediate browning products formed prior to generation of brown pigments. These intermediate browning products may be acted as precursors of brown pigments (Lertittkul *et al.*, 2007). Hence, the reduction of HMF content was underwent polymerization to form brown pigments as evidenced by the increase in BI during storage. However, HMF was not generated under low RH due to the restrict mobility of a reactant.

On the other hand, the HMF content was increasd in all samples that stored under 75% of RH and in both temperatures with storage time ($P<0.05$). At the end of storage under 4^{o}C, HMF content increased to 225.55 mg/kg and 85.49 mg/kg in a sample that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, respectively. High temperature during storage can promote the HMF formation. Therefore, palm sugar cake samples that stored under 30^{o}C presented a higher HMF content than those that stored under 4^{o}C. At the end of storage, HMF content was 235.42 mg/kg and 95.27 mg/kg in a sample that produced from palm sugar syrup with either using an open pan or a vacuum evaporator, respectively. The increase in HMF was coincidental with an increase in IBP and BI. This indicated that HMF might undergo polymerization to form brown pigments and thus HMF was still generated under the condition that contained sufficient water to promote Maillard reaction (Eichner and

Karel, 1972). The HMF content of all samples in this study was higher than the permitted maximum limit of 40 mg/kg as recommended by the Codex Alimentarius (Turhan *et al.*, 2008). However, samples that produced from palm sugar syrup with using a vacuum evaporator contained lower HMF than that produced from palm sugar syrup with using an open pan. Therefore, vacuum evaporation can be used as an alternative way to produce palm sugar syrup and palm sugar cake in order to reduce HMF content.

Changes in free amino group content of palm sugar cake during storage

At the early stage of Maillard reaction, terminal α-amino groups of peptides and ε-NH_2 groups of lysine react with the carbonyl functions of reducing sugars present in the reaction medium. The loss of available primary amino groups can be used as an indicator to indicate the Maillard reaction (Laroque *et al.*, 2008). The free amino group content in palm sugar cake was monitored during storage as shown in Figure 42 and 43. No change in free amino group content was found in all samples that stored under 11% of RH and 4^{o}C with storage time ($P>0.05$). The free amino group content of all samples that stored under 11% of RH and 30^{o}C decreased within the first month ($P<0.05$). Thereafter, the free amino group content was remained constant in all samples during storage ($P\geq0.05$). This can be explained by the Maillard reaction rate was controlled by diffusion rate of reactants and this reaction is relatively slow under low RH. The decrease of free amino group content within the first month was also corresponding to the decrease of reducing sugar within the first month. Under 75% of RH and in both temperatures, free amino group content was decreased in all samples during storage with storage time ($P<0.05$). Palm sugar cake that stored under 30^{o}C presented a lower free amino group content than those stored under 4^{o}C ($P<0.05$). The free amino group content tended to decrease during storage due to it acted as a substrate of Maillard reaction.

Changes in volatile flavour compounds of palm sugar cake during storage

Changes in volatile flavour compounds of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator were studied using Headspace Soild Phase Microextraction technique (HS-SPME) coupled with Gas chromatography-mass spectrometry (GC-MS). Volatile flavour compouds were commonly found in all samples, consisting of 2 alcohols, 1 ketone, 1 acid, 1 pyrrole, 10 furans and 6 pyrazines. Table 43-46 show the changes in volatile flavour compounds in palm sugar cake samples during storage.

The formation of volatile flavour compounds from Maillard reaction and Caramelisation were found in palm sugar cake including furan, pyrazine and pyrrole. These compounds have been reported in various plant syrup such as maple syrup, coconut syrup and birch syrup (Kallio, 1989; Akochi *et al.*, 1994; Akochi *et al.*, 1997; Purnomo, 2007). These volatile flavour compounds are typical flavours in palm sugar cake. Furan derivatives such as 2-furanmethanol, 2-furancarboxaldehyde, 2-furancarboxaldehyde, 2-acetylfuran, 5-methylfurfural, 5-hydroxymethylfurfural, 2-furan methane thiol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2-methyl dihydro-3(2H)-furanone, 2-methyl dihydro-2(3H)-furanone and 2,3-dihydro-3,5-dihydroxy-6M-pyran-4-one were found in all samples. These furans contribute to the sweet, caramel, cook sugar or burnt sugar. Furans account for the caramel-like odour of heated carbohydrates (Ho *et al.*, 2007). The formation of furans can take place through two pathways such as lipid peroxidation and degradation of carbohydrates involved Maillard reaction. In this study, furan is known to be formed by the degradation of carbohydrates. Furfural is formed from the Amadori intermediate of the Maillard reaction by 1,2-enolization followed by a deamination and dehydration and can be formed via Caramelisation (Nursten, 1980; Meynier and Mottram, 1995). Pyrazine derivatives were detected in all palm sugar cake samples including to 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, methyl pyrazine, 2-methoxy-6-methylpyrazine and 2-ethyl-3 5-dimethylpyrazine. All these pyrazines are correlated with sensory attributes, such as nutty, sweet and roasty

odour which consequently contribute to typical aromas of palm sugar cake (Ho *et al.*, 2007). The presence of pyrazines in palm sugar cake indicated that not only Caramelisation, but also the Maillard reaction took place during the production of palm sugar cake (Apriyantono *et al.*, 2002). Normally, alkyl pyrazine are commonly found in heated food. One of the main routes of pyrazines formation is through the Strecker degradation of amino acids in the presence of dicarbonyl compounds. In addition to the Strecker aldehyde, this reaction yields an aminocarbonyl compounds, and the condensation of these two molecules results in an alkyl pyrazine (Amrani-Hemaimi *et al.*, 1995; Hwang *et al.*, 1995; Meynier and Mottram, 1995). One pyrrole (acetylpyrrol) was identified in palm sugar cake. This pyrrole tends to contribute to sweet or burnt sugar attributes. There are two pathways to form a pyrrole. First pathway is the interaction between an amino acid and a 3-dexoyhezosone through the Strecker degradation followed by dehydration and ring closure. The second pathway is the reaction of a furan with an amine or an amino acids (Rizzie, 1974; Hwang *et al.*, 1995). In addition, storage under high temperature (30^{o}C) was promoted a higher formation of furan, pyrazine and pyrrole than under low temperature during storage (4^{o}C).

Phenethyl alcohol or benzene ethanol had been reported to possess a sweet or rose aroma. It is derived from L-phenylalanine through metabolic reaction of yeast (Soufleros *et al.*, 2004). The 3-hydroxy-2-butanone was reported to be responsible for sweet odour (Cheetham, 2002). This compound is the main volatile flavour compounds in palm sap as reported by Taiapaiboon (2004). The 3-hydroxy-2-butanone was remained constant in all samples that stored under 11% of RH. However, 3-hydroxy-2-butanone of samples that stored under 75% of RH tended to decrease with storage temperature and time. Acetic acid can be found in palm sugar cake. The acetic acid was remained constant in all samples that stored under 11% of RH. However, acetic acid of samples that stored under 75% of RH tended to increase with storage temperature and time. This probably due to the α-dicarbonyl compounds are unstable and undergo a cleavage reaction (at the C-C bond), resulting in acetic acid during Maillard reaction (Martins and Van Boekel, 2005).

Table 43. Changes in volatile flavour compounds (ppb) of palm sugar cake produced from palm sugar syrup with using an open pan and stored under 11% of RH during storage for 12 months

RT[A]	RI[B]	Volatile flavour compounds	Attribute[C]	Storage temperature (°C) and storage time (month)						
				0 month	4 months		8 months		12 months	
					4°C	30°C	4°C	30°C	4°C	30°C
		Furans								
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	3284	3343	3354	3321	3657	3346	3288
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	3375	3382	3380	3371	3383	3321	3342
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	316	316	362	344	331	303	331
12.88	2344	5-hydroxymethylfurfural	caramel	2767	2745	2767	2719	2697	2758	2729
8.81	1608	5-methylfurfural	sweet, caramel, nutty	7135	7193	7153	7193	7180	7168	7140
8.35	1537	2-acetylfuran	sweet, caramel	1084	1046	1088	1044	1092	1082	1036
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	917	908	937	925	929	920	925
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	157	156	157	165	155	165	165
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	786	731	741	751	722	754	733
11.72	2577	2,3-dihydro-3,5-dihydroxy-6M-pyran-4-one	caramel, sweet, burnt sugar	1178	1156	1152	1135	1149	1185	1188
		Pyrazines								
5.81	1292	Methyl pyrazine	nutty, roasty	2275	2275	2266	2272	2234	2255	2271
6.84	1314	2,3-dimethylpyrazine	roasted nut, sweet	428	419	433	465	429	450	404
6.50	1309	2,5-dimethylpyrazine	sweet, roasty	457	424	427	444	465	428	448
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	6729	6738	6730	6755	6741	6738	6731
7.82	1470	2-ethyl-3,5-dimethylpyrazine	roasty	2056	2068	2061	2075	2045	2063	1977
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	652	622	626	644	686	644	651
		Alcohols								
10.36	1951	Phenethyl alcohol	sweet	198	160	161	160	162	166	156
8.81	1587	2,3-butanediol	fruity	343	342	344	355	303	366	343
		Acid								
7.84	1473	Acetic acid	sour	4048	4045	4120	4205	4176	4104	4079
		Ketone								
6.00	1307	3-hydroxy-2-butanone	sweet	454	468	471	483	464	454	433
		Pyrrole								
10.62	2023	Acetylpyrrole	sweet, burnt sugar	652	635	650	643	668	658	623

Note: [A] RT refers to retention time (min); [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24}), [C] Reference:http://www.thegoodscentscompany.com/rawmatex.html

Table 44. Changes in volatile flavour compounds (ppb) of palm sugar cake produced from palm sugar syrup with using a vacuum evaporator and stored under 11% of RH during storage for 12 months

RT[A]	RI[B]	Volatile flavour compounds	Attribute[C]	Storage temperature (°C) and storage time (month)						
				0 month	4 months		8 months		12 months	
					4°C	30°C	4°C	30°C	4°C	30°C
		Furans								
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	1920	1906	1907	1960	1920	1960	1921
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	1668	1688	1689	1656	1694	1669	1652
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	295	270	288	263	249	255	271
12.88	2344	5-hydroxymethylfurfural	caramel	1287	1242	1265	1228	1249	1187	1242
8.81	1608	5-methylfurfural	sweet, caramel, nutty	3467	3429	3420	3395	3327	3330	3323
8.35	1537	2-acetylfuran	sweet, caramel	442	472	427	437	447	456	445
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	476	445	441	459	491	462	481
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	107	101	105	113	116	105	106
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	440	410	414	411	462	413	431
11.72	2577	2,3-dihydro-3,5-dihydroxy-6M-pyran-4-one	caramel, sweet, burnt sugar	886	862	885	870	826	823	837
		Pyrazines								
5.81	1292	Methyl pyrazine	nutty, roasty	1504	1578	1544	1480	1523	1506	1534
6.84	1314	2,3-dimethylpyrazine	roasted nut, sweet	223	250	241	218	232	220	224
6.50	1309	2,5-dimethylpyrazine	sweet, roasty	204	227	205	209	228	236	204
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	4073	4087	4003	4040	4017	4040	4025
7.82	1470	2-ethyl-3,5-dimethylpyrazine	roasty	1647	1599	1642	1603	1666	1683	1661
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	323	325	308	298	333	322	323
		Alcohols								
10.36	1951	Phenethyl alcohol	sweet	101	144	108	99	128	91	108
8.81	1587	2,3-butanediol	fruity	202	186	196	220	205	207	200
		Acid								
7.84	1473	Acetic acid	sour	3442	3490	3450	3472	3437	3492	3432
		Ketone								
6.00	1307	3-hydroxy-2-butanone	sweet	779	725	708	710	708	700	695
		Pyrrole								
10.62	2023	Acetylpyrrole	sweet, burnt sugar	323	362	346	329	328	321	318

Note: [A] RT refers to retention time (min); [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24}), [C] Reference:http://www.thegoodscentscompany.com/rawmatex.html

Table 45. Changes in volatile flavour compounds (ppb) of palm sugar cake produced from palm sugar syrup with using an open pan and stored under 75% of RH during storage for 4 weeks

RT[A]	RI[B]	Volatile flavour compounds	Attribute[C]	Storage temperature (°C) and storage time (week)				
				0 week	2 weeks		4 weeks	
					4°C	30°C	4°C	30°C
		Furans						
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	3284	3499	3589	3660	3777
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	3375	3460	3536	3450	3623
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	316	324	351	352	427
12.88	2344	5-hydroxymethylfurfural	caramel	2767	2667	2687	2693	2769
8.81	1608	5-methylfurfural	sweet, caramel, nutty	7135	7115	7191	7113	7228
8.35	1537	2-acetylfuran	sweet, caramel	1084	1124	1244	1124	1212
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	917	986	1093	1005	1121
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	157	171	204	197	215
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	786	869	974	911	1011
11.72	2577	2,3-dihydro-3,5-dihydroxy-6M-pyran-4-one	caramel, sweet, burnt sugar	1178	1233	1309	1295	1341
		Pyrazines						
5.81	1292	Methyl pyrazine	nutty, roasty	2275	2353	2422	2352	2595
6.84	1314	2,3-dimethylpyrazine	roasted nut, sweet	428	450	589	521	623
6.50	1309	2,5-dimethylpyrazine	sweet, roasty	457	447	505	524	730
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	6729	6871	6921	6915	7032
7.82	1470	2-ethyl-3,5-dimethylpyrazine	roasty	2056	2145	2217	2203	2286
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	652	653	728	724	975
		Alcohols						
10.36	1951	Phenethyl alcohol	sweet	198	198	223	224	265
8.81	1587	2,3-butanediol	fruity	343	350	352	363	327
		Acid						
7.84	1473	Acetic acid	sour	4048	4123	4267	4285	4416
		Ketone						
6.00	1307	3-hydroxy-2-butanone	sweet	454	437	393	419	319
		Pyrrole						
10.62	2023	Acetylpyrrole	sweet, burnt sugar	652	684	721	699	789

Note: [A] RT refers to retention time (min); [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24}), [C] Reference:http://www.thegoodscentscompany.com/rawmatex.html

Table 46. Changes in volatile flavour compounds (ppb) of palm sugar cake produced from palm sugar syrup with using a vacuum evaporator and stored under 75% of RH during storage for 4 weeks

RT[A]	RI[B]	Volatile flavour compounds	Attribute[C]	Storage temperature (°C) and storage time (week)				
				0 week	2 weeks		4 weeks	
					4°C	30°C	4°C	30°C
		Furans						
9.21	1683	2-furanmethanol	cooked sugar, burnt sugar	1920	1991	2074	2040	2123
8.05	1496	2-furancarboxaldehyde	cooked sugar, burnt sugar	1668	1773	1856	1812	1896
8.14	1507	3-furancarboxaldehyde	cooked sugar, burnt sugar	295	304	329	303.97	361
12.88	2344	5-hydroxymethylfurfural	caramel	1287	1156	1346	1147	1351
8.81	1608	5-methylfurfural	sweet, caramel, nutty	3467	3343	3504	3314	3786
8.35	1537	2-acetylfuran	sweet, caramel	442	489	544	504	563
10.79	2071	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel, sweet, burnt sugar	476	454	500	475	562
5.75	1286	2-methyl dihydro-3(2H)-furanone	caramel, sweet, burnt sugar	107	479	505	484	584
9.18	1677	2-methyl dihydro-2(3H)-furanone	caramel, sweet, burnt sugar	440	136	214	137	278
11.72	2577	2,3-dihydro-3,5-dihydroxy-6M-pyran-4-one	caramel, sweet, burnt sugar	886	948	1052	1006	1242
		Pyrazines						
5.81	1292	Methyl pyrazine	nutty, roasty	1504	1612	1627	1641	1728
6.84	1314	2,3-dimethylpyrazine	roasted nut, sweet	223	293	398	355	425
6.50	1309	2,5-dimethylpyrazine	sweet, roasty	204	304	322	338	411
6.57	1310	2,6-dimethylpyrazine	sweet, roasty	4073	4172	7170	4280	4321
7.82	1470	2-ethyl-3,5-dimethylpyrazine	roasty	1647	1685	1709	1731	2038
10.69	2043	2-methoxy-6-methylpyrazine	sweet, roasty	323	385	425	402	516
		Alcohols						
10.36	1951	Phenethyl alcohol	sweet	101	157	231	219	280
8.81	1587	2,3-butanediol	fruity	202	195	204	228	261
		Acid						
7.84	1473	Acetic acid	sour	3442	2476	3517	3553	3611
		Ketone						
6.00	1307	3-hydroxy-2-butanone	sweet	779.38	708.62	675	686	662
		Pyrrole						
10.62	2023	Acetylpyrrole	sweet, burnt sugar	323	388	437	417	511

Note: [A] RT refers to retention time (min); [B] RI refers to retention index that was based on a series of alkane (C_{10}-C_{24}), [C] Reference:http://www.thegoodscentscompany.com/rawmatex.html

5.5 Conclusion

The addition of sucrose and glucose syrup showed a pronounced effect on the properties of palm sugar cake. The addition of sucrose and glucose syrup can improve the properties of palm sugar cake such as a reduction of dark colour as indicated by lower IBP, BI and HMF content, an increased Tg and crystallinity. The addition of sucrose increased hardness and decreased solubility of palm sugar cake due to sucrose can promote sugar crystallisation. On the other hand, the addition of glucose syrup decreased hardness and increased solubility of palm sugar cake due to glucose syrup can retard sugar crystallisation, resulting in high amorphous fraction in a product. Additionally, palm sugar cake that produced from palm sugar syrup with using a vacuum evaporator presented better properties as evidenced by lower a*, BI, HMF content and higher in hardness, crystallinity and Tg. Thus, palm sugar syrup produced by a vacuum evaporator can be used as an alternative raw material to produce palm sugar cake.

In addition, storage temperature and time affected the properties of palm sugar cake. Low temperature (4^{o}C) during storage can retard dark colour of palm sugar cake as indicated by lower L*, a*, and BI. High RH (75% of RH) during storage promotes a decrease in Tg, crystallinity and hardness and after that a sample became watery or liquefied within the first month of storage. On the other hand, under low RH (11% of RH) during storage, the properties of palm sugar cake such as hardness and crystallinity was remained constant and, resulting in the sample solidified during storage for 12 months. Thus, the properties of palm sugar cake can be improved by using palm sugar syrup produced by a vacuum evaporator combination with storage under low temperature and low RH.

CHAPTER 6

MOISTURE ADSORPTION ISOTHERM AND GLASS TRANSITION TEMPERATURE OF PALM SUGAR CAKE

6.1 Abstract

The aim of this study was to determine the effect of storage temperature (20^oC and 30^oC) on moisture adsorption isotherm (MSI) characteristic, equilibrium moisture content (EMC) and glass transition temperature (Tg) of palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator. It was found that MSI of all palm sugar cake samples that stored under both storage temperatures was shown Type-III isotherms. This type can be found in a crystalline product. However, storage temperature did not affect on EMC when a sample stored under 11-75% of RH ($P \geq 0.05$). The EMC of all samples that stored under 20^oC was higher than those stored under 30^oC and 85% of RH ($P < 0.05$). In addition, higher EMC was found in a sample that produced from palm sugar syrup with using an open pan when compared to samples that produced from palm sugar syrup with using a vacuum evaporator ($P < 0.05$). Storage temperature did not affect on the Tg of a samples. There was no significant differences in Tg of a sample that stored under 11-51% RH ($P \geq 0.05$). However, the Tg of a sample decreased with increasing RH in a range of 75-85%. Palm sugar cake that produced from palm sugar syrup with using a vacuum evaporator presented a higher Tg than that produced from palm sugar syrup with using an open pan ($P < 0.05$). In addition, a decrease in crystallinity and increase in IBP and BI during storage was detected in a sample that stored under high RH (75-85%).

6.2 Introduction

Sugar cake is made from various materials, mainly plant sap. Plant sap such as palm sap, coconut sap and maple sap are commonly used to produce sugar cake (Apriyantono *et al.,* 2002; Pattnayak and Misra, 2004; Rao and Das, 2009; Aider *et al.,* 2006; Aider *et al.,* 2007). This form of sugar based product is easy to transport and store and also provides a good source of sugar which is used as an ingredient. In the southern part of Thailand particularly in Songkhla province, palm sugar cake was originally made from the sugary sap of the Palmyra palm (*Borassus flabellifer* Linn.) (Phaichamnan *et al.,* 2010). In general, palm sap is heated during palm sugar cake production in order to remove water from the sap until its concentrated (the total soluble solid is more than 80^{o}Brix). After the temperature of this sample reaches approximately 120^{o}C, it is removed from the heat and stirred. The stirring process is continued until the solution begins to crystallise and stiffen. At this stage, it can be poured into a mould. Normally, palm sugar cake deteriorates fast and become watery within one or two weeks due to it has hygroscopic nature. In addition, a production of invert sugar through microbial degradation further increases the hygroscopicity of the palm sugar cake (Rao *et al.,* 2006). For the ease of handling, packaging and storage, sugar cake in a solid form is becoming popular. However, palm sugar cake product becomes watery and deteriorates during storage by moulds and yeasts. Knowledge of moisture sorption isotherms in palm sugar cake product at different storage temperature could be used as a tool for its handling, storage and packaging system design. The moisture adsorption isotherms of sugar cake that produced by sugarcane juice and date syrup have been reported by Verma and Narain (1990) and Rao *et al.* (2006). However, an information on moisture sorption isotherm of palm sugar cake that produced from palmyra palm syrup is rarely investigated. Therefore, the quality of palm sugar cake product relating with glass transition temperature (Tg) are of interest since Tg is considered as a tool to understand many aspects of food processing, shelf life and food preservation (Tanon *et al.,* 2009; Syamaladevi *et al.,* 2009; Mosquera *et al.,* 2010). Therefore, the aim of this work was to

determine moisture adsorption isotherm (MSI) and glass transition temperature of palm sugar cake stored under two temperatures including 20^{o}C and 30^{o}C. Storage temperatures under 20^{o}C and 30^{o}C were respresented for the ambient temperature of temperate and tropical country, respectively. The changes in crystallinity and the browning development of palm sugar cake that produced from palm sugar syrup either using an open pan and a vacuum evaporator during storage under the range of 11-75% of RH were also monitored.

6.3 Materials and Methods

Chemicals

Lithium chloride, potassium acetate, magnesium chloride, magnesium nitrate, potassium carbonate, sodium chloride and potassium chloride were purchased from Prolabo (Paris, France).

Raw material

Palm sap was collected from a contact farm in Songkhla province. Natural palm sap was tapped and harvested after 12 h of collecting in an open container. During tapping, natural wood called Kiam (*Cotylelobium lanceotatum* Craih.) was added into the container since the beginning state of tapping. Palm sap was kept in an icebox (4^{o}C) during transportation (30 min). The sample was filtrated using cloth sheet at room temperature and kept under 4^{o}C until used.

Production of palm sugar syrup

Palm sap was concentrated using two methods either using an open pan or a vacuum evaporator. Palm sap (15 liters) was concentrated by using an open pan (at approximately 110^{o}C) and a vacuum evaporator (at 80^{o}C) until the total soluble solids reached 70^{o}Brix to obtain palm sugar syrup.

Production of palm sugar cake

Palm sugar cake was prepared from palm sugar syrup which was concentrated by two methods either an open pan or a vacuum evaporator. The mixture of palm sugar syrup sample, sucrose and glucose syrup was mixed in a ratio of 50:40:10. Then, the mixture was boiled using an open pan until the total soluble solid reached 80^{o}Brix (at temperature approximately 120^{o}C). After that, it was removed from heat and stirred until its became crystalline and then the sample was poured in thin (0.5 cm) slabs and allowed to cool at ambient temperature.

Moisture adsorption isotherm

The moisture adsorption isotherm (MSI) of palm sugar cake was monitored gravimetrically by exposing a sample to an atmosphere of known relative humidity at a constant temperature. A sample was stored in a desiccator over P_2O_5 for one week. After storage, a sample was considered as anhydrous stage. Anhydrous sample was transferred into a hermetic plastic box that contained saturated salt solutions with different relative humidities. This sample was kept under two storage temperatures either 20^{o}C or 30^{o}C over seven saturated solutions of LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, NaCl and KCl to get a specific RH as shown in Table 54 (Labuza, 1985). The sample weight was monitored everyday till its reached a constant value (mass

different<0.0005 g), where the equilibrium moisture content of the sample was assumed to be achieved. In each equilibrated stage, a sample was determined for moisture content and Tg.

Table 54. Relative humidity of saturated salt solution at 20°C and 30°C

Salt/Temperature	20°C	30°C
LiCl	11.31	11.28
CH_3COOK	23.13	21.61
$MgCl_2$	33.01	32.44
K_2CO_3	43.16	43.17
$Mg(NO_3)_2$	54.38	51.40
NaCl	75.47	75.09
KCl	85.11	83.62

Source: Labuza (1968)

Determination of moisture content

The moisture content was measured using a vacuum oven. About 2-5 g of the sample was placed in a pre-dried aluminum dish and dried in a vacuum oven at 60°C (pressure<70mmHg) for 6 h. The dried sample was placed in a desiccator and cooled for 0.5 h to room temperature. The weight was recorded and the percentage moisture based on the initial wet weight was calculated.

Measurement of glass transition temperature

The glass transition temperature (Tg) was determined by differential scanning calorimetry model DSC7 (Perkin Elmer, U.S.A.). Palm sugar cake approximately 5 mg was powdered using a mortar and weighted in an aluminum pan, hermetically sealed and then taken for DSC analysis. Firstly, a sample was cooled from 25°C to -40°C at the rate of 10°C/min. After that, a sample was heated at the rate 10°C/min from -40°C to 200°C followed by cooling to -40°C at the rate of 120°C/min and

then repeated run was performed. The reference was an empty pan, while dry ice was used for cooling sample. The mid point of the glass transition was considered as the characteristic temperature of the transition.

Measurement of crystallization

Change in crystallization in each sample that stored under 30^{o}C was monitored by XRD. A sample was removed from a plastic box that contained in each saturated salt solution with different RH at several intervals. The sample was rapidly dried in a vacuum oven (40^{o}C). The dried sample was powdered using a mortar. The crystallinity of palm sugar cake was identified by X-ray diffraction method. The powder sample was placed into a sample holder for the powder X-ray diffraction and the surface was smoothed with a glass slide. The measure was carried out with the powder X-ray diffraction meter using Cu radiation, under operational conditions of 40 kV of potency and 30 mA. A sample was scanned with 2θ being between 5^{o} and 40^{o}. Data acquisition was accomplished using a step size of 0.05° and a step time of 1 s. The crystallinity was calculated by intregrating total peak area.

Measurement of intermediate browning product and browning intensity

A samples stored under 30^{o}C was removed from a plastic box that contained saturated salt solution with different RH at several intervals in order to determine intermediate browning product (IBP) (absorbance at 280 nm) and browning intensity (BI) (absorbance at 420 nm). The absorbance was measured by using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan), as described by Takano (2005) and Kawai *et al.* (2005). Appropriate dilution (20-fold for IBP and 10-fold for BI) was made using distilled water to obtain reliable absorbance readings.

Statistical analysis

All analysis and measurements were performed in triplicates. The experimental design was a completely randomized design (CRD). The effect of RH (11-85%) on EMC and Tg in each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator and storage temperature and the effect of storage temperature (20^{o}C and 30^{o}C) on EMC and Tg in each palm sugar cake that produced from palm sugar syrup with either using an open pan or a vacuum evaporator were determined using CRD. The effect of processing method of palm sugar syrup on EMC and Tg in each RH was determined using CRD. The effect of storage time on crystallinity, IBP and BI in each RH was determined using CRD. Data was subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test (Steel and Torrie, 1980). Analysis was performed using a SPSS package (SPSS 6.0 for windows, SPSS Inc, Chicago, IL).

6.4 Results and Discussion

Natural of moisture adsorption isotherm

Moisture adsorption isotherm (MSI) can be used to describe the relationship between water activity (Aw) or relative humidity (RH) and the equilibrium moisture content (EMC, % db) of a given food at a constant temperature (Al-Muhtaseb *et al.,* 2002). Figure 58 shows MSI of the palm sugar cake that produced from palm sugar syrup either using an open pan or a vacuum evaporator and stored in both temperatures (20°C and 30°C). All palm sugar cake samples demonstrated Type III isotherm characteristics that referred to a crystalline product as stated by Brunauer-Emmett-Teller (BET) classification (Brunauer *et al.,* 1940; Rizvi, 1986). The MSI characteristic of palm sugar cake is similar to other high sugar rich food such as jaggery from sugarcane juice and date syrup (Rao *et al.,* 2006). As a result, a small increase in EMC of palm sugar

cake that stored under 11-54% of RH was detected ($P<0.05$). On the other hand, a sharp increase in EMC of palm sugar cake that stored under 75-85% of RH was observed ($P<0.05$). The type III of sorption shows very little moisture gain until the RH goes above the point where water begins to dissolve a crystal surface (such as in 75% and 85% RH for sucrose). Firstly, water molecules interact via hydrogen bonds with the hydroxyl groups on a crystal surface. However, under low RH (11-54% RH), the interaction of water with the sugar molecules is not strong enough to break the interactive forces of individual sugar molecules in a crystal. However, as the RH increased, the increase of overall water-sugar interactions caused disruption of the sugar-sugar interactions. Thus, water begins to penetrate into a crystal, dissolving sugar molecules and exposing new surfaces. Under these range of RH (75-85% RH), the moisture rises dramatically. Then, solution is being created, dissolution of sugar occurs, and a crystalline sugar is converted to amorphous state, resulting higher absorption of moisture. (Ayranci *et al.*, 1990; Bell and Labuza, 2000; Rao *et al.*, 2006).

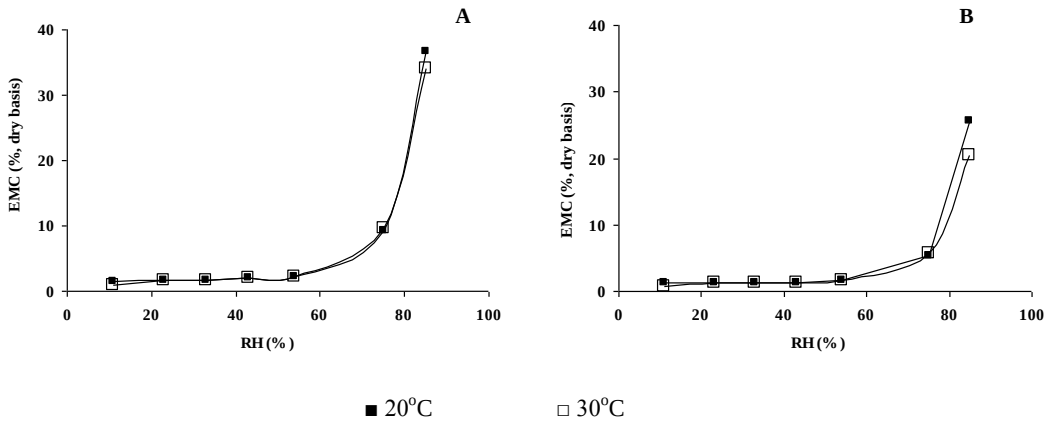


Figure 58. MSI of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B)

Storage temperature did not affect on EMC ($P\geq0.05$) with a range of 11-75% of RH. However, under 85% of RH, EMC of a sample that stored under 20°C was

higher than those stored under 30^{o}C ($P<0.05$). This result is similar to other food containing sugar based product such as jaggery and dried fruit (Rao *et al.,* 2006 and Goula *et al.,* 2008). This result is explained by high excitation state of water molecules at high temperature that caused a decreasing of an attractive force between water molecules and food components (Al-Muhtaseb *et al.,* 2002; Mohamed *et al.,* 2004). In addition, according to Palipane and Driscoll (1992) water molecules are activated to higher energy levels and break away from the water-binding sites of the food with increasing temperature. Thus, the decreasing in equilibrium moisture content are occurred.

The EMC of palm sugar cake that produced from palm sugar syrup with using an open pan was higher than that produced from palm sugar syrup with using a vacuum evaporator at constant RH. This result might be explained by the different types of sugar containing in palm sugar cake product. Palm sugar cake that produced from palm sugar syrup with using an open pan contained high reducing sugar content. This due to high processing temperature and a long time during processing can promote high inversion reaction. Normally, monosaccharide such as invert sugar is hygroscopic. Hence, palm sugar cake that contained high amount of reducing sugar is rapidly absorbed water into the structure. In addition, crystallinity in the sample that produced from palm sugar syrup with using an open pan was lower than that produced from palm sugar syrup with using a vacuum evaporator. Thus, water molecules are easily penetrated into the structure, resulting in more water was absorbed.

Glass transition temperature

Figure 59A and 59B show the Tg of palm sugar cake that produced from palm sugar syrup either using an open pan or a vacuum evaporator, respectively. There are no significant differences in Tg of palm sugar cake that stored under 11-51% RH ($P\geq0.05$). Tg decreased with increasing RH in a range of 75-85% RH ($P<0.05$). However, temperature seems to be not affected the Tg of palm sugar cake ($P\geq0.05$). Tg decreased with increasing RH due to the plasticizing effect of water. In general, all biomaterials can

be plasticized by water, and an increase in water content causes a decrease in Tg. Water plasticization can occur if a material is stored at relative humidities that lead to an increase in the water content of the material (Roos, 1995; Joupplia 2006). Moreover, Tg of palm sugar cake that produced from palm sugar syrup with using a vacuum evaporator was higher than those that produced from palm sugar syrup with using an open pan. This probably due to low reducing sugar content in a sample. Tg of palm sugar cake was depended on type and ratio of sugars. Low molecular weight sugars (glucose and fructose) had been shown to plasticize more easily than high molecular wight sugars such as sucrose (Roos, 1995; Joupplia 2006). Thus, high invert sugar (fructose and glucose) content in a sample that produced from palm sugar syrup with using an open pan also caused a higher decrease in Tg when compared to a sample that produced from palm sugar syrup with using a vacuum evaporator.

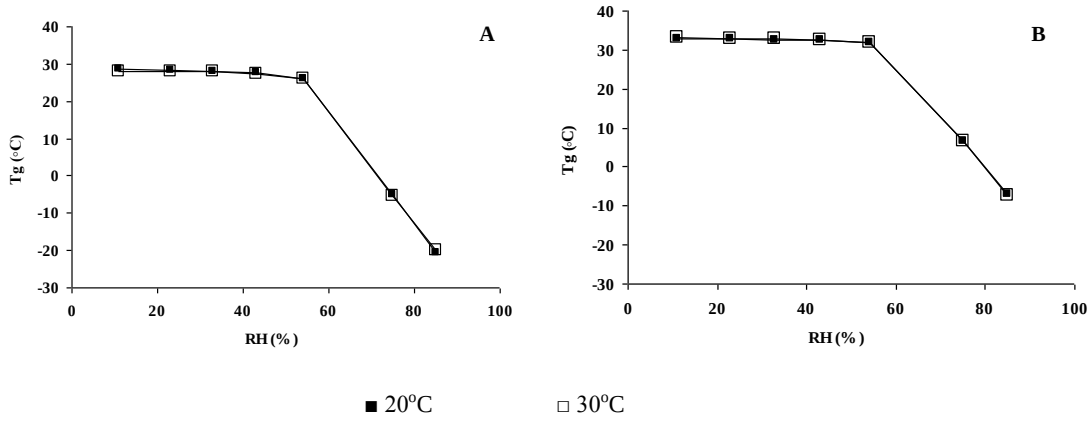


Figure 59. Tg as a function of RH of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B)

Changes in crystallinity of palm sugar cake during storage in different relative humidity

Crystallinity of palm sugar cake during storage under different relative humidities (RH) was shown in Figure 60. Crystallinity of all samples tended to decrease with increasing RH and storage time ($P<0.05$). Similar trend of crystallinity was found in palm sugar cake that stored under low RH including 11-51%.

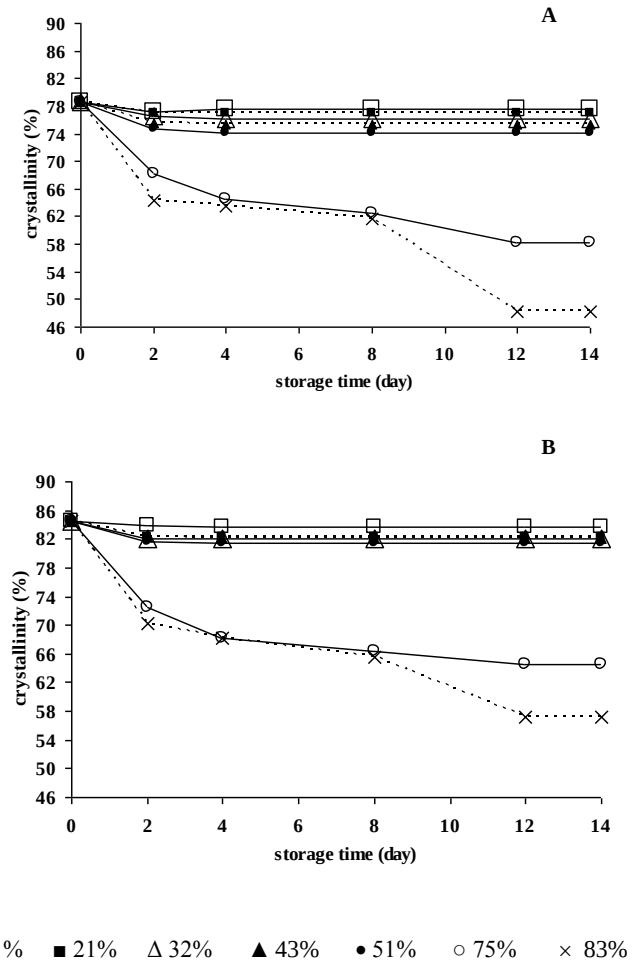


Figure 60. Changes in crystallinity of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) during storage in different relative humidities under 30^{o}C

Initial crystallinity of palm sugar cake that produced from palm sugar syrup either using an open pan or a vacuum evaporator was 78.54% and 84.53%, respectively. Crystallinity of all samples that stored under low RH including 11-51% slightly decreased from their initial values within the first two days during storage ($P<0.05$). Thereafter, no change ($P\geq0.05$) in crystallinity was found in all samples until the fourteen days of storage. Continuous decrease in crystallinity was found in a sample that stored under high RH including 75-83% until the twelve days of storage ($P<0.05$). Thereafter, no change ($P\geq0.05$) in crystallinity was found in all samples until the fourteen day of storage. The reduction of crystallinity during storage of palm sugar cake was due to the increase in moisture content in a product. Under high RH, water content of a sample was sharply increased as shown by MSI, resulting in the decrease in crystallinity and a sample becames watery and partial liquefied.

Changes in intermediate browning product and browning intensity during storage in different relative humidity

The Maillard reaction caused colour change in the palm sugar cake during storage. The accumulation of intermediate browning product (IBP) and browning intensity (BI) was monitored in a sample that stored under 30^{o}C as shown in Figure 61. RH in a range of 11-51% did not affect on IBP and BI ($P\geq0.05$). However, the IBP and BI increased with increasing RH in a range of 75-85% ($P<0.05$). No changes in IBP and BI were found in palm sugar cake that stored under low RH including 11-51% during storage time ($P\geq0.05$). On the other hand, continuous increase in IBP and BI was found in a sample that stored under high RH (75-85% RH) during storage time ($P\geq0.05$). Maillard reaction was clearly demonstrated to be affected by water content and water activity (Bell, 2007). Under low RH, a sample absorbed less water from an environment, and thus reactant mobility was restricted. While a sample gains a lot of water form an environment when it stored under high RH, resulting in a sufficient water to promote the Maillard reaction (Maio and Roos, 2006; Bell, 2007).

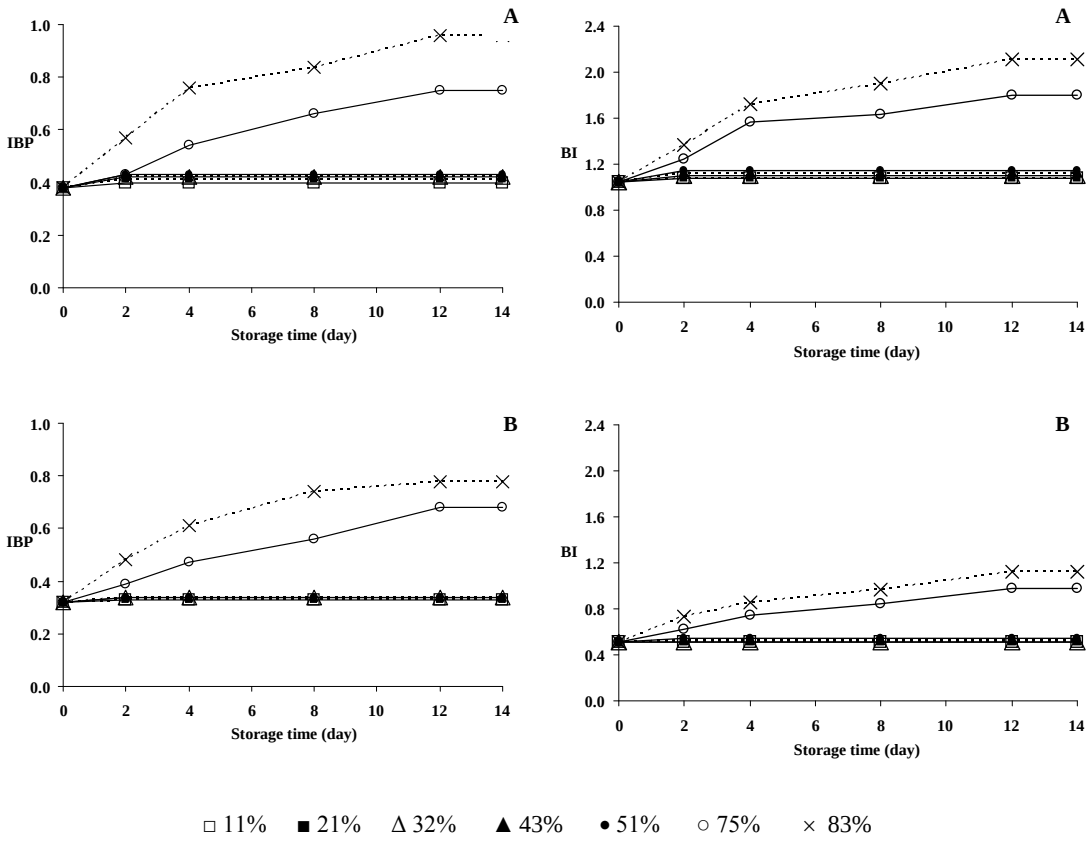


Figure 61. Changes in IBP and BI of palm sugar cake produced from palm sugar syrup with either using an open pan (A) or a vacuum evaporator (B) during storage in different relative humidities under 30^{o}C

6.5 Conclusion

The MSI for palm sugar cake are Type-III isotherms. Storage temperature did not affect in MSI characteristics, EMC and Tg of palm sugar cake (P≥0.05). Under low RH (11-51% RH), palm sugar cake absorbed less water while high amount of moisture content was absorbed in a sample that stored under high RH (75-85% RH). Low RH (11-51% RH) did not affect on Tg, crystallinity, IBP and BI of palm sugar cake. On the other hand, high RH (75-85% RH) influenced a decrease in Tg, crystallinity and an

increase in IBP and BI. Thus, high RH (75-85% RH) is not suitable to store palm sugar cake due to it decreased in Tg and crystallinity and increased in brown colour.

CHAPTER 7

SUMMARY AND FUTURE WORKS

7.1 Summary

1. The properties of palm sap, palm sugar syrup and palm sugar cake samples collected in Songkhla province showed differed in physical, chemical and microbiological properties among samples ($P<0.05$). Low pH of all palm sap samples might be contributed to the activity of microorganisms due to they can use sugars and produce organic acids and ethanol. These organic acids cause the inversion reaction, resulting in an increment of reducing sugar content. The variation in palm sugar syrup properties was probably due to heating condition. The steps of the heating process affected the properties of the palm sugar syrup. Two steps of heating process had more effect on the HMF formation than the one step of heating process. The properties of palm sugar cake may depend on the heating process, the property of palm sap or syrup and the ingredient added. High reducing sugar content can promote nonenzymatic browning reactions and retard crystallisation of palm sugar cake. The addition of sucrose as a seeding ingredient can enhance the hardness and crystallinity of palm sugar cake.

2. Harvesting time of palm sap affected the brown colour of palm sugar syrup. The delay of harvesting time (24 h) can promote nonenzymatic browning reactions and inversion reaction of palm sugar syrup during the production and storage. HMF content was found higher than 40 mg/kg in palm sugar syrup that produced by palm sap after collection time for 24 h. This indicated that palm sap after harvesting time for 24 h was improper to produce palm sugar syrup when compared to palm sap after 6-18 h of harvesting time. In addition, low temperature during storage (4^{o}C) can be used to reduce the browning development of palm sugar syrup when compared to high storage temperature (30^{o}C). Thus, a suitable harvesting time of palm sap, as a raw material and

low temperature during storage can be retarded the browning development of palm sugar syrup during storage.

3. Processing method affected properties of palm sugar syrup. The reduction of brown colour during heating could be achieved by producing palm sugar syrup by a vacuum evaporator. Temperature and time during storage influenced the properties of palm sugar syrup that produced by an open pan and a vacuum evaporator. During storage, Maillard reaction took place in a sample that stored under 4^{o}C lower than those that stored under 30^{o}C. Only sample that produced by an open pan and stored under 30^{o}C contained higher HMF content (50.58 mg/kg) than the standard requirement. Thus, the combination of concentration process by a vacuum evaporator and low temperature during storage (4^{o}C) can be used to retard brown colour formation in palm sugar syrup during storage.

4. The MRPs and CPs of heated palm sap (12 h of harvesting) and palm sugar syrup production with using an open pan and a vacuum evaporator exhibited antioxidant activity via electron electron donating and radical scavenging. The browning development during heating process was coincidental with an increase in antioxidant activity. However, MRPs or CPs such as HMF showed a toxicological status, but the concentration of HMF is lower than the standard requirement. Thus, MRPs and CPs from palm sugar syrup that produced by both methods are a new source of antioxidant and these palm sugar syrup can be used possibly for application in food product afterward with safe.

5. The addition of sucrose and glucose syrup can improve the properties of palm sugar cake mainly the reduction of dark colour and the increment of Tg when compared to sample that produced from 100% palm sugar syrup. The addition of sucrose can promote sugar crystallisation, resulting in high hardness and crystallinity. On the other hand, the addition of glucose syrup caused a decrease in hardness of palm sugar cake. The highest overall acceptability score was observed in a sample that produced from 50% syrup, 40% sucrose and 10% glucose syrup. Temperature and time during storage affected the properties of palm sugar cake. Low temperature (4^{o}C) during storage

can retard dark colour formation in palm sugar cake. Low RH (11% of RH) during storage can maintain the properties of palm sugar cake such as hardness and crystallinity, resulting in a sample still solidified during 12 months of storage. On the other hand, high RH (75% of RH) during storage promotes the decrease in Tg, crystallinity and hardness and a sample became watery or liquefied within the first month of storage. In addition, the MSI for palm sugar cake samples was Type-III isotherms. Storage temperature did not affect in MSI characteristics, EMC and Tg of palm sugar cake ($P \geq 0.05$). High RH (75-85%) influenced the decrease in Tg, crystallinity and the increase in IBP and BI. Thus, high RH (75-85%) is not suitable to store palm sugar cake due to the Tg and crystallinity decreased and brown colour increased.

7.2 Future works

1. The suitable collection procedure of palm sap such as collected in a closed container or using aseptic collection method during collection should be studied to improve the properties of palm sap, since this sap can affect the properties of palm sugar syrup and palm sugar cake afterward.

2. The application of antioxidant of palm sugar syrup should be further studied, especially focus on its stability when it applies in food systems.

3. Cost for the production of palm sugar cake by palm sugar syrup obtained from an open pan and a vacuum evaporator should be further calculated and the technology transfer should be promoted.

4. Further study related to the properties of palm sugar cake should be focused on the relative humidity during storage as well as type of packaging used.

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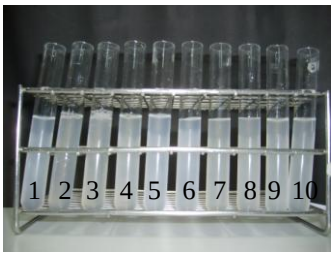
Appendix

A



B

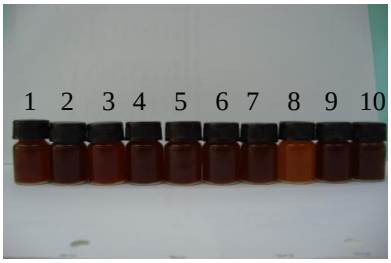


Figure 1. Ten samples of palm sap (A) and palm sugar syrup (B)

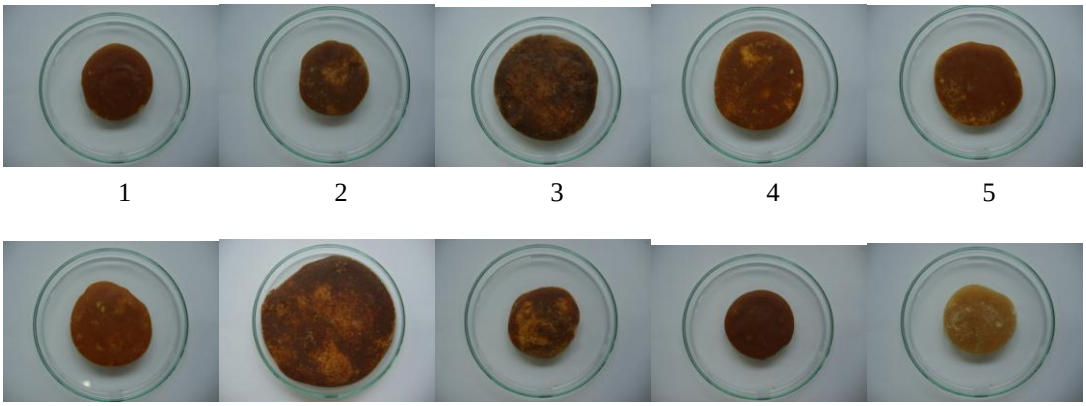


Figure 2. Ten samples of palm sugar cake

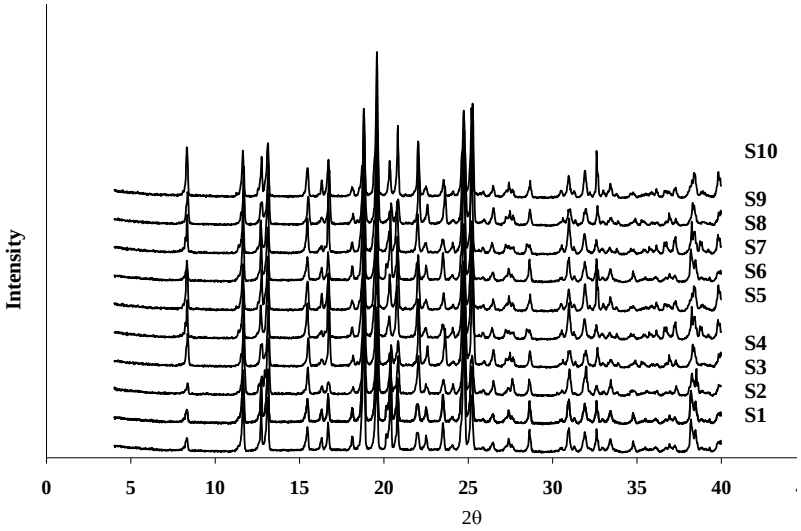


Figure 3. XRD patterns of ten palm sugar cake samples (S1-S10)

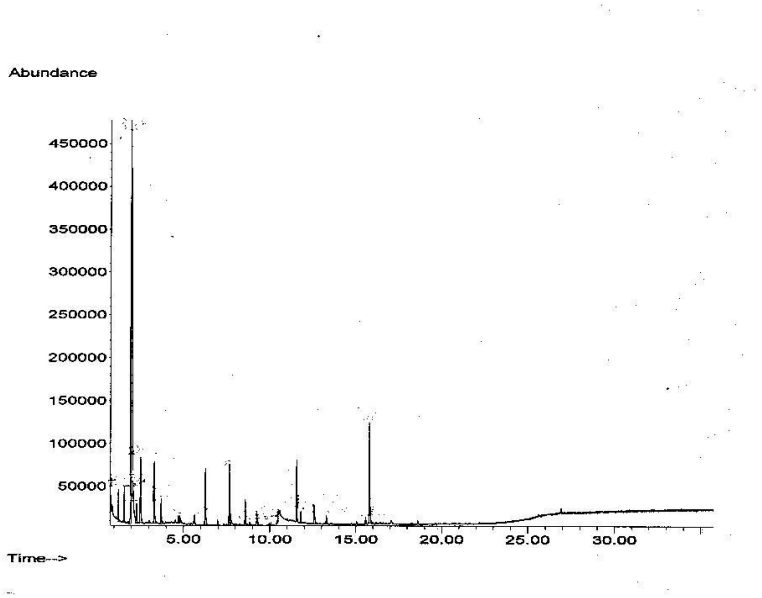


Figure 4. An example of chromatogram of volatile flavour compounds in palm sap

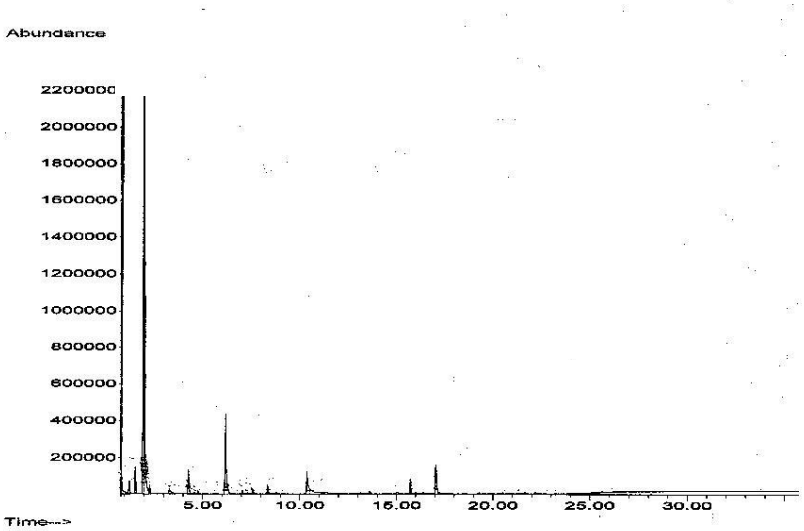


Figure 5. An example of chromatogram of volatile flavour compounds in palm sugar syrup

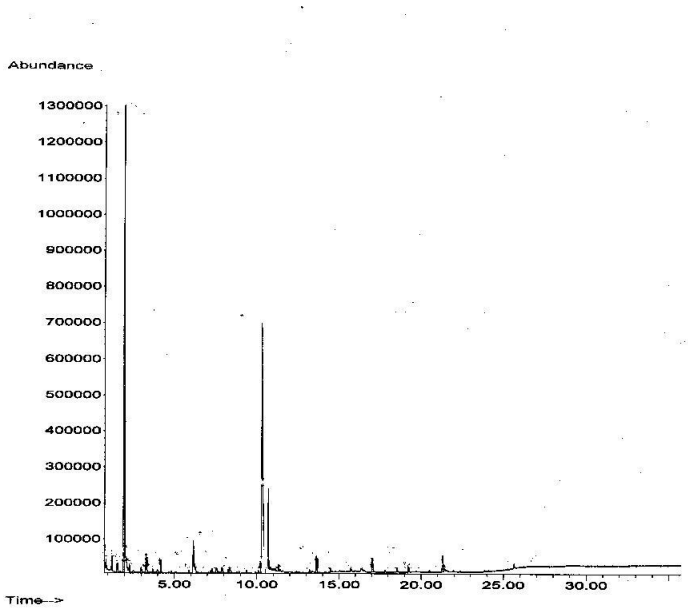
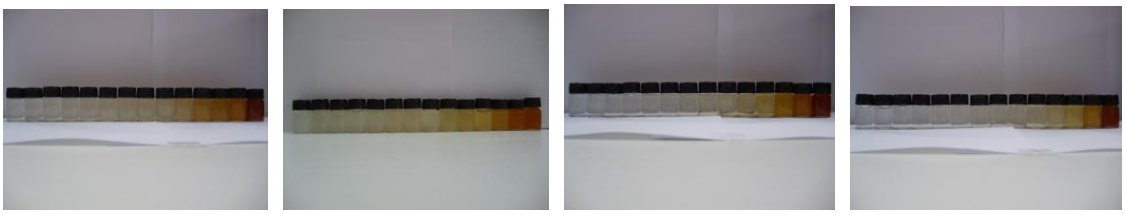


Figure 6. An example of chromatogram of volatile flavour compounds in palm sugar cake



6 h 12 h 18 h 24 h

Figure 7. Samples of palm sugar syrup produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h during the production

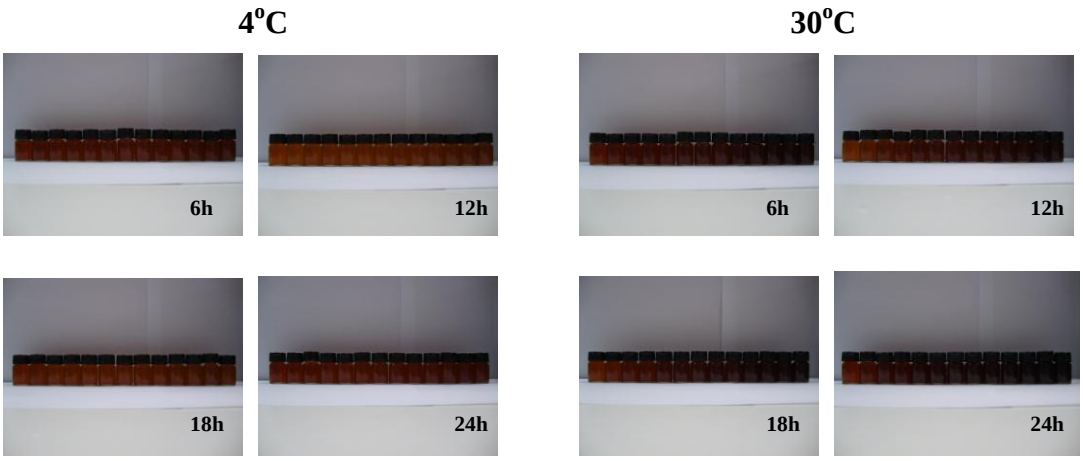


Figure 8. Samples of palm sugar syrup produced by palm sap after harvesting time for 6 h, 12 h, 18 h and 24 h during storage under 4°C and 30°C for 12 months

Open pan Vacuum evaporator at 70°C Vacuum evaporator at 80°C

Figure 9. Samples of palm sugar syrup produced by an open pan and a vacuum evaporator at 70°C and 80°C during the production

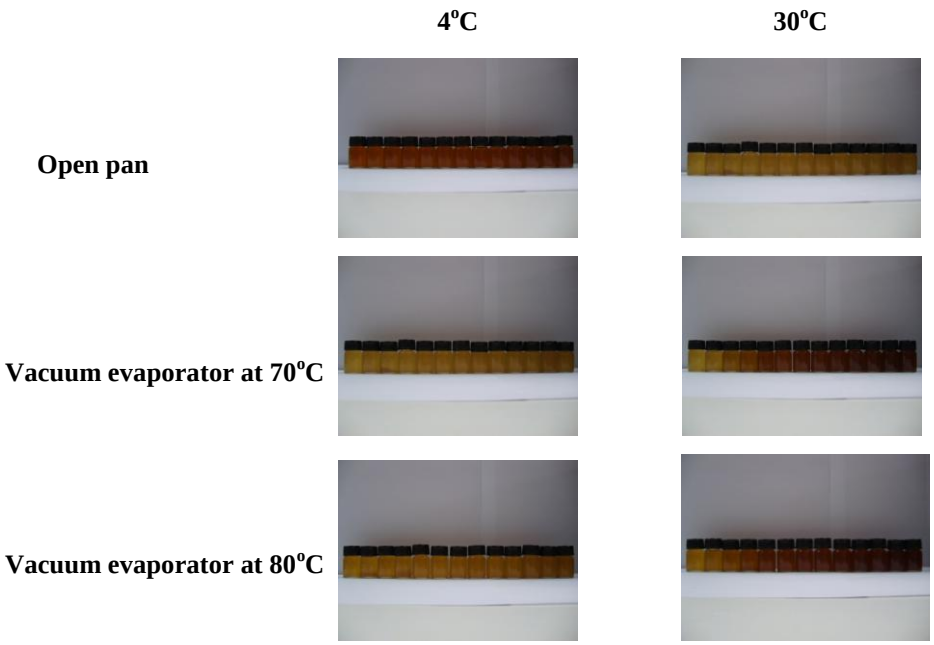


Figure 10. Samples of palm sugar syrup produced by an open pan and a vacuum evaporator at 70°C and 80°C during storage under 4°C and 30°C for 12 months

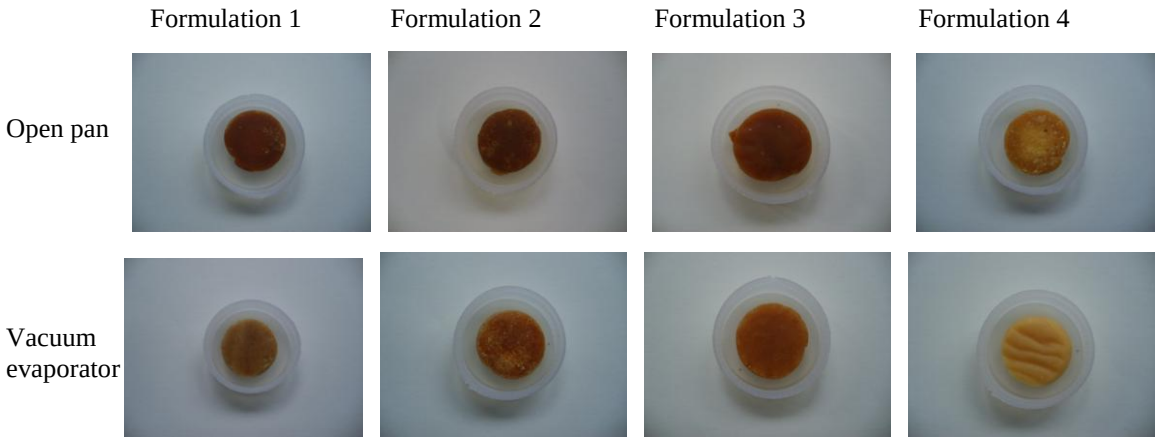


Figure 11. Samples of palm sugar cake produced from palm sugar syrup with either using an open pan (120°C) and a vacuum evaporator (80°C)

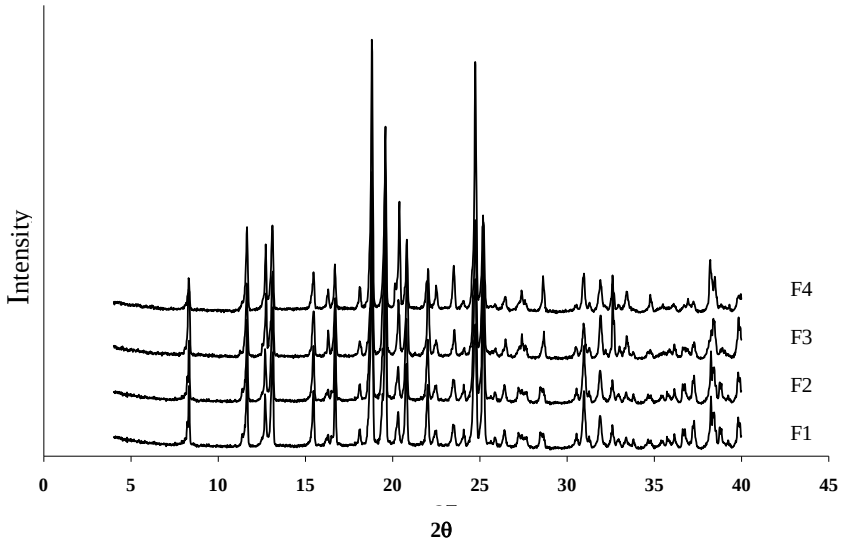


Figure 12. XRD patterns of four formulations (F1-F4) of palm sugar cake produced from palm sugar syrup with using an open pan (120^{o}C)

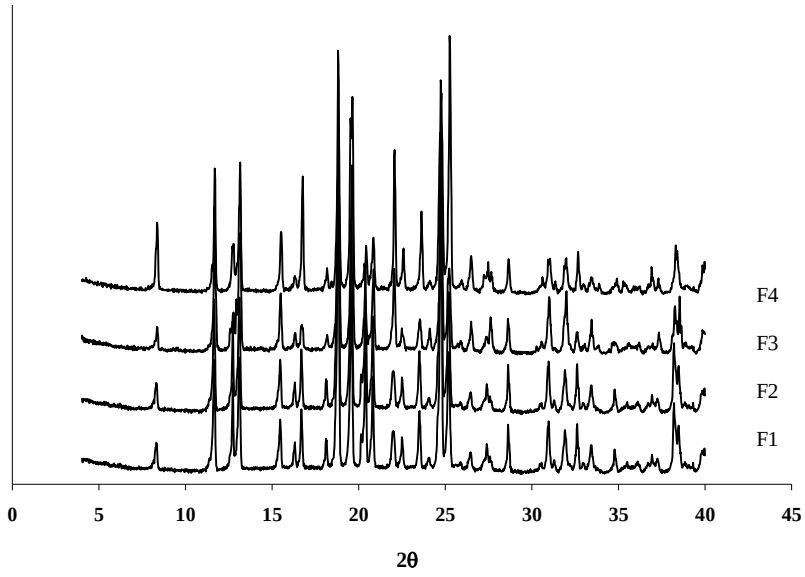


Figure 13. XRD patterns of four formulations (F1-F4) of palm sugar cake produced from palm sugar syrup with using a vacuum evaporator (80^{o}C)

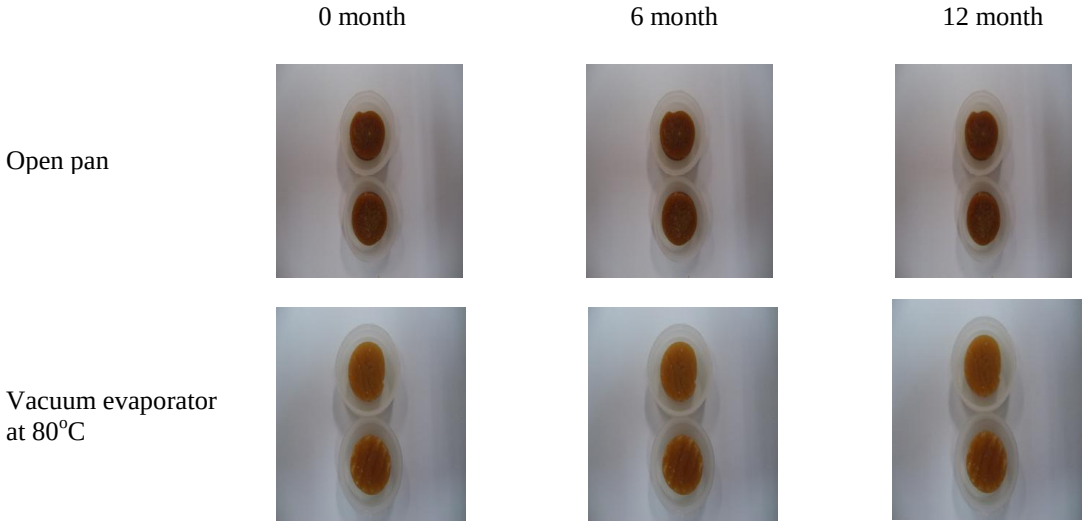


Figure 14. Samples of palm sugar cake produced from palm sugar syrup with either using an open pan (120°C) or a vacuum evaporator (80°C) and stored under 11% of RH, 4°C for 12 months

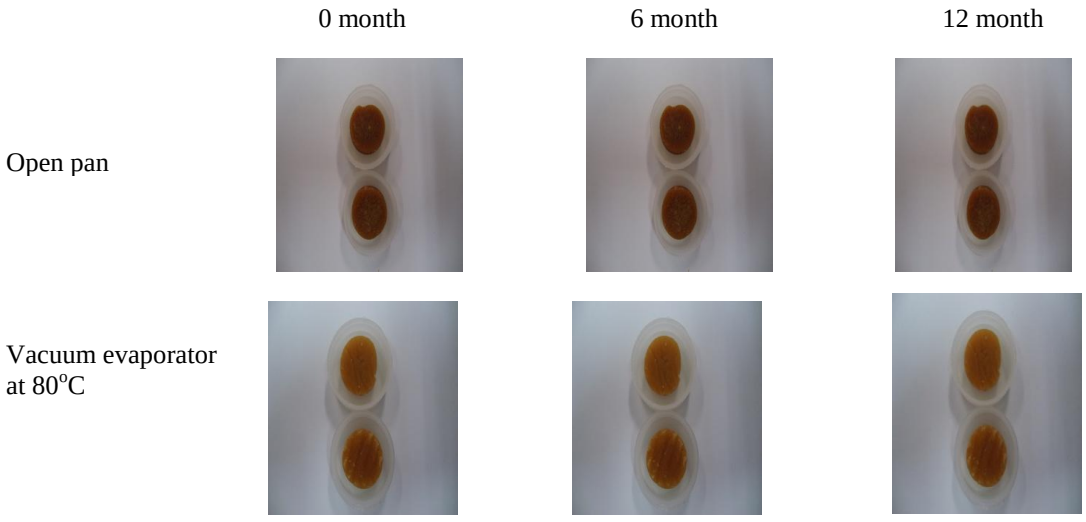


Figure 15. Samples of palm sugar cake produced from palm sugar syrup with either using an open pan (120°C) or a vacuum evaporator (80°C) and stored under 11% of RH, 30°C for 12 months

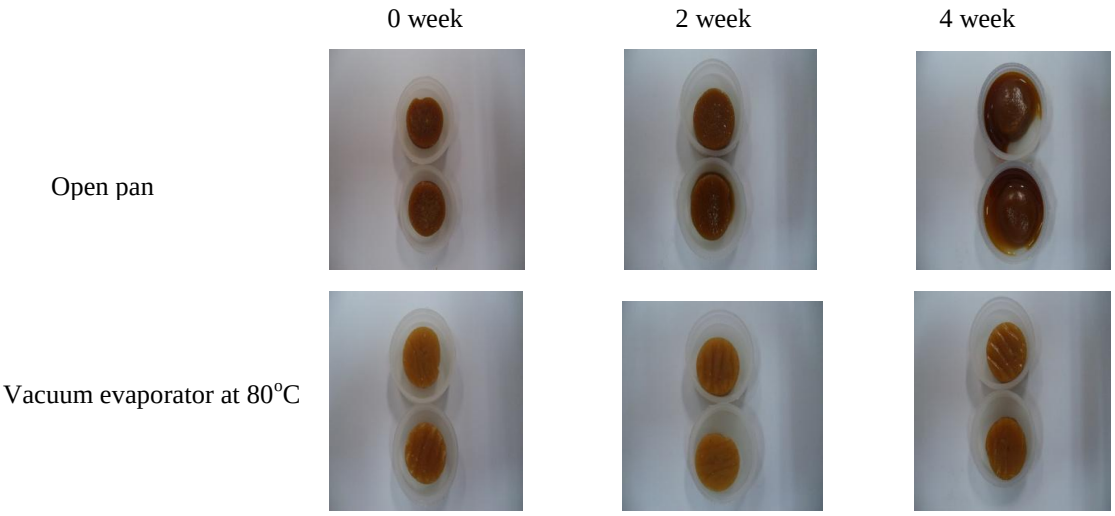


Figure 16. Samples of palm sugar cake produced from palm sugar syrup with either using an open pan (120°C) or a vacuum evaporator (80°C) and stored under 75% of RH, 4°C for 4 weeks

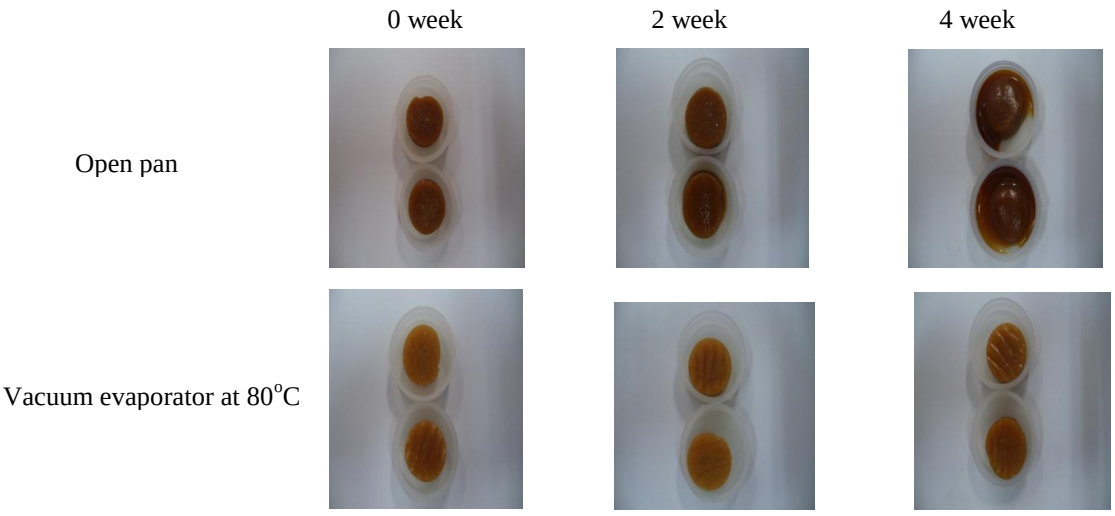


Figure 17. Samples of palm sugar cake produced from palm sugar syrup with either using an open pan (120°C) or a vacuum evaporator (80°C) and stored under 75% of RH, 30°C for 4 weeks

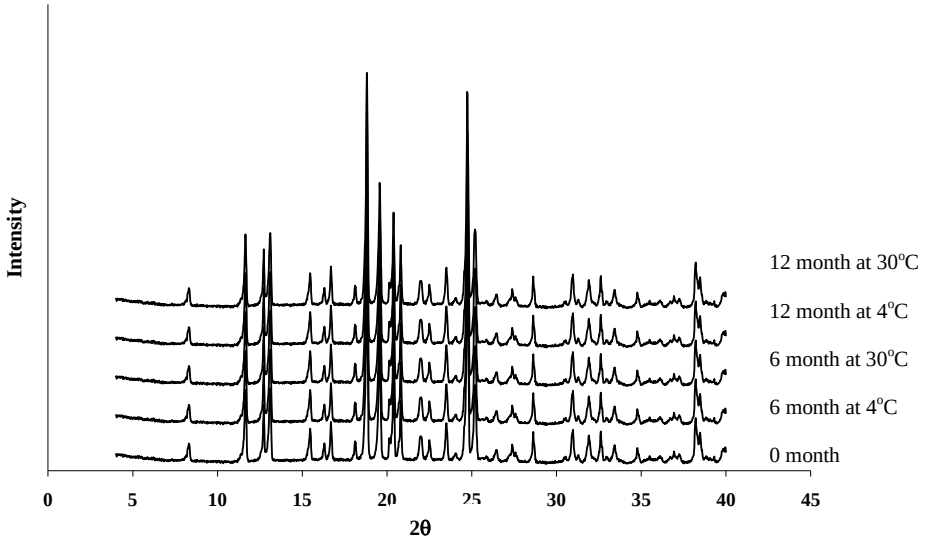


Figure 18. XRD patterns of palm sugar cake produced from palm sugar syrup with using an open pan (120°C) during storage under 11% of RH for 12 months

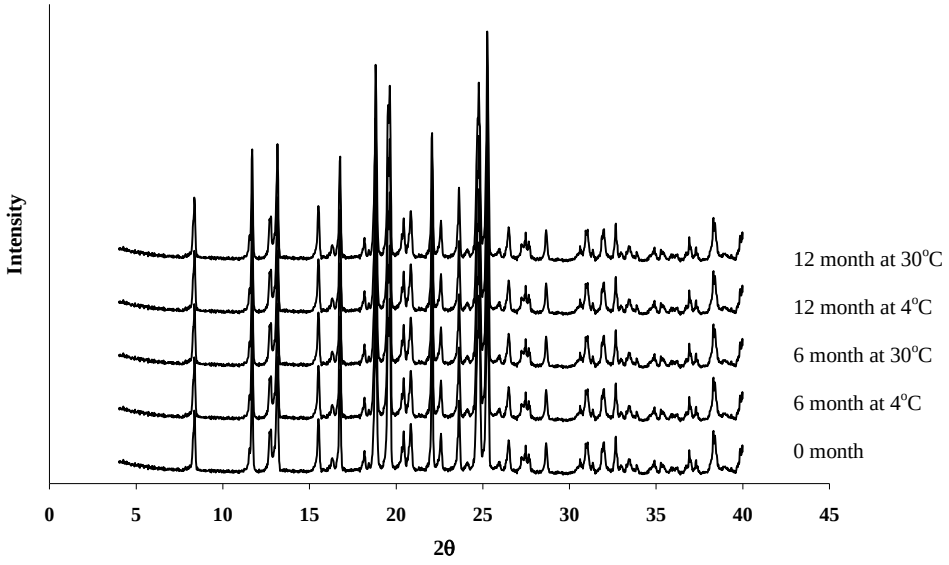


Figure 19. XRD patterns of palm sugar cake produced from palm sugar syrup with using a vacuum evaporator (80°C) during storage under 11% of RH for 12 months

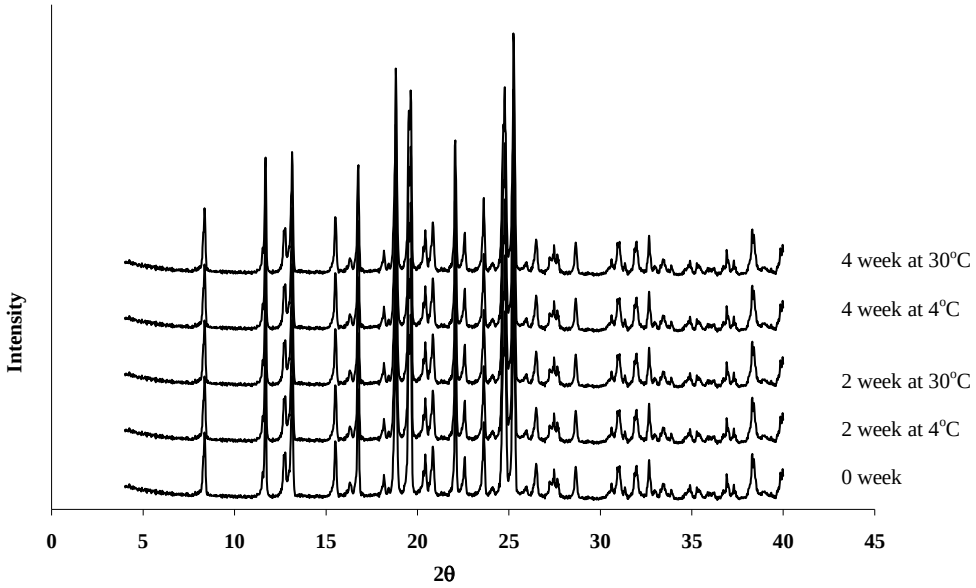


Figure 20. XRD patterns of palm sugar cake produced from palm sugar syrup with using an open pan (120°C) during storage under 75% of RH for 12 months

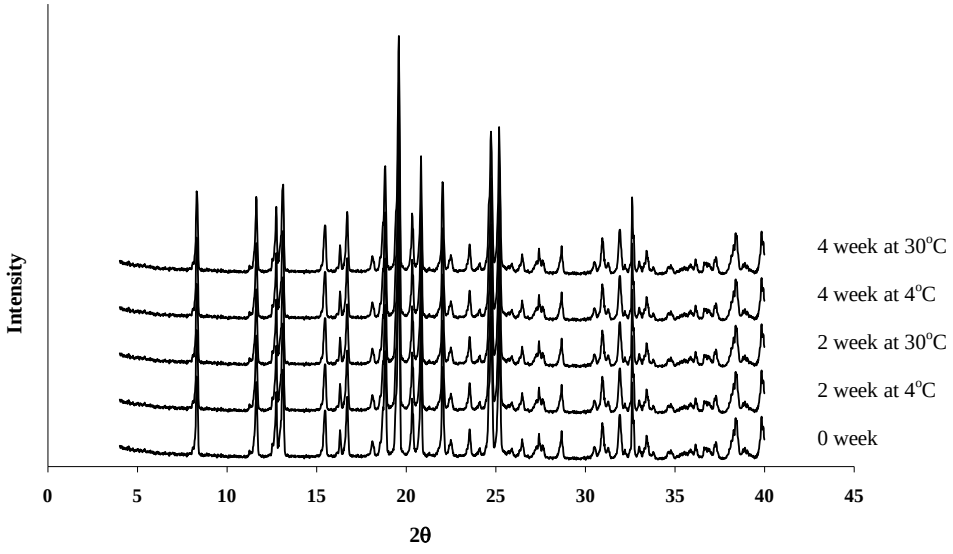


Figure 21. XRD patterns of palm sugar cake produced from palm sugar syrup with using a vacuum evaporator (80°C) during storage under 75% of RH for 12 months