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Characterization of palm sap harvested in Songkhla province, Southern Thailand

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Abstract:The purpose of this study was to characterise the quality of palm sap after harvested in Songkhla province, southern Thailand. Ten palm sap samples were analyzed. The results showed differed in physical and chemical quality 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 turbidity 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 0.27% to 0.93%. The total soluble solids ranged from 10.80 to 17.40^{O}Brix. Total and reducing sugars were varied in a range of 10.36% to 16.94% and 0.88% to 3.56%, respectively. The sucrose, glucose and fructose contents were found vary in a range from 9.29% to 17.44%, 0.50% to 1.85% and 0.50% to 1.81%, respectively. Protein content varied from 0.31-0.39 mg/g. Ethanol was also found in all samples that indicating the fermentation. All results indicated a large variation quality of palm sap although they harvested in the same production area in Songkhla province. The different quality of palm sap was mainly due to the fermentation of sugars by the activity of microorganisms during palm sap collecting time.

Keywords: palm sap, quality, flavour, fermentation, Thailand, *Borassus flabellifer* Linn.

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 or juice. 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 morning and 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.*)* and Payorm wood *(Shorea rofburthii G* Don*)* is commonly added to the collection receptacle because it can delay spoilage in

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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).

Normally, palm sap is a raw material to produce palm sugar syrup. Some factors affected the quality of palm sugar syrup such as processing method and quality of palm sap (Phaichamnan *et al.*, 2010). Naknean *et al.* (2009) studied the effect of processing method (open pan and vacuum evaporator) on quality of palm sugar syrup. This result suggested that concentration by vacuum evaporator was an improvement method for palm sugar syrup production since this method can be minimized the loss of quality due to less time and the degradation of product due to heating process. However, another factor such as quality of palm sap should be concerned. Since the composition and quality of palm sap is found to vary with place, time and duration of tapping. Low quality of palm sap caused the dark colour of palm syrup, due to more acids accumulation in the system can be induced browning reaction. At present, an information on physical and chemical properties of palm sap from *Borassus flabellifer* Linn. has been seldom reported. The aim of this research was to characterize the quality of palm sap harvested in Songkhla province, southern Thailand. In addition, more details of palm sap quality will be benefit for farmers, producers and finally consumers.

Materials and Methods

Sample collection

Palm sap (10 samples) was randomly collected from farmers in Songkhla province, in the southern of Thailand. Palm sap was harvested after 12 hours of collecting time with added natural wood (Kiam wood) during tapping process using bamboo tube. After that the bottles of palm sap was kept in an icebox (4^0C) during transportation (30 min) to the department of Food Technology, Prince of Songkla University, Hat-Yai Campus. The physical and chemical property of each sample was determined within a day. Before analyzing, the sample was filtrated by sheet cloth and kept at 4^0C until analysis.

Physical properties measurement

Colour measurements of samples were carried out using a Hunter Lab Colourflex colourimeter. Instrumental colour data was provided as CIE system in terms of L* (lightness), a*(redness and greenness) and b*(yellowness and blueness). The turbidity of palm sap was estimated by measuring the transmittance at 650 nm using a spectrophotometer as describe by Taipaiboon (2004).

Chemical properties measurement

The pH value was measured at ambient temperature with a pH meter (Satorious, USA) which calibrated with pH 4.0 and 7.0. Total acidity was determined by titration with NaOH and calculated in term of lactic acid as described by Rangana (1986). The total soluble solids of palm sugar syrup were determined as degree Brix using hand refractometer. Total sugars and reducing sugars were quantified by Lane and Eynon and Volumetric method; titration with Fehling reagents. The results were express as gram of glucose per 100 gram of sample (Rangana, 1986). Type and concentration of sugar was determined using HPLC (Shimadzu, CR6A Chromatopac) with Shim pack CLC NH_2 column and refractive index detector. The mobile phase used was the solution of acetonitrile and water (80:20), pumped at a flow rate of 1.5 ml/min and injection volume 20 µl. Samples were prepared by making appropriate dilutions with distilled water. All sample solutions were passed through a 0.45 µm syringe filter (Nylon) to remove particulates prior to HPLC analysis. The sugars of D-glucose, D-fructose and sucrose were used as external standards. The calibration curve of each sugar was plotted between peak areas and concentrations (Stuckel and Low, 1996). Protein content was analyzed by Bradford method according to Boyes *et al.* (1997).

Volatile flavour compounds (Adapted from Ho et al., 2006)

The 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). The sample (20 ml) was injected in a duran bottle and equilibrated at 80°C for 30 min in a water bath. A manual SPME holder containing fiber was inserted into a duran bottle and exposed to the sample headspace for 15 min at 80°C. 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°C. The SPME fiber was conditioned at 250°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°C. The GC oven

temperature was programmed from 40 to 230°C at the rate of 8°C/min and hold at 230°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°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.

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).

Results and Discussion

Table 1 showed physical qualities of 10 palm sap samples including L*, a*, b* and transmittance value. All physical qualities 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 (Gupa *et al.,* 1980). 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 oxidaes 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.

Turbidity of palm sap was determined by measurement the transmittance value at 650 nm. The transmittance values of 10 palm sap samples were found to vary from 39.56%-79.95%. In general, the presence of cell fragments has been found to be responsible for the immediate turbidity in fresh juice. Additionally, haze formation caused in turbidity of juice. The turbidity 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. 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). Furthermore, microorganisims are also responsible for turbidity 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 increase in turbidity of palm sap.

Chemical quality parameters such as pH, total acidity, total soluble solids, reducing sugars as well as fructose, glucose, sucrose and total sugars content were determined as can be seen in Table 2. The pH and total acidity of all palm sap samples were

significantly different among the samples ($P<0.05$). The pH values of all palm sap samples varied from 4.19 to 5.23 while total acidity found in a range from 0.027% to 0.093%., as calculated base on lactic acid equivalence. 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.

Total soluble solids of 10 palm sap samples varied from 10.8°Brix to 17.4°Brix. The variation of total soluble solids of palm sap depends on different source of palm sap and fermentation of sugar caused by microorganisms (Iwuoha and Eke, 1996).

Protein content of all palm sap samples was found in a range of 0.31-0.39 mg/g. 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). In general protein acts as a substrate of Maillard reaction that occurring during the production of palm sugar syrup. High protein content presented in palm sap influenced on the quality of palm sugar syrup afterward.

Total sugars, reducing sugars as well as fructose and glucose contents were analyzed in this study and showed significant different across samples ($P<0.05$). Total sugars were varied in a range of 10.36%-16.94%. Reducing sugars of all palm sap samples were found to vary between 0.88% and 3.56%. Glucose content was ranged from 0.50% to 1.85% and fructose content was ranged from 0.50% to 1.81%, respectively (Table 3). The sucrose content was found to vary between 9.29% and 17.44% (Table 3). This might be due to 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 synthesized by microorganisms. 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 sap. High reducing sugars content presented in palm sap also influence on the browning colour of palm sugar syrup afterward, due to Maillard reaction.

Volatile flavour compounds in ten palm sap 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 10 palm sap samples were obtained. Volatile flavour compounds were commonly found in all samples, consisting of alcohols, aldehydes, esters, ketones, acids, and terpenes as shown in Table 4. Ethanol was mainly presented in all samples. Ethanol in these categories 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; Tesfaye *et al.,* 2002).

Microbiological analysis at different stages of palm sap fermentation was done by Uzochukwu *et al.* (1991, 1994 and 1997). They reported that the

Table 1. Physical quality of 10 palm sap samples

Sample/Quality	L*	a*	b*	Transmittance value (%)
1	87.53 ± 0.01^{a}	1.46 ± 0.01^{g}	13.10 ± 0.05^{h}	79.95 ± 0.76
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}

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 2. Chemical quality of 10 palm sap samples

Sample/ Quality	pH	Total acidity (%)	Total soluble solid (^{O}Brix)	Reducing sugars (%)	Total sugars (%)	Protein (mg/g)
1	5.10 ± 0.05^{b}	0.03 ± 0.01^{e}	10.67 ± 0.42^{i}	0.99 ± 0.01^{f}	10.81 ± 0.07^{h}	0.33 ± 0.01^{ce}
2	4.49 ± 0.01^{f}	0.06 ± 0.00^{c}	15.93 ± 0.12^{c}	1.85 ± 0.03^{c}	14.32 ± 0.57^{e}	0.34 ± 0.01^{c}
3	4.60 ± 0.01^{e}	0.05 ± 0.01^{d}	16.57 ± 0.06^{b}	1.74 ± 0.09^{ce}	18.94 ± 0.06^{a}	0.34 ± 0.00^{c}
4	5.23 ± 0.06^{a}	0.03 ± 0.00^{e}	12.07 ± 0.12^{f}	0.88 ± 0.01^{f}	11.72 ± 0.19^{g}	0.32 ± 0.00^{ef}
5	4.78 ± 0.04^{d}	0.03 ± 0.01^{e}	12.40 ± 0.00^{e}	1.15 ± 0.01^{f}	12.60 ± 0.27^{f}	0.31 ± 0.00^{f}
6	4.90 ± 0.01^{c}	0.05 ± 0.01^{d}	11.07 ± 0.12^{g}	1.02 ± 0.01^{f}	10.36 ± 0.10^{h}	0.39 ± 0.00^{a}
7	4.19 ± 0.01^{fg}	0.08 ± 0.01^{b}	16.00 ± 0.00^{c}	2.63 ± 0.34^{b}	15.68 ± 0.20^{c}	0.31 ± 0.01^{f}
8	4.53 ± 0.01^{h}	0.09 ± 0.01^{a}	16.33 ± 0.12^{b}	3.56 ± 0.53^{a}	15.80 ± 0.20^{c}	0.31 ± 0.02^{f}
9	4.46 ± 0.03^{g}	0.08 ± 0.01^{b}	17.33 ± 0.12^{a}	2.54 ± 0.10^{b}	16.94 ± 0.81^{b}	0.34 ± 0.01^{c}
10	4.89 ± 0.01^{c}	0.03 ± 0.01^{e}	12.00 ± 0.00^{f}	1.39 ± 0.15^{ef}	11.62 ± 0.08^{g}	0.36 ± 0.01^{b}

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. Type of sugars and their concentrations of 10 palm sap samples

Sample/Quality	Fructose (%)	Glucose (%)	Sucrose (%)	Ratio of fructose:glucose:sucrose
1	0.50 ± 0.01^{h}	0.50 ± 0.02^{g}	9.40 ± 0.22^{h}	1:1:19
2	0.95 ± 0.01^{e}	0.96 ± 0.02^{c}	12.24 ± 0.63^{e}	1:1:13
3	0.80 ± 0.02^{f}	0.75 ± 0.02^{e}	17.44 ± 0.53^{a}	1:1:23
4	0.52 ± 0.01^{h}	0.51 ± 0.02^{g}	10.58 ± 0.37^{g}	1:1:21
5	0.62 ± 0.02^{g}	0.59 ± 0.01^{f}	11.47 ± 0.21^{f}	1:1:19
6	0.48 ± 0.02^{h}	0.53 ± 0.02^{g}	9.29 ± 0.15^{h}	1:1:19
7	1.29 ± 0.02^{b}	1.23 ± 0.08^{b}	13.25 ± 0.44^{c}	1:1:11
8	1.81 ± 0.03^{a}	1.85 ± 0.01^{a}	12.41 ± 0.13^{e}	1:1:9
9	1.23 ± 0.03^{c}	1.22 ± 0.01^{b}	14.04 ± 0.12^{b}	1:1:12
10	0.50 ± 0.01^{h}	0.51 ± 0.04^{g}	10.48 ± 0.10^{g}	1:1:21

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 4. Volatile flavour compounds of 10 palm sap samples

Volatile flavour compounds	Attribute [A]	Area[B] (%)/ Sample No									
		1	2	3	4	5	6	7	8	9	10
Alcohols											
Ethanol	alcoholic	57.68	59.82	55.71	71.46	52.92	67.44	54.83	54.20	62.21	57.86
Isoamyl alcohol	fermented	13.24	17.09	10.89	12.17	15.94	10.81	9.98	10.48	10.87	15.39
Aldehydes											
Acetaldehyde	fruity	0.26	1.01	nd	nd	nd	nd	nd	nd	0.43	nd
Acids											
Acetic acid	sour	nd	1.23	1.73	nd	nd	1.10	10.22	3.93	7.34	nd
Ketones											
3-Hydroxy-2-butanone	sweet	1.74	1.28	12.99	0.69	1.16	2.59	6.33	1.47	1.63	1.24
6-Methyl-5-hepten-2-one	citrus, herbal	1.48	1.18	nd	nd	nd	nd	nd	nd	nd	nd
2-Nonanone	fruity	nd	0.51	2.11	nd	nd	0.50	0.87	0.34	0.32	0.37
2-Heptanone	cheesy	nd	0.44	nd	nd	nd	nd	0.81	0.27	0.26	nd
2-Undecanone	fruity	nd	nd	3.65	nd	nd	nd	0.46	nd	nd	nd
2,3-Butanedione	powerful, buttery	1.97	nd	nd	1.90	2.41	nd	nd	0.77	0.94	nd
2,3-Pentanedione	fruity, sweet	0.3	1.40	4.24	nd	nd	nd	nd	0.12	nd	0.54
Esters											
Isoamyl acetate	fruity, sweet	nd	2.06	nd	nd	0.66	1.34	1.01	0.54	1.16	nd
Ethyl acetate	sweet, fruity	12.91	9.40	7.01	10.22	22.5	10.92	6.10	10.63	7.94	20.56
Ethyl hexanoate	fruity, sweet	nd	0.49	nd	nd	nd	3.26	0.33	0.49	0.46	nd
Ethyl octanoate	fruity, pineapple	1.41	2.10	nd	nd	0.76	1.19	1.83	8.42	3.23	nd

AReference:http://www.thegoodscentscompany.com/rawmatex.html,http://www.flavornet.org/flavornet.html,http://.pherobase.com/search.php, B Percent mean area ration was calculated by dividing peak area by total area of all peaks.

organisms found in palm sap in the early stage of fermentation (sugar 12%, pH 7-7.2) are mostly entirely Leuconostocs and Lactobacilli as well as a small proportion of incompletely identified fructan-producing bacteria which produced no acid in pure culture in sterile palm sap. Thus, the Leuconostocs and Lactobacilli produce glucans likely to be dextran. Dextran synthesis leads to a net production of fructose. When fructose is available in wine and lactic acid bacteria are able to grow, they can produce equimolar amounts of lactic and acetic acids from fructose and this could constitute a serious source of acetic acid in wine. 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-odour (Cheetham, 2002). This volatile flavour compound in palm sugar sap as reported by Taiapaiboon (2004). In addition 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 aroma. This compound is derived from isoamyl alcohol and acetyl coenzyme A by alcohol acetyl transferase in yeast (Inoue *et al.,* 1994).

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 13 physical and chemical properties from 10 palm sap samples (Figure 1.). Two principal components (PC1 and PC2) were calculated. They accounted for 79.98% of the variability in the original data as can be seen in Figure 1. The graphical PCA illustrated high positive relationship between L* value and turbidity (measured in terms of transmittance value). The results showed that the decrease in transmittance value might be due to the accumulation of undissolved particle as mentioned previously and caused in the decrease in L* 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 and total sugars. 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

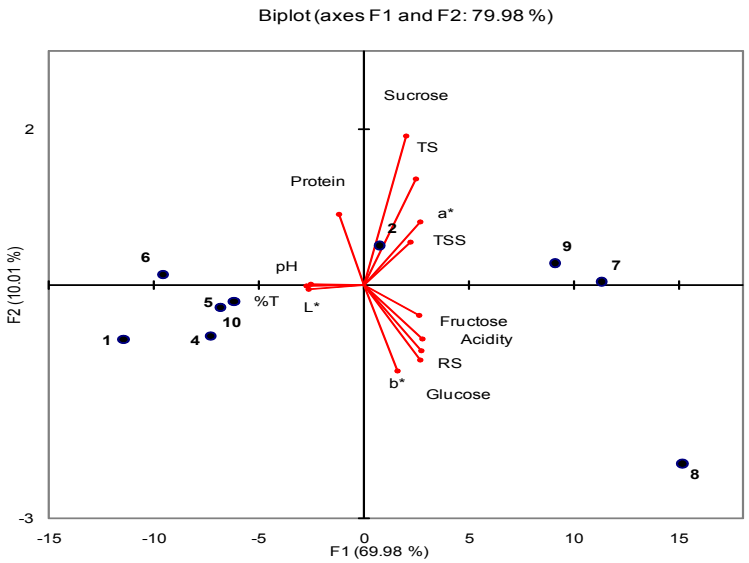

Figure 1. Biplot PC1 and PC2 of the properties of palm sap 10 samples

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, 4, 5, 6 and 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, 3, 7, 8 and 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.

Conclusion

The results presented natural variation of palm sap quality harvested in Songkhla province, in the southern Thailand. These variations may come from genetic and metabolite characteristics of the tree, environment factors, the collecting time, microbial load, personal hygiene and sanitary equipment. Microorganisms seem to be the main affect on quality of palm sap due to they can use sugar and produce organic acids and alcohol, mainly ethanol. These organic acids cause the inversion reaction. Ethanol is off-flavour in palm sap and resulting in the unacceptable from consumers.

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