

Quality Profiles of Pasteurized Palm Sap (*Borassus flabellifer* Linn.) Collected from Different Regions in Thailand

Phisut NAKNAEN^{1,*} and Mutita MEENUNE²

¹Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, Bangkok 10110, Thailand

²Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Songkhla 90112, Thailand

(*Corresponding author's e-mail: n.phisut@yahoo.com)

Received: 23 November 2014, Revised: 19 January 2015, Accepted: 9 February 2015

Abstract

Palm sap is obtained by tapping the inflorescence of the palmyra palm, and is usually used in terms of pasteurized palm sap. So far, this product has been produced by each producer using their own experience, and using conventional methods. Therefore, the purpose of this study was to characterize the quality of pasteurized palm sap collected from 3 provinces, including Bangkok, Phetchaburi, and Ayutthaya. Five pasteurized palm sap samples were randomly collected from each province, and analyzed for their physical, chemical and microbiological qualities. The results showed a range of L*, a* and b* values between 44.12 to 78.67, 0.40 to 2.61, and 10.47 to 19.33, respectively. The transmittance value ranged from 27.32 to 81.30 %. The pH value varied from 5.00 to 8.17, while total acidity ranged from 0.01 to 0.23 %. The total soluble solids ranged from 14.60 °Brix to 24.10 °Brix. Total and reducing sugars varied in a range of 14.23 to 24.10 %, and 0.25 to 8.47 %, respectively. The sucrose, glucose and fructose contents were found in a range from 13.67 to 19.05 %, 0.14 to 4.85 %, and 0.17 to 4.17 %, respectively. Phenolic content varied from 0.64 to 1.03 mg/g. The total viable count, and the yeast and mold count, of all samples were higher than the allowed maximum limit, as recommended by Thai Community Product Standards. The results indicated a large variation in quality of pasteurized palm sap among the 3 provinces ($p < 0.05$).

Keywords: Palm sap, pasteurization, quality, Bangkok, Phetchaburi, Ayutthaya

Introduction

Palmyra palm (*Borassus flabellifer* Linn.) can be found in tropical countries such as India, Thailand, Myanmar, Sri Lanka, and Cambodia. In Thailand, palmyra palm trees are dense in the southern part of Thailand, from the Phetchaburi to Songkhla provinces. In addition, they can be found in other parts, such as in the Phitsanulok, Buriram, and Ayutthaya provinces. 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 grown inflorescences with a very sharp sickle or knife at the apex of the palm tree. The sap collector cuts the outer end of the inflorescence to collect the sap. Each inflorescence is 25 to 30 cm in length, and 2.0 - 2.5 cm in diameter. Normally, sap is collected twice a day from each inflorescence, either in the morning or in the evening. Three to 6 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). During the collecting process, pieces of wood, such as Kiam wood (in Songkhla province), Takian wood (in Ayutthaya province), or Payorm wood (in Phetchaburi province), are generally added to the container, to prevent fermentation from microorganisms [1,2].

However, farmers stop tapping the sap in the rainy season, because there is little yield and, also, there may be water contamination of the sap in the open containers. After each harvesting season, inflorescences are removed, by using a sickle or knife, to allow new inflorescences to grow.

The main product of the Palmyra palm tree is sap, or juice. Palm sap is usually used in the form of fresh palm sap. However, fresh palm sap is sensitive to rapid spontaneous fermentation by naturally occurring yeast and bacteria. Consequently, it is transformed into an alcoholic beverage within some hours at ambient temperatures. Thus, preservation techniques, such as pasteurization and concentration, are applied to prolong the shelf life of fresh palm sap, and this leads to the creation of new products, such as pasteurized palm sap, palm sugar syrup, and palm sugar cake. Among palm sap products, pasteurized palm sap is well utilized.

Pasteurized palm sap is normally produced by heating palm sap at boiling temperatures. It has been reported that thermal deterioration can take place during thermal processing, and this can affect the quality of a product, especially the color, flavor, and nutritional value [2-5]. To date, this product has been produced by each producer using their own experience, and using conventional methods. In addition, the specific qualities of other products remain undefined. There is a lack of standard procedures in controlling critical factors influencing the final product quality during the traditional methods of production.

The quality of pasteurized palm sap varies across producers, depending on individual production techniques. The differences are based on personal hygiene, sanitary facilities, harvesting conditions, heating temperatures, heating time, and storage conditions. Until now, scientific data has rarely been reported on the properties of pasteurized palm sap in Thailand, even though it is a commercial product. Therefore, the aim of this work was to characterize the properties of pasteurized palm sap produced in various areas of central part of Thailand, such as in the Bangkok, Ayutthaya and Phetchaburi provinces. The data produced could be used as a guideline to differentiate the quality of pasteurized palm sap.

Materials and methods

Sample collection

Pasteurized palm sap samples (15 samples) were collected from producers in the Phetchaburi, Ayutthaya and Bangkok provinces. Five pasteurized palm sap samples were randomly collected from each province. After that, the bottles of pasteurized palm sap were kept in an icebox (4 °C) during transportation to the Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, Bangkok, Thailand (approximately 2 h from Ayutthaya, 2.5 h from Phetchaburi, and 0.5 - 1 h from Bangkok). The physical, chemical, and microbiological qualities of each sample were determined within a day of collection. The harvesting procedures, processing conditions, and sanitation practices of the samples collected from the Ayutthaya and Phetchaburi provinces are presented in **Table 1**.

Physical quality measurement

The color measurements of the samples were carried out using a Hunter Lab Colorflex colorimeter (illuminant D65, 2.5 inches of port size and 10° of observation angle). Instrumental color data was provided as a CIE system in terms of L* (lightness), a*(redness and greenness) and b*(yellowness and blueness). The clarity of the palm sap was estimated by measuring the transmittance at 650 nm using a spectrophotometer (Shimadzu, Kyoto, Japan). The browning index (BI) of the samples was measured according to the method of Ajandouz *et al.* [6]. Appropriate dilution of the samples was made using distilled water and the absorbance was measured at 420 nm. The absorbance was measured by using a UV-160 spectrophotometer.

Table 1 Harvesting procedures and processing conditions of the pasteurized palm sap collected from the Ayutthaya and Phetchaburi provinces from the survey data.

| Harvesting procedures/ Processing conditions | Ayutthaya | Phetchaburi |
|--|---|--|
| Collection time of palm sap | 10 - 12 h | 10 - 12 h |
| Container for palm sap collection | Bamboo tube | Bamboo tube |
| Antimicrobial agent added during palm sap collection | Lime powder (2 - 3 g) and Takian wood (2 - 3 g) | Payorm wood (3 - 5 g) |
| Delay time before pasteurization | 1 - 2 h | 1 - 2 h |
| Pasteurization process | Boiling temperature (100 °C) and approximately 30 min | Boiling temperature (100 °C) and approximately 1 h |
| Delay time before packaging | Immediately after pasteurization | Approximately 30 min |
| Packaging for pasteurized palm sap | Plastic bottles or plastic bags | Plastic and glass bottles |
| Temperature during the sale | Approximately 4 - 10 °C | Ambient temperature |

Chemical properties measurement

Determination of pH, total acidity and total soluble solid

The pH value was measured at an ambient temperature with a pH meter (Satorious, USA) which was calibrated with a pH of 4.0 and 7.0. The total acidity was determined by titration with NaOH and calculated in term of lactic acid, as described by Rangana [7]. The total soluble solid (TSS) was determined as degree °Brix using a hand refractometer.

Determination of sugar

The total and reducing sugars were quantified by titration with Fehling reagents, according to the Lane and Eynon volumetric method. The results were expressed as grams of glucose per 100 gram of sample [7]. The type and concentration of sugar were determined using an HPLC (Agilent 1100 series) with a Zorbax Carbohydrate column and refractive index detector. The mobile phase was a solution of acetonitrile and water (80:20), pumped at a flow rate of 1.5 ml/min. 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 external standards. The calibration curve of each sugar was plotted between peak areas and concentrations [8].

Determination of phenolic content

The quantification of the phenolic content in each sample was carried out according to the method of Balange and Benjakul [9]. The appropriate diluted sample (0.5 ml) was mixed with 0.5 ml of distilled water. Thereafter, 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of 2 % sodium carbonate solution were added. The mixture was mixed thoroughly and placed in the dark for 40 min. After that, the absorbance was recorded at 725 nm, and the phenolic content was calculated from the standard curve of gallic acid.

Determination of antioxidant activity

DPPH radical-scavenging activity was determined by DPPH assay, as described by Binson *et al.* [10], with a slight modification. An appropriate diluted 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.

Determination of 5-hydroxymethylfurfural (HMF)

For the determination of the 5-hydroxymethylfurfural (HMF), the sample was centrifuged at 5,000 rpm for 15 min. The supernatant was used to measure the HMF content. To determine the HMF content, 2 ml of a sample (appropriate dilution) was introduced into the tube. Two ml of 12 % trichloroacetic acid and 2 ml of 0.025 M thiobabitoric acid were subsequently added and mixed thoroughly. The tube with the sample was then placed in a water bath at 40 °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 the HMF was utilized to quantify the HMF concentration [7].

Microbiological quality measurement

The total viable count (TVC), mold and yeast count (M&Y) and lactic acid bacteria (LAB) were analyzed. Each sample was aseptically taken and a serial dilution in 0.1 g/100 g peptone water conducted for the microbial counts. Pour plating on Plate Count Agar (Merck KGaA, Darmstadt, Germany) was performed for the TVC, overlaid with the same medium, and the plates were incubated at 35 - 37 °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 the mold and yeast count, and the plates were incubated at 20 - 25 °C for 5 days. The lactic acid bacteria count was analyzed using the pour plate technique with MRS agar, and the plates were incubated at 37 °C for 2 days [11].

Statistical analysis

All analysis and measurements of each collected sample were performed in triplicate. The experimental design was a completely randomized design (CRD). The data was subjected to analysis of variance (ANOVA). A comparison of means was carried out by Duncan's multiple-range test [12]. Analysis was performed using an SPSS package. Principle Component analysis (PCA) was applied to observe the relationship among all the property indicators from fifteen pasteurized palm sap samples by using XLSTAT software (www.XLSTAT.com).

Results and discussion

Table 2 shows the physical properties of pasteurized palm sap samples, including L*, a*, b*, transmittance value and browning index. The results indicate a large variation in the quality of pasteurized palm sap among the provinces ($p < 0.05$). Normally, fresh palm sap is oyster white in color and translucent, with a nearly neutral pH [13]. However, pasteurized palm sap samples tend to show a red color shade, as indicated by the positive a* values. The red shade of pasteurized palm sap was mainly affected by the harvesting procedures and pasteurization process. During the collecting process, Takian wood, Payorm wood, and lime paste were commonly added in the collection receptacle to prevent fermentation from microorganisms. The pigment of Takian wood, Payorm wood, or even lime paste could dissolve the palm sap during collection, leading to the red shade of the palm sap [14,15]. In addition, an enzymatic browning reaction can take place during the collecting of palm sap [16,17]. Polyphenol oxidase is responsible for this reaction. This enzyme catalyzes the hydroxylation of monophenols (from the metabolite of the plant and Kiam wood) to *o*-diphenols, and the oxidation reaction of *o*-diphenols to *o*-quinones. Quinones are very reactive compounds, which strongly interact with other molecules, leading to a high level of pigment with a high molecular weight and marked red to brown coloring [18]. Moreover, a Maillard reaction could take place during pasteurization and storage, resulting in the brown color formation.

From the results, the highest a* value was found in samples collected from Ayutthaya province. We found that a combination of lime powder and Takian wood was usually used as an antimicrobial agent and added in the bamboo tube during the harvesting of palm sap in Ayutthaya province. However, only Payorm wood was used during the harvesting of palm sap in Phetchaburi province. It is well known that nonenzymatic browning reactions are very much dependent on pH [19-21]. The pH of a system significantly influences both the reaction rate and the type of product formed. At high pH, more colour

intensity is produced, by both Maillard reaction and Caramelization [21]. The amount of unprotonated amino groups, which is considered to be a reactive species, obviously increases 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 [22,23]. Hence, the higher pH levels of samples collected from Ayutthaya province might enhance the browning development in the samples during pasteurization and storage. The lowest browning index was observed in all samples collected in Phetchaburi province. According to our survey data, the producers in Phetchaburi province usually added sucrose to the palm sap before pasteurization to adjust the sweetness. The reducing sugar content in palm sap can be reduced by the addition of sucrose. Since sucrose is a non-reducing sugar, therefore, it cannot participate in a Maillard reaction, leading to the reduction in brown color formation.

Table 2 Physical quality profiles of pasteurized palm sap.

| Physical quality | Ranges ¹ | Ranges ² | Ranges ³ |
|-------------------------|---------------------|---------------------|---------------------|
| L* | 44.12 - 73.51 | 46.56 - 53.11 | 74.57 - 78.67 |
| a* | 0.48 - 1.14 | 1.45 - 2.61 | 0.40 - 1.35 |
| b* | 11.08 - 13.56 | 10.47 - 12.40 | 17.86 - 19.33 |
| Transmittance value (%) | 32.43 - 75.29 | 27.32 - 33.15 | 76.22 - 81.30 |
| Browning index | 0.31 - 1.02 | 1.08 - 1.28 | 0.69 - 0.74 |

Note: ¹Quality of pasteurized palm sap collected from Bangkok province.

²Quality of pasteurized palm sap collected from Ayutthaya province.

³Quality of pasteurized palm sap collected from Phetchaburi province.

The clarity of the palm sap was measured in terms of the transmittance value (%). More clarified juice was found with a high transmittance value. In general, the presence of cell fragments has been found to be responsible for the clarity of fresh juice. Additionally, haze formation causes a reduction in the clarity of juice. The clarity of palm sap depends greatly on its protein concentration and the polyphenol compounds, which are dissolved from wood (Kiam, Takian or Payorm woods), and are present in natural palm sap itself [2]. An interaction between protein and polyphenol can be induced and, therefore, a large colloid size or haze can be developed [24,25]. The clarity of pasteurized palm sap is also mainly influenced by the harvesting procedures of palm sap and the processing conditions. High amounts of Takian wood and Payorm wood are responsible for the turbidity of palm sap, due to the polyphenol present in wood that can dissolve into palm sap. In addition, lime powder is not fully soluble in palm sap and causes turbidity in it. The results showed that the samples collected from Ayutthaya province had the lowest clarity. This result could be because of the use of lime powder and Takian wood as antimicrobial agents during the collection of the palm sap. The highest clarity was found in samples collected from Phetchaburi province. According to the survey data from some producers in Phetchaburi province, palm sap was pasteurized and then placed at ambient temperatures to allow the undissolved particles to settle down. After that, the supernatant was packed in bottles. Thus, samples collected from Phetchaburi province showed greater clarification than those from Ayutthaya province, which were packed immediately after pasturization.

The pH of all pasteurized palm sap samples was significantly different among the samples ($p < 0.05$), while total acidity showed a narrow range. Pasteurized palm sap collected from Ayutthaya province yielded a high pH, while lower pH values were found in all samples collected from Phetchaburi province (**Table 3**). Normally, natural palm sap showed a neutral pH of approximately 7, as reported by Jitbunjerdkul [26] and Lasekan *et al.* [27]. However the growth of microorganisms during collection caused a decrease in the pH, due to the conversion of sugars to acids [1]. The alkaline pH found in all samples collected from Ayutthaya province might be due to the addition of lime powder to the bamboo tube added before harvesting in order to retard the growth of microorganisms.

Table 3 Chemical quality profiles of pasteurized palm sap.

| Chemical quality | Ranges ¹ | Ranges ² | Ranges ³ |
|---|---------------------|---------------------|---------------------|
| pH | 5.03 - 8.11 | 7.46 - 8.17 | 5.00 - 6.30 |
| Total acidity (%) | 0.11 - 0.23 | 0.11 - 0.22 | 0.22 - 0.23 |
| Total soluble solid (°Brix) | 14.60 - 20.30 | 17.50 - 19.40 | 22.00 - 24.10 |
| Total sugar (%)* | 14.23 - 20.15 | 17.46 - 19.44 | 22.04 - 24.10 |
| Reducing sugar (%)* | 0.25 - 6.37 | 0.37 - 0.82 | 7.56 - 8.47 |
| Sucrose content (%)* | 14.38 - 19.05 | 16.71 - 18.62 | 13.67 - 16.54 |
| Glucose content (%)* | 0.14 - 3.45 | 0.20 - 0.49 | 3.62 - 4.85 |
| Fructose content (%)* | 0.17 - 3.21 | 0.21 - 0.37 | 3.24 - 4.17 |
| Polyphenol content (mg/g)* | 0.65 - 1.03 | 0.75 - 0.89 | 0.64 - 0.81 |
| HMF (mg/kg)* | 4.24 - 10.22 | 4.32 - 6.34 | 10.42 - 13.45 |
| DPPH radical scavenging activity (μmol TE/g)* | 5.43 - 9.67 | 5.77 - 7.58 | 9.05 - 11.24 |

Note: ¹Quality of pasteurized palm sap collected from Bangkok province.

²Quality of pasteurized palm sap collected from Ayutthaya province.

³Quality of pasteurized palm sap collected from Phetchaburi province.

*These values were calculated in terms of dry basis.

The total soluble solid (TSS) of all pasteurized palm sap samples varied from 14.60 °Brix to 24.10 °Brix (**Table 3**). Mainly, the initial TSS of palm sap and the processing conditions affected the TSS of pasteurized palm sap. Normally, palm sap contains TSS at approximately 10 - 18 °Brix. Microorganism contamination during collection is responsible for the low TSS of palm sap, due to the effects of the sugar fermenting process [1]. In addition, processing conditions, such as the processing temperature and processing time, also influenced the TSS of pasteurized palm sap. During pasteurization palm sap was boiled in an open container, resulting in the removal of water, especially at high processing temperatures and long processing times. The highest TSS was found in all samples collected from Phetchaburi province. This result could be explained by the long processing time (approximately 1 h) used during palm sap pasteurization. All samples collected from Ayutthaya province were boiled for approximately 30 min, according to our survey data. The samples collected from Bangkok province showed a large variation in the pH and TSS. This could be because the pasteurized palm sap sold in Bangkok province was obtained as fresh palm sap from various producers in areas near and around Bangkok province.

The total sugar, the reducing sugar, and the fructose and glucose contents of all pasteurized palm sap samples were analyzed in this study, and showed significant differences across the samples ($p < 0.05$). A positive correlation between the total sugar and TSS was observed, suggesting that the highest proportion of the soluble solid in pasteurized palm sap were sugars. All samples were boiled to produce pasteurized palm sap, thus, the processing temperature and times were the main factors affecting the total sugar content in pasteurized palm sap. The samples collected from Phetchaburi province showed the longest processing time, resulting in the production of increased total sugar content.

The most abundant of the sugars found in palm sap was sucrose. The presence of fructose and glucose can come from its natural state and the inversion reaction caused by invertase activity and acidic conditions. The occurrence of invertase in palm sap was due to its natural presence, and it was also synthesized by microorganisms. Sucrose can be converted to glucose and fructose by invertase via microorganisms, and, thus, yield organic acids and alcohols in palm sap. The fermenting organisms, particularly *Saccharomyces cerevisiae* and *S. carlsbergensis*, are considered as primary sources of invertase [28]. In addition, the inversion of sucrose could take place significantly during thermal pasteurization. The highest reducing sugar content, as well as the glucose and fructose content, was found in all samples collected from Phetchaburi province (**Table 3**). This result suggests that the low pH of

palm sap and a long heating time could promote the inversion reaction of sucrose. A low reducing sugar content was observed in all samples collected from Ayutthaya province, indicating that alkaline conditions and a short heating time could minimize the formation of glucose and fructose. A high content of reducing sugars present in the pasteurized palm sap influenced the brown color development of a sample during storage via a Maillard reaction. However, the browning index of the samples collected from Ayutthaya was higher than those collected from Phetchaburi, although higher reducing sugar content was detected in the samples collected from Phetchaburi province when compared to the samples collected from Ayutthaya province. This result might be explained by the effect of pH being a dominant factor to promote nonenzymatic browning reactions during heating when compared to the effect of reducing sugar content.

Phenolic compounds are one of the most important compounds in plants. In addition, they can be found in plant sap [29,30]. The presence of phenolic compounds in palm sap can occur naturally, or be added from external sources, such as from Payorn wood and Takian wood, during collection. Low amounts of phenolic content were found in all samples collected from Ayutthaya province. This result might be due to the farmers in Ayutthaya province using both Takian wood (2 - 3 g) and red lime paste (2 - 3 g) as antimicrobial agents during the collection of palm sap. High amounts of Takian or Kiam wood (4 - 5 g) were inserted into the bamboo tube for palm sap collected from Phetchaburi province.

Chemical indicators for assessing the quality of over-processed foods have proved to be useful. The HMF is commonly used as an indication of Maillard reaction and sugar pyrolysis intermediates formed during thermal processing in food products [31,32]. Furan compounds have been evaluated as an indicator of the severity of heat treatment, including temperature and time, in several food products, including honey, fruit juices, coffee, breakfast cereals, breads, and baby cereals. HMF is not present in fresh, untreated foods, but it rapidly accumulates in sugar-rich foods during heating at high temperatures for longer times. The accumulation of HMF is considered undesirable in thermally processed foods, and its presence in food is the focus of some potential toxicological concerns [33]. A lower HMF content was found in all samples collected from Ayutthaya province compared to those collected from Phetchaburi province. This result might be because all samples collected from Phetchaburi province contained the highest reducing sugar content and the lowest pH. Theoretically, sucrose is hydrolyzed, particularly at high temperature and low pH. Sugars decompose into furans or HMF by 2 possible pathways: caramelization and Maillard reaction. In caramelization, reducing carbohydrates directly suffer 1,2-enolisation, dehydration and cyclization reactions [32]. In the Maillard reaction, the Amadori product is subjected to enolization and subsequent dehydration of the sugar moiety and the release of an intact amino acid. The Maillard reaction is favored in foods with a high carbohydrate content, temperatures above 50 °C and a pH of 4 - 7. However, the caramelization reaction needs more drastic conditions, such as higher temperatures (> 120 °C) [32]. Thus, the low reducing sugar content and alkaline medium found in all samples collected from Ayutthaya province could minimize the formation of HMF.

Among antioxidant evaluation methods, the DPPH assay has been most widely used. The DPPH assay is relatively simple and stable, and the DPPH is available commercially in a usable format. The literature suggested that a DPPH assay would be an easy and accurate method with regard to measuring the antioxidant capacity of fruit and vegetable juices or extracts [34]. The DPPH assay is based on the reduction by antioxidants of the purple DPPH radical to a corresponding pale yellow, and is measured by colorimetry at 517 nm [35]. The highest DPPH radical scavenging activity was observed in all samples collected from Phetchaburi province. Phenolic compounds present in palm sap are responsible for antioxidant activity. The antioxidant activity of phenolic compounds is clearly related to free radical-scavenging and hydrogen-donation ability [36]. Moreover, Maillard reaction products (MRPs) and caramelization products (CPs) formed during pasteurization also showed antioxidant activity. CPs and MRPs showed DPPH radical scavenging activity, because they were able to reduce the DPPH radical to yellow-colored diphenylpicrylhydrazine [37,38]. MRPs and CPs could function as electron donors. The hydroxyl groups of MRPs or CPs play an important role in reducing such activity. Hence, the high phenolic and HMF contents found in all samples collected in Phetchaburi province were responsible for the high DPPH radical scavenging activity. HMF can be generally classified as MRPS and CPS and, thus,

presents antioxidant activity. However, though HMF is not a good indicator for antioxidant activity, it is normally claimed to be an indicator of heat stress for sugar based product.

Table 4 Microbiological quality profiles of pasteurized palm sap.

| Microbiological quality | Ranges ¹ | Ranges ² | Ranges ³ |
|-------------------------|---|---|---|
| TVC (cfu/g) | 1.40×10^6 - 8.60×10^6 | 1.10×10^3 - 9.50×10^3 | 4.20×10^5 - 7.20×10^5 |
| M&Y (cfu/g) | 1.20×10^8 - 7.90×10^8 | 1.70×10^4 - 6.80×10^6 | 1.00×10^8 - 5.30×10^8 |
| LAB (cfu/g) | 4.30×10^4 - 2.10×10^5 | 6.30×10^2 - 1.10×10^3 | 1.20×10^3 - 1.90×10^4 |

Note: ¹Quality of pasteurized palm sap collected from Bangkok province.

²Quality of pasteurized palm sap collected from Ayutthaya province.

³Quality of pasteurized palm sap collected from Phetchaburi province.

The largest variation in the quality profiles was found in all samples collected in the Bangkok province. It could be seen that the quality of the pasteurized palm sap collected in the Bangkok province were made up of the quality profiles of samples collected from the Phetchaburi and Ayutthaya provinces. According to our survey, pasteurized palm sap sold in the Bangkok province was generally received from producers near Bangkok province, such as from the Phetchaburi, Ayutthaya, Nonthaburi and Chachoengsao provinces. This resulted in the large variation in quality. Delays in the times of the pasteurization process can occur due to the distances involved. In such cases, greater fermentation processes can take place.

Table 4 shows the microbiological quality of pasteurized palm sap samples, including TVC, M&Y, and LAB. The Thai Community Product Standards [39] require that TVC and M&Y in pasteurized palm sap samples shall not be more than 500 and 100 cfu/g, respectively. According to these criteria, all the pasteurized palm sap samples in this study did not meet these standards. The microbial load was generally reduced after pasteurization. However, some spoilage microorganisms may have survived and developed, leading to a limited shelf life. In addition, inadequate sanitation and hygiene practices could affect the safety and quality of the product. According to the survey data, poor sanitation and facilities are the main factors affecting the microbial load of pasteurized palm sap. The highest microbial loads were detected in samples collected from Bangkok province. As mentioned previously, all the samples collected from Bangkok province were received from various areas. This involved long transportation times and improper transportation conditions, such as insufficient ice in iceboxes, or lack of temperature control during transportation and storage. These might induce an increase in microbial loads for pasteurized palm sap. In addition, the survey data revealed that almost all samples sold in the Ayutthaya and Phetchaburi provinces had short selling periods (approximately 1 - 3 days) compared with those sold in Bangkok province (3 - 7 days).

Pasteurized palm sap samples showed large variations in quality ($p < 0.05$). The data analysis of 12 qualities from 15 samples was plotted using a multivariate technique-Principal Component Analysis (PCA). Three main principle components (PCs) characterized the quality profile of the pasteurized palm sap samples collected from the three provinces (86.92 % of all variance): PC1 (57.99 %); PC2 (15.89 %); and PC3 (13.55 %). The graph of the PCA illustrates a high positive relationship between L^* value and clarity (measured in term of transmittance value). On the other hand, for the color parameters, a negative correlation of L^* with a^* and BI was found. This suggests that the enzymatic browning of palm sap, pigment from wood, and higher pH of palm sap caused the decrease in the L^* value and an increase in the a^* value and BI. In addition, the pH value showed a negative relationship with reducing sugars, including fructose and glucose, and the HMF content. This suggested that the formation of reducing sugar and HMF took place largely under acidic conditions. In addition, a positive relationship was found in the HMF content, polyphenol content and DPPH radical scavenging activity, suggesting that MRPs and CPS, such as HMF and phenolic compounds, had hydrogen-donating activity.

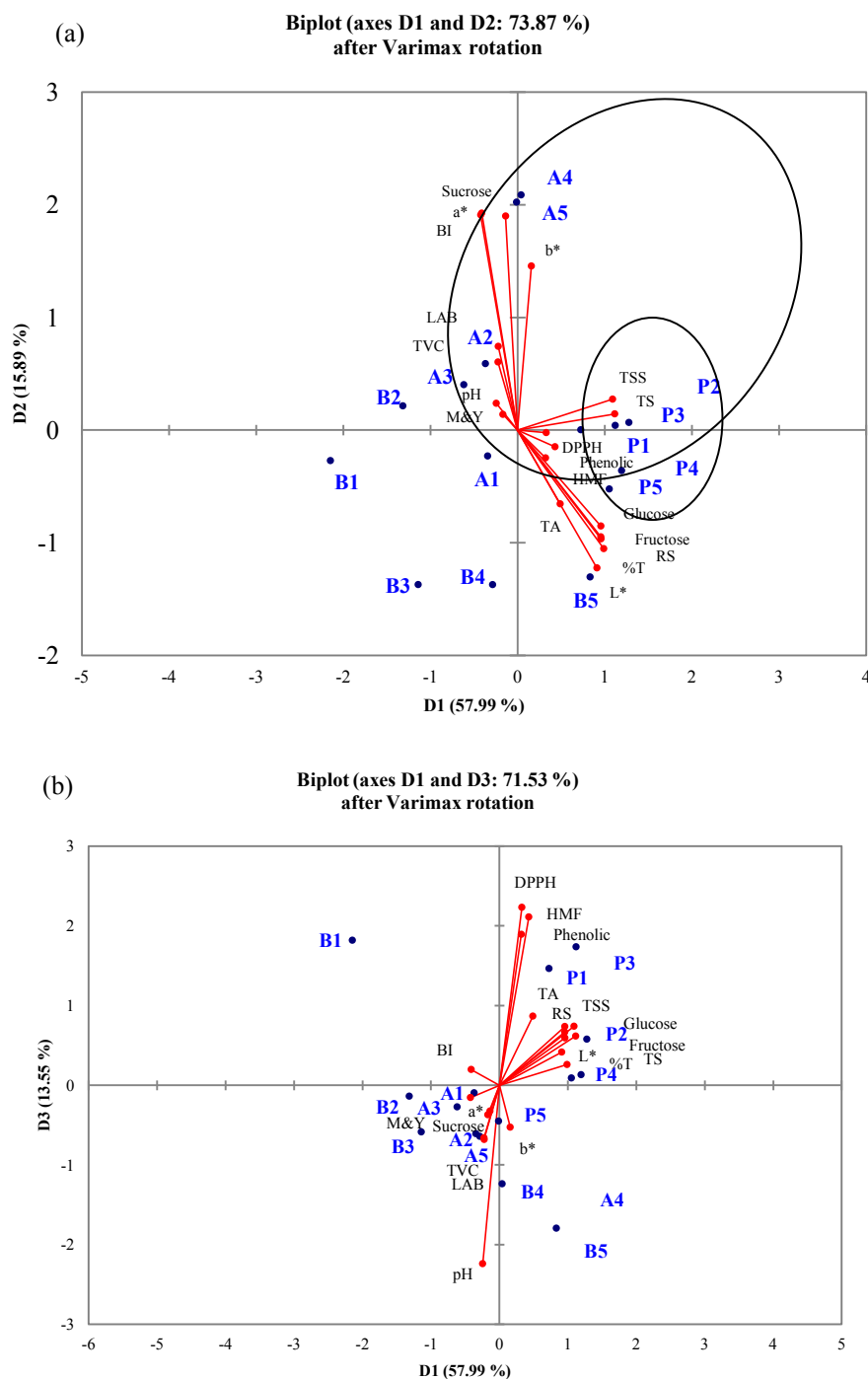


Figure 1 Biplot PC1 and PC2 (a) and biplot PC1 and PC3 (b) of the qualities of fifteen pasteurized palm sap samples.
Note: P1 - P5 refer to the samples collected from Phetchaburi province. A1 - A5 refer to the samples collected from Ayutthaya province. B1 - B5 refer to the samples collected from Phetchaburi province.

All samples collected from Phetchaburi province are located in the left part of the score plot. These samples are correlated with high reducing sugar content, indicating that a high rate of inversion reaction took place during collection and production. On the other hand, almost all samples collected from Ayutthaya province appeared on the right part of score plot, showing that these samples had high sucrose content. These results could suggest that alkaline conditions, caused by the addition of red lime paste, could minimize the loss of sucrose. Furthermore, the largest variation in the quality profiles was observed in the samples collected from Bangkok province; this is confirmed by the distribution of all samples in the score plot.

Conclusions

The physical, chemical, and microbiological properties of pasteurized palm sap differed among the samples, as the products were produced locally and individually by each producer. They were not in agreement with the Thai Community Product Standards. The quality of the pasteurized palm sap is affected by factors such as harvesting procedures, processing conditions, and sanitation practices. The producers in each province used different harvesting procedures, leading to varied initial quality of the palm sap. In addition, Maillard reactions and inversion reactions also took place, particularly during pasteurization, and these resulted in browning and the loss of sucrose in products. The microbial loads of all pasteurized palm sap samples were higher than the standards recommended by the Thai Community Product Standards. Therefore, adequate sanitation and good hygiene practices need to be integrated during the collecting, processing, packaging, and storing of palm sap. This must be done in order to prevent all potential hazards, especially the growth of microorganisms; this would result in an improvement in the quality and safety of pasteurized palm sap.

Acknowledgements

Financial support from Srinakharinwirot University for this research is gratefully acknowledged.

References

- [1] P Naknaen, M Meenune and G Roudaut. Characterization of palm sap harvested in Songkhla province, Southern Thailand. *Int. Food Res. J.* 2010; **17**, 977-86.
- [2] M Paichamnan, W Porsri and M Meenune. Quality profile of palm sugar concentrate produced in Songkhla province, Thailand. *Int. Food Res. J.* 2010; **17**, 425-32.
- [3] M Rattanathanalerk, N Chiewchan and W Srichumpoung. Effect of thermal processing on the quality loss of pineapple juice. *J. Food Eng.* 2005; **66**, 259-65.
- [4] E Akochi-K, I Alli and S Kermasha. Characterization of pyrazines formed during the processing of maple syrup. *J. Agric. Food Chem.* 1997; **45**, 3368-73.
- [5] A Apriyantono, A Nurhayati, Y Lidya, S Budiyo and T Soekarto. Rate of browning reaction during preparation of coconut and palm sugar. *Int. Congr. Ser.* 2002; **1245**, 275-8.
- [6] EH Ajandouz, LS Tchiakpe, F Ore, A Benajibas and A Puigserver. Effect of pH on caramelisation and Maillard reaction kinetics in fructose-lysine model systems. *J. Food Sci.* 2001; **66**, 926-32.
- [7] S Ranganna. *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*. McGraw-Hill, New Delhi, 1986.
- [8] P Naknean. Improvement in Shelf Life and Safety of Pasteurized Palm Sap (*Borassus flabellifer* Linn.) by the Addition of Nisin. *J. Food Saf.* 2013; **33**, 515-25.
- [9] P Naknean, N Juntorn and T Yimyuan. Influence of clarifying agents on the quality of pasteurised palmyra palm sap (*Borassus flabellifer* Linn.). *Int. J. Food Sci. Tech.* 2014; **49**, 1175-83.
- [10] W Binsan, S Benjakul, W Visessanguan, S Roytrakul, M Tanaka and H Kishimura. Antioxidative activity of Mungoong, an extract paste, from the cephalothorax of white shrimp (*Litopenaeus vannamei*). *Food Chem.* 2008; **106**, 185-93.
- [11] I Kiss. *Testing Methods in Food Microbiology*. Akademiai Kiado, Budapest, 1984.

- [12] RGD Steel and J Torrie. *Principle and Procedures of Statistics: A Biometrical Approach*. McGraw-Hill, New York, 1980.
- [13] RC Gupta, VK Jain and G Shanker. Palm sap as a potential starting material for vinegar production. *Res. Ind.* 1980; **25**, 5-7.
- [14] R Jamfa. 2002, Production of Syrup from Palm Sap. M. Sc. Dissertation. Narasuan University, Thailand.
- [15] P Naknaen, M Meenune and G Roudaut. Changes in physical and chemical properties during the production of palm sugar syrup by open pan and vacuum evaporator. *Asian J. Food Agro-Ind.* 2009; **2**, 448-56.
- [16] S Taiapai boon. 2004, Effect of High Pressure and Heat Treatment on Quality of Palm Sap. M. Sc. Dissertation. Prince of Songkla University, Thailand.
- [17] S. Loetkitsomboon. 2004, Effect of membrane filtration and heat treatment on quality of palm sap, M Sc. Dissertation. Prince of Songkla University, Thailand.
- [18] NAM Eskin. *Biochemistry of Foods*. Academic Press, New York, 1990.
- [19] EH Ajandouz and A Puigserver. Nonenzymatic browning reaction of essential amino acid: Effect of pH on caramelization and Maillard reaction kinetics. *J. Agric. Food Chem.* 1999; **45**, 1256-63.
- [20] J Kim and Y Lee. Antioxidant activity of Maillard reaction products derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time. *Food Chem.* 2009; **116**, 227-32.
- [21] S Oh, Y Lee, J Kim, J Kim, J Lee, MR Kim, H Yook and M Byun. Effect of pH on non-enzymatic browning reaction during γ -irradiation processing using sugar and sugar-glycine solutions. *Food Chem.* 2006; **94**, 420-7.
- [22] SIFS Martins and MAJS Van Boekel. Kinetics of the glucose/glycine Maillard reaction pathways: Influences of pH and reactant initial concentrations. *Food Chem.* 2005; **92**, 437-48.
- [23] VA Yayalayan, AA Ismail and S Mandeville. Quantitative determination of the effect of pH and temperature on the keto form of D-fructose by FT-IR spectroscopy. *Carbohydr. Res.* 1993; **248**, 355-60.
- [24] S Kermasha, M Goetghebeur and J Dumont. Determination of phenolic compound profiles in maple products by high-performance liquid chromatography. *J. Agric. Food Chem.* 1995; **43**, 708-16.
- [25] JK Siebert, VN Troukhanova and YP Lynn. Nature of polyphenol-protein interactions. *J. Agric. Food Chem.* 1996; **44**, 80-5.
- [26] S Jitbunjerdkul. Effect of antimicrobial agents on the quality of palm sugar products. *Songklanakarin J. Sci. Tech.* 1989. **11**, 161-5.
- [27] O Lasekan, A Buettner and M Christlbaure. Investigation of important odorant of palm wine (*Elaeis guineensis*). *Food Chem.* 2007; **105**, 15-23.
- [28] I Singh, S Solomon, AH Shrivastava, RK Singw and J Singw. Post-harvest quality deterioration of Cane juice: Physio-biochemical indicators. *Sugar Tech.* 2006; **8**, 128-31.
- [29] I Deslauriers. 2000, Recovery, Separation and Characterization of Phenolic Compounds and Flavonoids from Maple Products. M. Sc. Dissertation. McGill University, USA.
- [30] M Theriault, S Caillet, S Kermasha and M Lacroix. Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products. *Food Chem.* 2006; **98**, 490-501.
- [31] E Capuno, A Ferrigno, I Acampa, A Serpen, OC Acar and V Gokmen. Effect of flour type on Maillard reaction and acrylamide formation during toasting of bread crisp model systems and mitigation strategies. *Food Res. Int.* 2009; **42**, 1295-302.
- [32] LW Kroh. Caramelization in food and beverages. *Food Chem.* 1994; **51**, 373-9.
- [33] E Teixido, FJ Santos, L Puignou and MT Galceran. Analysis of 5-hydroxymethylfurfural in foods by gas chromatography-mass spectrometry. *J. Chromatogr. A* 2006; **1135**, 85-90.
- [34] D Huang, B Ou and RL Prior. The chemistry behind antioxidant capacity assay. *J. Agric. Food Chem.* 2005; **53**, 1841-56.
- [35] P Molyneux. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *J. Sci. Tech.* 2004; **26**, 211-9.

- [36] M Kukuc, S Kolayli, S Karaoglu, E Ulusoy, C Baltaci and F Candan. Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chem.* 2007; **100**, 526-34.
- [37] V Phongkanpai, S Benjakul and M Tanaka. Effect of pH on antioxidative activity and other characteristics of caramelization products. *J. Food Biochem.* 2006; **30**, 174-86.
- [38] W Lertittikul, S Benjakul and M Tanaka. Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein-glucose model system as influenced by pH. *Food Chem.* 2007; **100**, 669-77.
- [39] Thai Industrial Standards Institute Ministry. *Palmyra Palm and Palmyra Palm Products*. Bangkok, Thailand, 2003.