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

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Abstract

The aim of this study was to monitor the changes in the properties 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 room temperature (30°C) for 12 months at monthly intervals. During storage, Maillard reaction took place in samples stored under 4°C lower than those stored under 30°C. This was shown by lower a^* values, intermediate browning products (IBP), browning intensity (BI) and HMF content, and higher L^* values, fructose, glucose and free amino group contents during storage for 12 months. HMF, a possible mutagen formed by nonenzymatic browning during the heating and storage of sugar based products, seems of particular interest and concern. Only the sample produced by an open pan and stored under 30°C contained HMF content (50.58 mg/kg) higher than the permitted maximum limit (40 mg/kg as recommended by the Codex Alimentarious). However, other samples still contained HMF in agreement with this standard. In addition, the microbiological quality and total soluble solids of all samples complied with Thai legislation standards for palm sugar syrup during 12 months of storage.

Introduction

Palmyra palm trees are widely grown in Africa, South Asia, South America, Australia and in other tropical countries (Morton, 1988). In Southern Thailand, these trees are widely grown and very popular in Petchaburi and Songkhla provinces. They are planted on the dykes of rice fields for shading the rice and tapping the palm sap for cooking. The unique flavour of palm sugar syrup has made it popular as an ingredient in confectionery and baking products. In addition, emphasis on the consumption of natural foods has resulted in the use of palm sugar concentrate as an alternative sweetener. Palm sugar syrup is obtained by heating fresh palm sap obtained from the Palmyra palm tree (*Borassus flabellifer* Linn.) until it is concentrated. This syrup is popular among Thai consumers; however it has not yet been produced for industrial purposes.

Traditionally, palm sugar syrup is produced by evaporating the palm sap in an open pan, and heated, using a wood fired stove, until it becomes concentrated. The producer then determines the quality of the final product by the intensity of its brown colour, and the thickness and viscosity of the liquid during the on-going process. The product is removed from the pan to cool down and kept in a container for selling. Heat from the process alters its unique flavour and colour. This heat causes the dark colour and affects the quality during storage. An alternative way, vacuum evaporation under low temperatures, can be used to reduce the thermal degradation of food properties resulting from the decrease in processing temperature and time.

Since quality is supremely important in food, thermal deterioration has to be controlled during processing and storage. Nonenzymatic browning 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. Therefore, the study of the processing method and storage temperature that influence on nonenzymatic browning reactions are very important. To date, there is no published information on changes in properties during the storage of palm sugar syrup resulting from the processing method and storage temperatures. Therefore, this work was carried out to monitor the changes in the properties of palm sugar syrup produced by an open pan and a vacuum evaporator during storage.

Material and Methods

Chemicals

D-glucose, D-fructose and sucrose were

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purchased from Fluka (Messerchmittstr, Switzerland). Acetonitrile and water (HPLC grade) were obtained from Prolabo (Paris, France). Sodium hydroxide, trichloroacetic acid and sodium sulfite were obtained from Merck (Darmstadt, Germany). 2,4,6-Trinitrobenzenesulfonic acid (TNBS), L-leucine, hydroxymethylfurfural and thiobarbituric acid were purchased from Sigma-Aldrich (St.Louis. MO, USA).

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°C) during transportation (30 min). The sample was filtered using cloth sheet at room temperature and kept at 4°C until used.

Production of palm sugar syrup

Palm sap was concentrated using two methods; an open pan and a vacuum evaporator. Palm sap (15 liters) was concentrated by using an open pan (at approximately 110°C) and a vacuum evaporator (at 70 and 80°C) until its total soluble solids reached 70°Brix to obtain palm sugar syrup. Each palm sugar syrup sample was stored under 4°C and 30°C in a closed polypropylene plastic cup for 12 months. The physical and chemical properties of each sample were determined at one month intervals. The microbiological loads were analysed at 6 month intervals.

Determination of the physical properties

Measurement of colour

Measurements of the colour of the samples were carried out using a Hunter Lab Colourflex colourimeter. 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 turbidity

The turbidity of palm sugar syrup was determined by measuring the transmittance at 650 nm using a spectrophotometer (Shimadzu, Kyoto, Japan) as described by Phaichamnan *et al.* (2010).

Determination of browning

The browning determination was monitored by measuring the absorbance value at 280 nm for the intermediate browning products (IBP) and 420 nm for browning intensity (BI). Absorbance was measured by using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan), described by Naknean *et al.* (2009). Prior to the determination of absorbance, samples were diluted with distilled water to obtain reliable absorbance readings.

Determination of the chemical properties

Measurement of total soluble solids

The total soluble solid content of the palm sugar syrup was determined as degree Brix using a hand refractometer.

Moisture content and water activity

Moisture content was measured by using a vacuum oven. Water activity was measured at room temperature using a water activity meter (Novasina, Thermostanter).

pH and total acidity measurement

The pH value was measured at an ambient temperature (30°C) with a pH meter (Saterious, USA) which was calibrated with standard buffer at pH 4.0 and 7.0. The palm sugar syrup was diluted with distilled water and titrated with 0.01N NaOH using a few drops of 1% phenolphthalein solution as an indicator. The result was calculated as a percentage of lactic acid (Rangana, 1986).

5-hydroxymethylfurfural (HMF) content

HMF content was determined according to the method of Keeney and Bassette (1959).

Type and concentration of sugars by HPLC

The type and concentration of sugars were determined using HPLC (Shimadzu, CR6A Chromatopac) with a Hypersil NH_2 column and refractive index detector according to Naknean *et al.* (2009).

Free amino group content

Free amino group content was determined according to the method of Benjakul and Morrissey (1997).

Determination of microbiological properties

The microbiological properties of palm sugar syrup, including total microbial count, yeast and mold count and osmophilic yeast, were analysed at 6 month intervals. Pour plating on Plate Count Agar (Merck KGaA, Darmstadt, Germany) was performed for the total microbial count. 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. The osmophilic yeast count was also analysed using the spread plate technique on osmophilic potato dextrose agar (Kiss, 1984).

Statistical analysis

All analysis and measurement were performed in triplicate. 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 SPSS package (SPSS 11.0 for windows, SPSS Inc, Chicago, IL).

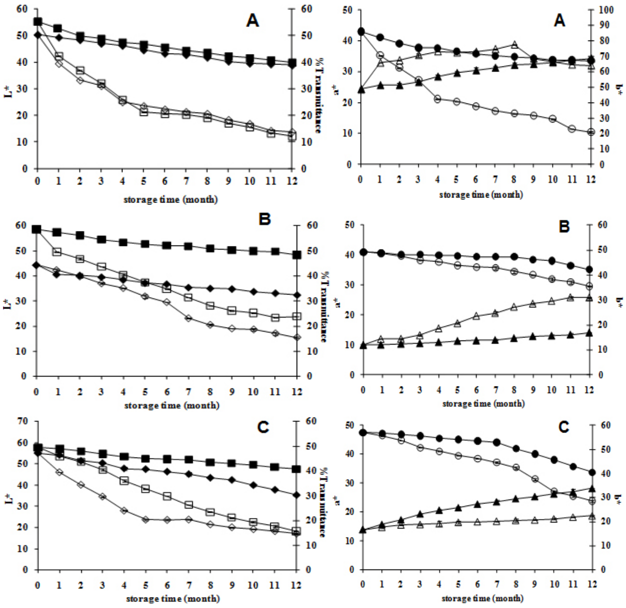
Result and Discussion

Changes in colour of palm sugar syrup during storage

The colour of a food is the first quality factor that the consumer appreciates and has a remarkable influence on its acceptance. Colour is also an indicator, such as of the natural transformation of fresh food (ripeness), and the changes that occur during its storage and processing. The change in the browning of palm sugar syrup was observed by using the CIE colour system (L^*, a^*, b^*).

The L^* value of palm sugar syrup was monitored during storage as depicted in Figure 1. The L^* value was significantly ($P < 0.05$) decreased by increasing the storage temperature and storage time. The L^* values were initially found to be 55.33, 58.64 and 57.78 for palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C, respectively. The L^* values decreased to 39.44, 48.40 and 47.59 for palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C and stored under 4°C until the end of storage, respectively. Additionally, L^* values were also reduced to 12.28, 23.81 and 18.30 for palm sugar syrups 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. The decreasing of this value indicates that the colour of palm sugar syrup changed to brown (Burdurlu and Karadeniz, 2003). At 4°C and 30°C, the decrease in L^* values of palm sugar syrup produced by an open pan was generally higher than that of palm sugar syrup produced by a vacuum evaporator. The greatest decrease in L^* values during storage occurred in palm sugar syrup samples stored at 30°C, followed by those stored at 4°C.

The a^* values can be used to evaluate browning of palm sugar syrup. The changes in a^* values of palm



■ L^*/4°C, □ L^*/30°C, ▲ a^*/4°C, Δ a^*/30°C, ♦ %T/4°C, ◊ %T/30°C, ● b^*/4°C, ○ b^*/30°C

Figure 1. Changes in L^*, a^*, b^* and %T values during storage of palm sugar syrup produced by an open pan (A), a vacuum evaporator at 70°C (B) and 80°C (C). Values represent mean ± standard deviation from triplicate determinations.

sugar syrup during storage are shown in Figure 1. The a^* values increased during storage from initial values of 24.35, 9.96 and 13.88 to 34.31, 14.07 and 18.73, respectively for palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C, and stored under 4°C until the end of storage, respectively. Moreover, the a^* values increased to 25.72 and 28.31 for palm sugar syrups produced by a vacuum evaporator at 70°C and 80°C and stored under 30°C until the end of storage, respectively. The a^* value significantly increased by increasing storage temperature and storage time ($P < 0.05$).

At 4°C and 30°C, the increase a^* values of palm sugar syrup produced by an open pan was generally higher than that of palm sugar syrup produced by a vacuum evaporator. Furthermore, palm sugar syrup samples stored under 30°C presented higher a^* values than those stored under 4°C. This was the case in the palm sugar syrup produced by open pan and vacuum evaporator at 70°C and 80°C.

Parameter b^* value indicates the variation between yellow and blue colour. Changes in b^* values during storage are presented in Figure 1. The b^* value significantly ($P < 0.05$) decreased by increasing the storage temperature and time. Initially the b^* values were found to be 86.14, 49.19 and 56.99 and these decreased to 67.17, 35.45 and 40.46, respectively for palm sugar syrups produced by an open pan and a vacuum evaporator 70°C and 80°C and stored under 4°C at the end of storage. The b^* values reduced to 20.84, 35.45 and 30.53 for palm sugar syrups 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. At 12 months storage, the b^* values of palm sugar syrups produced by an open pan decreased greater than that of palm sugar syrups produced by a vacuum evaporator. In addition, the greatest decrease in b^* value during storage occurred in the palm sugar syrup samples stored under 30°C, followed by those stored under 4°C.

Generally, the L^* and b^* values decrease while the a^* value increases during browning. This indicated that the colour of the palm sugar syrup became darker with more red component and less yellow components (Krapfenbauer *et al.*, 2006). The decrease in the L^* value is responsible for the dark colour and it may contribute to the nonenzymatic browning during storage (Maskan, 2006; Ibarz *et al.*, 1999; Rattanathanalerk *et al.*, 2005; Damasceno *et al.*, 2008). During the storage of sugar based products, Maillard reaction can take place because this reaction can progress at low temperatures. However, Caramelisation cannot occur since this reaction effectively takes place at temperatures of 120°C or above. Therefore, only Maillard reaction caused the browning of sugar based products in storage at ambient and low temperatures (Slade and Levine, 1991).

Moreover, the decrease in L^* values might be influenced by an increase in a^* values and a decrease in b^* values. As shown in the results, the storage temperature affected the colour of palm sugar syrup. The rate of Maillard reaction increased with increasing temperature and time (Martin *et al.*, 2001). Furthermore, temperature affects the activities of the reactants. The active form of sugar is considered to be an open chain, which is formed markedly with increasing temperature (Van Boekel and Martins, 2002). Moreover, the processing method is one of the parameters affecting the colour of palm sugar syrup during storage. Vacuum evaporation process operates under low temperature can reduce the rate of nonenzymatic browning during storage. Therefore, less colour formation was observed during storage when compared with palm sugar syrup produced by an open pan.

Changes in turbidity of palm sugar syrup during storage

Changes in transmittance values in palm sugar syrup during storage are shown in Figure 1. The transmittance value was significantly decreased by increasing the storage temperature and storage time ($P < 0.05$). Initial transmittance values of palm sugar syrup were 50.39 (for an open pan), 44.42 (for a vacuum evaporator at 70°C) and 47.16 (for a vacuum evaporator at 80°C). At the end of storage, the transmittance values declined to 38.86, 32.37 and 30.40 for palm sugar syrup produced by an open pan and a vacuum evaporator at 70°C and 80°C and stored under 4°C, respectively.

On the other hand, palm sugar syrup stored under 30°C showed lower transmittance values than those stored under 4°C, which were 13.86, 15.49 and 14.68 for palm sugar syrup produced by an open pan and by a vacuum evaporator at 70°C and 80°C, respectively. The results were in agreement with Gao *et al.* (1997) who studied the changes of turbidity in apple juice during storage and also obtained a decrease in this value. Lee *et al.* (2007) also reported an increase in the turbidity of clarified banana juice during storage at 4°C, 25°C and 37°C. The results showed that the decrease in transmittance value during storage was greatly enhanced by storing the banana juice at 30°C and was suppressed at 4°C.

In general, the presence of cell fragments has been found to be responsible for the immediate turbidity in fresh juice. Additionally, haze formation has caused turbidity in juice. The turbidity of palm sugar syrup depends greatly on its protein concentration and the polyphenol compounds, which are dissolved from Kiam wood and are presented in natural palm sap itself (Naknean *et al.*, 2010). Balange and Benjakul (2009) reported that the total phenolic content in Kiam wood (in its natural form) when extracted by water contained tannin at 29.33 mg/g of dried Kiam wood. The complex reaction 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 interaction has been 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. However, the hydrophobic interactions are generated through attraction between the aromatic structure of polyphenols and the nonpolar moiety in proteins (Katrine *et al.*, 2006). The rate of haze formation increased slightly at lower storage temperature (4°C), and more dramatically at 30°C for all palm sugar syrup samples. At higher temperatures the molecular mobility is higher. This allows more interaction to take place between the compounds involved in haze formation (Calderon *et al.*, 1968). Protein can react

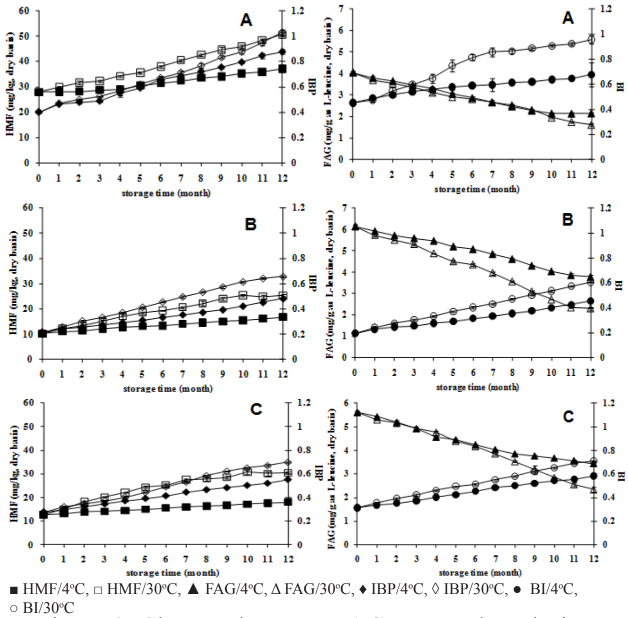


Figure 2. Changes in HMF, FAG, IBP and BI during storage of palm sugar syrup produced by an open pan (A), a vacuum evaporator at 70°C (B) and 80°C (C). Values represent mean ± standard deviation from triplicate determinations.

with phenol to form haze and that caused the decrease in transmittance values.

Changes in intermediate browning products (IBP) and browning intensity (BI) during storage

The Maillard reaction caused colour change in the palm sugar syrup during storage. The accumulation of IBP or BI was monitored as shown in Figure 2. The increase in UV absorbance at 280 nm is used to evaluate the intermediate degradation of products of nonenzymatic browning reaction. This is considered to indicate the formation of furfural compounds (presumably HMF) (Maio and Roos, 2006; Flink, 1983) and is used to detect products of the early stages of browning (Wijewickreme *et al*., 1997; Billaud *et al*., 2004). Moreover, the degree of browning, usually measured using the absorbance value at 420 nm, is often used to establish the extent of Maillard reaction. The increase in BI indicates the development of brown pigment in the final stage of Maillard reaction (Van Boekel, 1998).

A significant increase in IBP and BI during storage was found for palm sugar syrups produced by an open pan and a vacuum evaporator ($P < 0.05$). IBP and BI was significantly increased by increases in storage temperature and storage time ($P < 0.05$). At the beginning, the IBP of palm sugar syrups were 0.40 (for an open pan), 0.21 (for a vacuum evaporator at 70°C) and 0.28 (for a vacuum evaporator at 80°C). At the end of storage and at under 4°C, the IBP increased to 0.88, 0.48 and 0.55 for palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C, respectively. Likewise, an increase of the IBP was found in all samples stored under 30°C, which were 1.03, 0.66 and 0.70 for palm sugar syrup produced by an open pan and a vacuum evaporator at 70°C and 80°C, respectively. The increase in IBP was in accordance with an absorbance value at 420 nm. The initial BI of palm sugar syrups were 0.45 (for an open pan), 0.19 (for a vacuum evaporator at 70°C) and 0.31 (for a vacuum evaporator at 80°C). These were then increased to 0.68, 0.46 and 0.58 for palm sugar syrups produced by an open pan and a vacuum evaporator at 70 and 80°C at the end of storage at under 4°C, respectively. At the end of storage, samples stored at 30°C presented higher BI than those stored under 4°C, which were 0.96, 0.61 and 0.71 for palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C, respectively.

The results suggest that, the browning reactions during storage are greatly enhanced by storing palm sugar syrup at 30°C and were suppressed at 4°C. In addition, a similar relationship was found between the increase in IBP and BI. This suggested that an intermediate product was converted to a brown polymer and brown pigments were formed proportionately with the intermediate products generated. From the results it appears that some intermediate products might undergo conversion to the final brown compounds, while some intermediates are still being generated during storage (Ajandouz *et al*., 2001). Moreover, the increase in IBP and BI were coincidental with a decrease in L^* and a^* values as mentioned previously.

Changes in total soluble solid contents of palm sugar syrup during storage

The Thai Industrial Standards Institute Ministry of Industry (2003) has stated that the standard of total soluble solids (TSS) in palm sugar syrup shall not be less than 65°Brix. This is similar to The United States Department of Agriculture Standards (1980) which stated that the TSS content of the finished maple syrup shall not be less than 66°Brix, in order to prevent the micro-organisms growth during storage under room temperature. The total soluble solid content of all samples during storage is shown in Table 1. The initial TSS of all palm sugar syrup samples was approximately 70°Brix. The TSS of all palm sugar syrup samples remained constant during the 12 months of storage. Since, a closed polypropylene plastic cup was used to packed palm sugar syrup samples and inhibited water loss during storage. There was no significant difference in the TSS between samples stored under 4°C and 30°C.

Table 1. Changes in total soluble solid of palm sugar syrup during storage

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	70.23 ± 0.25 ns	70.23 ± 0.25 ns	70.07 ± 0.12 ns	70.07 ± 0.12 ns	70.07 ± 0.12 ns	70.07 ± 0.12 ns
1	70.07 ± 0.12 ns	70.07 ± 0.12 ns	70.07 ± 0.12 ns	70.03 ± 0.06 ns	70.03 ± 0.06 ns	70.03 ± 0.06 ns
2	69.93 ± 0.12 ns	70.07 ± 0.12 ns	70.02 ± 0.03 ns	70.03 ± 0.06 ns	70.03 ± 0.06 ns	69.97 ± 0.06 ns
3	69.87 ± 0.12 ns	70.07 ± 0.12 ns	70.03 ± 0.06 ns	70.08 ± 0.13 ns	70.03 ± 0.06 ns	69.93 ± 0.12 ns
4	69.90 ± 0.10 ns	70.07 ± 0.12 ns	70.07 ± 0.12 ns	70.10 ± 0.06 ns	70.03 ± 0.06 ns	70.00 ± 0.10 ns
5	69.93 ± 0.12 ns	70.07 ± 0.12 ns	70.07 ± 0.12 ns	70.03 ± 0.06 ns	70.07 ± 0.12 ns	70.07 ± 0.10 ns
6	69.93 ± 0.12 ns	70.07 ± 0.12 ns	70.02 ± 0.03 ns	70.10 ± 0.10 ns	69.97 ± 0.15 ns	70.10 ± 0.10 ns
7	69.87 ± 0.12 ns	70.07 ± 0.12 ns	70.03 ± 0.06 ns	70.20 ± 0.26 ns	70.23 ± 0.45 ns	70.07 ± 0.06 ns
8	69.90 ± 0.10 ns	70.03 ± 0.15 ns	70.07 ± 0.12 ns	70.03 ± 0.06 ns	70.03 ± 0.06 ns	70.10 ± 0.10 ns
9	69.93 ± 0.12 ns	69.93 ± 0.12 ns	70.07 ± 0.12 ns	69.93 ± 0.15 ns	70.03 ± 0.06 ns	70.07 ± 0.12 ns
10	69.93 ± 0.12 ns	69.97 ± 0.06 ns	70.02 ± 0.03 ns	70.03 ± 0.06 ns	70.10 ± 0.01 ns	70.07 ± 0.12 ns
11	69.87 ± 0.12 ns	69.93 ± 0.12 ns	70.03 ± 0.06 ns	70.03 ± 0.06 ns	70.07 ± 0.12 ns	70.13 ± 0.06 ns
12	70.03 ± 0.15 ns	70.00 ± 0.20 ns	70.07 ± 0.06 ns	70.10 ± 0.10 ns	70.07 ± 0.06 ns	70.10 ± 0.10 ns

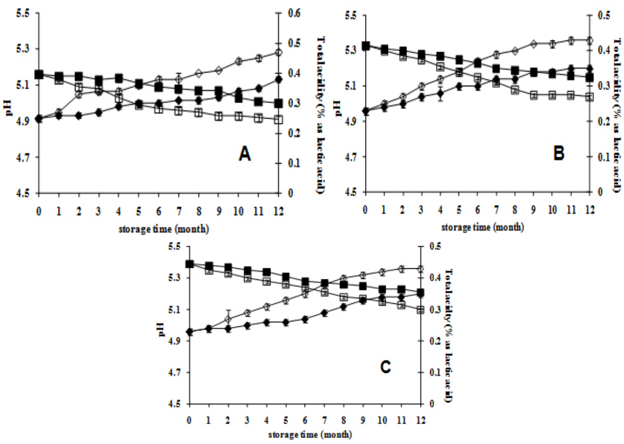
Each value is the mean of triplicate determinations ± standard deviation.
ns refers to not significant between two temperatures during storage (P > 0.05).

Initially, the TSS of palm sugar syrups were 70.23°Brix (for an open pan), 70.03°Brix (for a vacuum evaporator at 70°C) and 70.00°Brix (for a vacuum evaporator at 80°C). At the end of storage under 4°C, the TSS remained constant and were 70.00°Brix, 70.03°Brix and 70.00°Brix for palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C, respectively. Similarly, the TSS remained constant in all samples stored under 30°C, which were 70.03°Brix, 70.00°Brix and 70.00°Brix for palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C, respectively. From the results, it can be seen that all palm sugar syrup samples met the standard and stable during storage for 12 months either storage at 4°C or 30°C.

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

Moisture content and water activity of palm sugar syrup can be used as an indicator to evaluate the microbiological properties of sugar based products. Both moisture content and water activity are highly important for the shelf life of syrup during storage. In general, a high moisture content and water activity causes the syrup to ferment, spoil and lose flavour, with ensuring syrup-quality loss (Costa *et al.*, 1999). There were no significant differences in moisture content and water activity during storage in all the palm sugar syrup samples ($P \geq 0.05$).

Initially, moisture content (MC) and water activity (A_w) of all palm sugar syrup samples were as follows: 25.91% (MC), 0.79 (A_w) (for an open pan); 25.07% (MC), 0.79 (A_w) (for a vacuum evaporator at 70°C); and 25.34% (MC), 0.79 (A_w) (for a vacuum evaporator at 80°C). At the end of storage at under 4°C, the MC levels remained constant which were 25.85%, 25.56% and 25.18% for palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C, respectively. Similarly, the MC was found to remain constant in all samples stored under 30°C, which were 25.47%, 25.37% and 25.45% for palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C, respectively. At the end of storage, Aw in all samples also remained constant which were 0.80.



■ pH/4°C, □ pH/30°C, ♦ Total acidity/4°C, ◊ Total acidity/30°C

Figure 3. Changes in pH and total acidity during storage of palm sugar syrup produced by an open pan (A), a vacuum evaporator at 70°C (B) and 80°C (C). Values represent mean ± standard deviation from triplicate determinations.

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

Changes in pH and total acidity were observed during the 12 months of storage as shown in Figure 3. A significant decrease in the pH values and increase in total acidity was monitored for palm sugar syrups produced by an open pan and a vacuum evaporator during storage ($P < 0.05$). The greatest decrease in pH value and increase in total acidity during storage occurred in palm sugar syrup stored at 30°C, followed by the samples stored at 4°C. Initially 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 syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C and stored at 4°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 syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C and stored at 30°C until the end of storage, respectively.

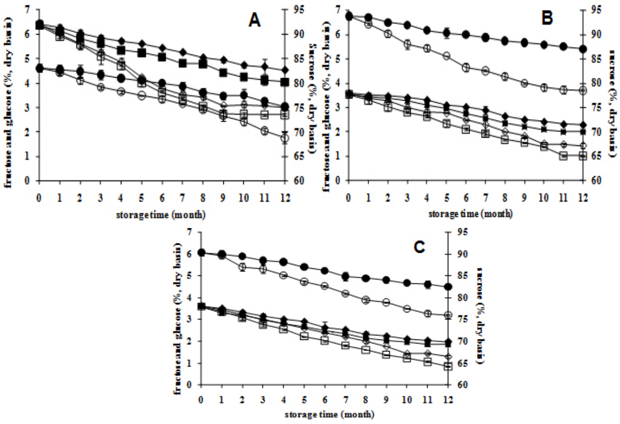
The total acidity increased during storage. This rose from initial values of 0.25%, 0.23% and 0.23% to 0.38%, 0.35% and 0.35% for palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C and stored under 4°C until the end of storage, respectively. Moreover, total acidity increased to 0.47%, 0.43% and 0.43% for palm sugar syrups 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 was probably due to chemical reaction and the growth of microorganisms. The reduction in pH values and increase in total acidity occurring in Maillard reaction were 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). In addition, Beck *et al.* (1990) reported that the decrease in pH observed during Maillard reaction could be attributed to the reaction of amines to form compounds of lower basicity and to the degradation of sugars into acids. This is in accordance with Carabasa-Giriber and Ibarz-Ribas 2000. They monitored the changes in pH in a model system of fructose-glutamic acid, fructose-asparagine, glucose-glutamic acid and glucose-asparagine during Maillard reaction.

Moreover, the pH value is indeed a useful index of possible microbial contamination (Conti *et al.*, 2007). Microorganism contaminants contribute to lower pH and higher total acidity of palm sugar syrup. Some microorganisms that survived after processing, such as osmophilic yeasts, can grow and produce organic acids. The hot season in southern Thailand favors the rapid growth of osmophilic yeasts such as *Saccharomyces rouxii* that produce organic acids as secondary metabolites. In addition, high storage temperatures can accelerate the rate of Maillard reaction and the growth of microorganisms that are responsible for the decrease in pH values and increases in total acidity.

Changes in HMF content of palm sugar syrup during storage

HMF has been found to be a well known indicator of the heating process and/or storage of sugar based product because of its toxicological status (Mendoza *et al.*, 2004). In addition, it has been used as a quality assurance parameter in food industry (Cammerer *et al.*, 1999). Changes in HMF content during storage of all palm sugar syrup samples are presented in Figure 2. An increase in the HMF content as storage times increased was observed in all samples ($P < 0.05$). The initial HMF content of palm sugar syrups were 26.05 mg/kg (for an open pan), 10.22 mg/kg (for a vacuum evaporator at 70°C) and 12.76 mg/kg (for a vacuum evaporator at 80°C). At the end of storage the HMF contents increased to 37.15 mg/kg, 16.74 mg/kg and 18.01 mg/kg for palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C and stored at 4°C, respectively. Meanwhile, palm sugar syrup samples stored at 30°C showed a higher HMF content than those stored under 4°C, which were 50.58 mg/kg, 25.27 mg/kg and 30.48 mg/kg for palm sugar syrup produced by an open pan and a vacuum evaporator at 70°C and 80°C, respectively. At 4°C and 30°C, an increase in the HMF content of palm sugar syrup produced by an open pan was generally higher than that of the palm sugar syrups produced by a vacuum evaporator. The greatest increase in HMF content during storage occurred in the samples of palm sugar syrup stored at 30°C, followed by those stored at 4°C for all samples. The increase in HMF was coincidental with an increase in IBP. Furthermore, the HMF content of all samples was lower than the permitted maximum limit of 40 mg/kg as recommended by the Codex Alimentarius and Council of European Union directive concerning honey (Turhan *et al.*, 2008).

During the storage of palm sugar syrup, HMF can be formed. In the acid medium of this product, the dehydration of carbohydrates, especially hexose, led to the formation of HMF. Moreover, Maillard reaction can also take place, giving rise to Amadori compounds forming during the first step of the reaction, and HMF as a consequence of further reaction (Mendoza *et al.*, 2004). Therefore, the considerable variations of HMF found in the samples may be an indicator of storage. Normally, the HMF content depends greatly on processing method, degree of heating, the acidic conditions and the storage conditions (Fallico *et al.*, 2008).



■ Fructose/4°C, □ Fructose/30°C, ♦ Glucose/4°C, ◊ Glucose/30°C, ● Sucrose/4°C, ○ Sucrose/30°C

Figure 4. Changes in fructose, glucose and sucrose during storage of palm sugar syrup produced by an open pan (A), a vacuum evaporator at 70°C (B) and 80°C (C). Values represent mean ± standard deviation from triplicate determinations.

Changes in sugar content of palm sugar syrup during storage

Sugar is a major component in palm sap and palm sugar syrup. Changes in the fructose, glucose and sucrose contents of palm sugar syrup are shown in Figure 4. The fructose, glucose and sucrose were initially found to be: 6.36%, 6.43%, 83.03% for open pan; 3.54%, 3.59%, 93.82% for vacuum evaporator at 70°C and 3.59%, 3.63%, 90.37% for vacuum evaporator at 80°C. A significant decrease in fructose, glucose and sucrose contents during storage was observed for palm sugar syrup produced by an open pan and a vacuum evaporator at 70°C and 80°C ($P < 0.05$). The fructose, glucose and sucrose were significantly decreased by increasing the storage temperature and storage time ($P < 0.05$). At the end of storage, the fructose, glucose and sucrose contents of samples stored under 4°C decreased to: 4.04%, 4.52%, 75.17% for an open pan; 2.00%, 2.30%, 87.05% for a vacuum evaporator at 70°C; and 1.85%, 1.98%, 85.51% for a vacuum evaporator at 80°C. In addition, the fructose, glucose and sucrose contents of samples 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°C.

Normally, most of the total carbohydrate fraction of syrup is in the form of sucrose, with varying amounts of invert sugars (glucose and fructose) present. The latter is primarily due to the function of microbial contamination of sap during storage prior to processing. In some cases, the inversion of sucrose to invert sugars may continue after packing into retail containers, especially if the syrup is low in TSS and fermentation occurs. A level of sucrose hydrolysis may also occur during thermal processing, depending on the processing method (Perkin and Van den Berg, 2009). From the results, the glucose and fructose of the samples produced by an open pan were higher than those produced by a vacuum evaporator. On the other hand, the sucrose of the samples produced by an open pan was lower than those produced by a vacuum evaporator. Furthermore, the samples produced by the vacuum evaporator at 70°C contained a higher level of sucrose than that produced by the vacuum evaporator at 80°C.

Palm sugar syrup produced by a vacuum evaporator can reduce the loss of sucrose more than in palm sugar syrup produced by an open pan. This is probably due to the process using a lower temperature and shorter time. Moreover, this process can also minimize sucrose inversion, therefore lowering the reducing sugar content compared with the process of palm sugar syrup production 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 (Naknean *et al*., 2009).

At 4°C and 30°C, the decrease in fructose, glucose and sucrose contents of palm sugar syrup produced by an open pan was generally higher than palm sugar syrups produced by a vacuum evaporator. The greatest decrease in fructose, glucose and sucrose during storage occurred in palm sugar syrup samples stored at 30°C, followed by those stored at 4°C. A decrease in fructose and glucose with storage times in each process can be used to indicate Maillard reaction. A decrease in sucrose refers to the inversion of sucrose that took place during the storage of palm sugar syrup.

Generally, the browning rate is influenced by the type of reducing sugars involved in the reaction. The reactivity of reducing sugars has been reported to decrease in the following order: aldopentose>aldohexose>ketohexose>disaccharide (Spark, 1969). Yeboah *et al*. (1999) found that the aldehyde group of the acylic form of aldose was more electrophilic than the keto group of the acylic form of ketose. As a result, all palm sugar syrup samples contained lower levels of fructose than glucose. However, Maillard reaction products derived from fructose showed more BI than those from glucose, presumably because fructose had a higher proportion of the open chain form than glucose (Benjakul *et al*., 2005). Thus, an amino acid-sugar complex could be formed more easily. However, Naranjo *et al.* (1998) found that glucose, which was more electrophilic, could react faster than fructose in a casein-sugar system. The differences in the reaction rates of sugar observed in many studies were possibly due to the different compositions of amino acids and conformations of protein, as well as the conditions used in different studies (Benjakul *et al*., 2005).

Changes in free amino group (FAG) content of palm sugar syrup 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. 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 FAG content can be used to evaluate Maillard reaction in palm sugar syrup during storage. The FAG content in palm sugar syrup was monitored during storage as shown in Figure 2. From the result, a significant decrease in FAG content was observed for palm sugar syrup produced by an open pan and a vacuum evaporator at 70°C and 80°C ($P < 0.05$). Initially the FAG contents were found to be 4.02 mg/g, 6.13 mg/g and 5.61 mg/g and then decreased to 2.15 mg/g, 3.79 mg/g and 3.44 mg/g in the palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C and stored under 4°C until the end of storage. The FAG contents declined to 1.61 mg/g, 2.30 mg/g and 2.34 mg/g for palm sugar syrups 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. At 4°C and 30°C, the decrease in the FAG content of palm sugar syrup produced by an open pan was generally higher than in palm sugar syrup produced by a vacuum evaporator. In addition, palm sugar syrup stored at 30°C contained a lower FAG content than one stored under 4°C in both palm sugar syrups produced by an open pan and a vacuum evaporator at 70°C and 80°C. The FAG content tended to decrease during storage since it declined during Maillard reaction.

From the results, the decrease in the free amino group, fructose and glucose were in accordance with the increase in IBP, BI and HMF. This indicated that the interaction between free amino group and sugars through the glycation process took place during the storage of palm sugar syrup. As a result, intermediate products were formed and further converted to brown pigment, as observed by the increase in BI (Benjakul *et al*., 2005).

Changes in microbial loads of palm sugar syrup during storage

The Thai Industrial Standards Institute Ministry of Industry (2003) requires that the total microbial and the yeast and mold counts in palm sugar syrup

Table 2. Changes in total microbial count (cfu/g) of palm sugar syrup during storage

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 3. Changes in yeast and mold (cfu/g) of palm sugar syrup during storage

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 4. Changes in osmophilic yeast (cfu/g) of palm sugar syrup during storage

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

shall not be more than 5 x 10^2 CFU/g and 100 CFU/g, respectively. Normally, microorganisms present in the palm sap are destroyed by the heating process during concentration. However, syrup could become contaminated from the air or equipment. In addition, a few microorganisms, especially osmophilic yeasts, may be found to survive and to grow after processing and during storage (Dumont *et al*., 1993). The counting of total microbial count and yeast and mold count showed that all samples at the end of storage had less than 10 CFU/g (Table 2 and 3). The osmophilic yeast of samples stored under 4°C showed 40 CFU/g (for an open pan and a vacuum evaporator at 80°C) and 50 CFU/g (for a vacuum evaporator at 70°C). The osmophilic yeast of samples stored under 30°C showed 70 CFU/g (for an open pan and a vacuum evaporator at 80°C) and 80 CFU/g (for a vacuum evaporator at 70°C) at the end of storage (Table 4). The total microbial count and yeast and mold count were well below the maximum limit values required by The Thai Industrial Standards Institute Ministry of Industry (2003).

Thus it can be stated that the quality of all the palm sugar syrup samples produced in this study was good. They were in accordance with national and international standards. Furthermore, cold temperature storage (4°C) restricted or delayed the growth of microorganisms and thus reduced potential for acid production and product spoilage. However, all palm sugar syrup samples can be stored at room temperature and be safe for consumers. In general, the microbes currently reported to be found in sugar based product, such as syrup and honey, 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 the honey industry, because they can grow even at a limited level of water activity (Aw approximately 0.65-0.80). Moreover, osmophilic yeast may survive after heating as sugar can protect its spore as reported by Phaichamnan *et al.* (2010). Thus the growth of osmophilic yeast is another factor affecting the decrease of pH values and increases in the total acidity of syrup during storage as mentioned previously (Snowdon and Cliver, 1996).

Conclusion

This study provided information on the processing of palm sugar syrup and storage conditions that could influence the properties of palm sugar syrup. Palm sugar syrup produced by a vacuum evaporator retained desired quality attributes in syrup better than palm sugar syrup produced by heating with an open pan. Moreover, the loss of quality of palm sugar syrup due to nonenzymatic browning reaction increased with increases in storage temperature and time. The results obtained from the different treatments suggest that nonenzymatic browning of palm sugar syrup during storage could be reduced. This could be done by using vacuum evaporator for the production of palm sugar syrup and storing at low temperatures. Furthermore, the microbial loads of all samples complied with Thai legislation standards for palm sugar syrup at the end of storage.

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