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# Differentiation between adulterated and non-adulterated palm sap using physical and chemical properties combined with discriminant analysis

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**Abstract**

Pasteurized palm sap is well known juice in many tropical countries. It has unique flavor and taste but is not available throughout year. Consequently there are many palm sap manufacturers trying to increase their production quantities by addition of other substances such as water and other cheaper syrups or sugar. The objective of this research was to discriminate the commercially pasteurized palm sap samples into adulterated and non-adulterated groups using their physical and chemical properties combined with canonical discriminant analysis. The result found that six from nine variables including total soluble solid, reducing sugar, titratable acidity, pH, $L^*$, and $b^*$ values were selected for the discriminant analysis. A good discrimination between the non-adulterated and adulterated samples was attained by applying one canonical discriminant function, which provided 100% of the 14 palm sap samples correctly classified. While $L^*$ and $b^*$ values as well as total soluble solid were found to be the most important predictor variables for the classification of the adulterated palm sap samples, the reducing sugar, titratable acidity, and pH value were of the most significance for that of the non-adulterated ones.



## Introduction

Palmyra palm (*Borassus flavellifer* Linn.) sap is a well known local product in many tropical countries such as Thailand, Malaysia, Myanmar, Indonesia, India, and Combodia (Davis and Johnson, 1987; Naknean *et al*., 2010). Pasteurized sap is expensive juice compared to other fruit juices due to unique characteristic taste and flavor. Palm sap is harvested by tapping the palmyra inflorescences and collected in bamboo or plastic containers for twice per day in the morning and evening. Consequently, it is left in the containers for 12-24 h before pasteurization. Fresh palm sap is translucent and whitish and has pH of 6.7-7.2 (Faparsui and Barsir, 1971; Lasekan and Abbas, 2010; Naknean *et al*., 2010). Its quality undergoes gradual changes during harvesting time due to fermentation and browning reaction. For examples, the sap's pH gradually decreases from almost 7 to 4.2-5.8, titratable acidity slowly increases from around 0.02 to 1.0% (w/v) or higher of lactic acid, and color becomes dimmer white (Thai Community Product Standard, 2003; Naknean *et al*., 2010). Major compositions of the sap are mono- and disaccharides and most of them are sucrose. The sap also contains small amount of reducing sugars such as glucose and fructose. Several researchers reveal that the sap has total sugar content of 130-180 mg/mL, reducing sugar content of 9-35 mg/mL and total soluble solid of 11-17 $^o$Brix depending on variety of the Palmyra palm, growing area, and weather conditions (Davis and Johnson, 1987; Naknean *et al*., 2014).

As a result of shortage of the palm sap during raining season, some manufacturers try to increase their production quantities by adding syrup made from other cheaper sugars. Sucrose and coconut sugar are often used for this purpose because they are easier available throughout the year and cheaper than sugar from Palmyra palm. Although coconut sugar contains glucose, fructose and sucrose similar to those of the palm sap, the uniquely sensorial quality between the palm sap and coconut sugar is different. Consumers who are not familiar with the sap cannot differentiate between non-adulterated and adulterated saps. Besides the other sugars and syrup, a clarifying agent such as lime substance is added during pasteurization of the sap to neutralize acidic taste naturally occurring from fermentation during harvesting and to precipitate haze naturally occurring from interaction between protein and polyphenol substances. The polyphenol substances are contaminated in the sap due to addition of the Kiam or Payorm woods containing tannic acid or tannin to retard microorganism growth during harvesting the sap (Chanthacum and Beuchat,

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1997; Phaichamnan *et al*., 2010). Intentional addition of various substances to the palm sap not only cause decrease in its quality but also may harm consumers' health. Therefore, discrimination between adulterated and non-adulterated palm saps using their properties is important for the consumers. Recently, the non-adulterated palm sap and the adulterated one with sucrose and coconut sugar are differentiated using transmission coefficients of microwave properties at 2.45 GHz. The researchers report that the transmission coefficients of the adulterated palm sap at lower total soluble solid than 24 $^{o}$Brix are higher than those of the non-adulterated one (Kaewsawat *et al*., 2012; Kanahna *et al.*, 2013).

Canonical discriminant analysis is a type of multivariate analysis methods and is used to select suitable predictor variables that could distinguish between two or more groups by determination of linear combinations of the variables, which provided the maximum discrimination between groups (Huberty, 1994; Johnson and Wichern, 2002). The canonical discriminant analysis was applied to discriminate origins of roasted sesame oils (Korean, Chinese, and Indian oils). The researchers reported that the sesame oil could be separated into 3 groups of its sources based on its composition contents including linoleic acid, oleic acid, palmitic acid, sesamin, sesamolin, and sesamol (Jeon *et al*., 2013). Moreover, it could be applied to correctly differentiate between three lettuce cultivars in association with their compositions of fructose, citric acid, vitamin C, nitrate and hue angle (López *et al*., 2014). Several researchers have revealed various physical, chemical, microbiological and sensory qualities of the palm sap as mentioned above. However, no published work has used those properties combined with canonical discriminant analysis to differentiate between the intentional adulterated and non-adulterated palm saps. The objectives of this study were to examine the physical and chemical properties of commercial palmyra palm saps obtained from various manufacturers and to discriminate them into adulterated and non-adulterated groups using their properties in association with canonical discriminant analysis.

## Materials and Methods

*Materials*

Phenol, sulfuric acid ($H_2SO_4$), and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). Phenolphthalein and sodium potassium tartrate were purchased from APS Fine Chem (Sydney, Australia). Disodium hydrogen arsenate heptahydrate was purchased from Sigma-aldrich (St. Louis, MO, USA). Glucose, fructose, sucrose, copper sulfate pentahydrate ammonium molybdate, and other chemicals of analytical grade were purchased from Ajax Fine Chem Pty, Ltd (NSW, Australia).

*Collection of palm sap samples*

Pasteurized palm sap samples randomly obtained from fourteen manufacturers (S1-S14) in Phetchaburi province, Thailand were randomly collected for 3 bottles from each of them and kept in ice box during transportation to the faculty of Agricultural Technology, Phetchaburi Rajabhat University and were used to evaluate their properties for attaining a canonical discriminant function. After that all pasteurized palm saps were kept in a refrigerator at 4-8$^{o}$C until analyses within the collected day. Their compositions and pasteurization process of the palm sap samples from each manufacturer were acquired by interviewing and observation at the production places in order to categorize which one was adulterated or non-adulterated with other substances. The adulterated palm sap was defined as the palm sap intentionally added with other substances such as sugars from other sources including sucrose, coconut sugar or glucose syrup, lime substance, and water.

The pasteurization process of all manufacturers was basically started with filtration of the harvested palm sap using a sheet cloth and then it was boiled in a large wok on a wood fired stove for 15 to 30 min. After transferred to a container, the pasteurized palm sap was left to cool down at ambient temperature before filling to bottles. Four additional palm sap samples for validation of the canonical discriminant function were purchased from the manufacturers different from the above fourteen manufacturers in Phetchaburi province.

*Determination of titratable acidity*

Titratable acidity of all palm sap samples was determined according to titration method described by Nielsen (2003). The palm sap sample was titrated with 0.01 mol/L NaOH and 1% (w/v) phenolphthalein solution as an indicator. The titratable acidity was expressed as percent (% w/v) of lactic acid.

*Determination of reducing sugar content*

The reducing sugar content of all palm sap samples was determined according to Somogyi-Nelson method (Fournier, 2001). The absorbance at 500 nm of the mixture of diluted sample and reactant solution was evaluated using a spectrophotometer (Jasco, Tokyo, Japan) and the reducing sugar content

was obtained from a calibration curve of glucose solution as a standard.

*Determination of total sugar content*

The total sugar content was determined according to the phenol-sulfuric acid assay as described by Dubois *et al*. (1956). The absorbance at 490 nm of the mixture of the diluted sample and reactant solution was measured using the spectrophotometer and the total sugar content was obtained from a calibration curve of the mixture of glucose, fructose, and sucrose solution as a standard at the ratio of 1:1:8, respectively.

*Determination of total soluble solid and pH value*

The total soluble solid was measured using a hand refractometer (ATAGO CO., LTD, Tokyo, Japan) and pH value was evaluated using a pH meter (Sartorius, Goettingen, Germany).

*Determination of color values*

The color of the palmyrah palm juice was measured using a colorimeter (ColorFlex EZ, Reston, VA, USA). Data of the color value were obtained in the terms of CIE *"L*"* (lightness), *"a*"* (redness and greenness), and *"b*"* (yellowness and greenness). Chroma value was calculated as follows:

$$\text{Chroma value} = (a^{*2}+b^{*2})^{1/2} \quad (1)$$

*Statistical analysis*

Each measurement was conducted in triplicates. Data analysis was done using the SPSS statistics software package (IBM Corporation, NY, USA) at a significant level of 0.05 for analysis of variance (ANOVA) and Duncan's multiple range tests to determine mean difference of the physical and chemical properties of the sources of palm sap samples. Canonical discriminant analysis was also performed with the same software to classify the sources of palm sap samples.

Discriminant function is linear combination of considered predictor variables and derived from equation (2).

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + \ldots + a_nX_n \quad (2)$$

Where $Y$ = discriminant score

$a_i$ = discriminant coefficient for variable i and $i = 1,\ldots, n$

$a_0$ = canonical discriminant constant

$X_i$ = predictor variable i and $i = 1,\ldots, n$

$n$ = numbers of predictor variables affecting $Y$

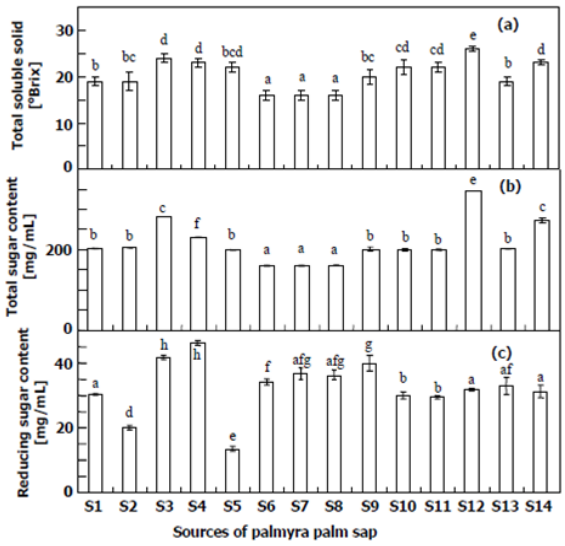


Figure 1. Variation in (a) total soluble solid, (b) total sugar, and (c) reducing sugar contents in palmyra palm sap samples obtained from fourteen different manufacturers

In the research, the palm sap samples were categorized into 2 groups of non-adulterated and adulterated ones. Mean difference of the discriminant scores between both groups was evaluated by independent t-test at a significant level of 0.05.

## Results and Discussion

Pasteurized palm sap samples obtained from 14 commercial manufacturers in Phetchaburi province coded from S1 to S14 were analyzed for their physical and chemical properties. The pasteurized palm sap samples coded S6, S7, and S8 were considered as non-adulterated ones due to no addition of any substance during pasteurization, but the other samples coded from S1 to S5 and from S9 to S14 were adulterated by addition of some water, other cheaper sweeteners such as sucrose and coconut sugar, and lime substance as a clarifying agent during production.

*Total soluble solid, total sugar and reducing sugar*

According to Figure 1(a) and (b) the total soluble solid and sugar contents of all palm sap samples were varied from 15.8 to 26.4 °Brix and from 160 to 350 mg/mL, respectively. While the non-adulterated palm sap samples coded S6 to S8 showed the total soluble solid content of 16 to 17 °Brix and the total sugar of 160 to 170 mg/mL, the adulterated samples showed higher contents of both values ($p < 0.05$). The results of the present study were in agreement with those reported by Dalibard (1999) and Naknean *et al*. (2010). Some of the adulterated samples coded S3, S4, S5, S12, and S14 showed significantly high contents of total sugar and total soluble solid, which were attributed to addition of other cheaper and easier

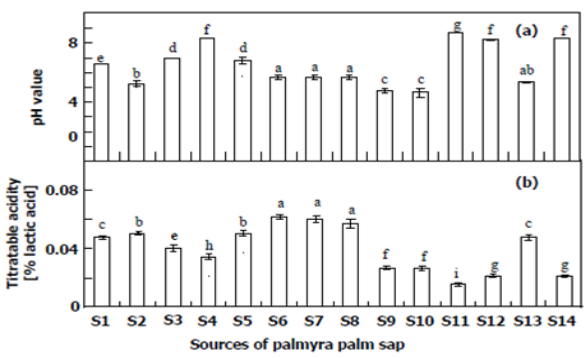


Figure 2. Difference in (a) pH values and (b) titratable acidity of palm sap samples obtained from fourteen different manufacturers

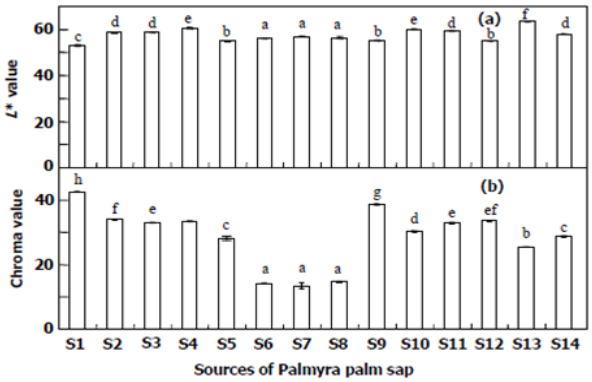


Figure 3. Variation in (a) $L^*$ value and (b) chroma value in palmyra palm sap samples obtained from fourteen different manufacturers

available sweeteners such as sucrose, coconut sugar, glucose syrup. In addition, all adulterated palm sap samples had higher total sugar content than 200 mg/mL, which were higher than average contents of the pasteurized palm sap reported by the other researches (Ho *et al*., 2008; Naknean *et al*., 2010; Phaichamnam *et al.*, 2010). However, both adulterated and non-adulterated palm sap samples contained the total soluble solid content in agreement with a regulated value in Thailand (>12 $^{o}$Brix) (Thai Community Product Standard, 2003). Figure 1(c) shows variation of total reducing sugar contents of the adulterated and non-adulterated palm sap samples. The reducing sugar content of the non-adulterated palm sap coded S6 to S8 was ranged from 13 to 18 mg/mL, which was in accordance with the report from the other researchers (Naknean *et al.*, 2014). On the contrary, the adulterated palm sap samples contained quite various reducing sugar contents ranged from 5 to 23 mg/mL, which were attributed to intentional addition of other sweeteners either sucrose or coconut sugar during pasteurization and water. Although the major sugar composition in the palm sap and coconut sugar was sucrose, which was responsible for about 80% or more by weight of total sugar contents and almost all reducing sugars in both palm sap and coconut sugar were glucose and fructose (Gupta *et al.*, 1980; Apriyantono *et al*., 2002; Naknean *et al*., 2014), the quality attributes such as flavor, aroma, and taste of the non-adulterated palm sap and adulterated one were different.

*Titratable acidity and pH*

The 14 pasteurized palm sap samples exhibited variation of pH values and titratable acidity as shown in Figure 2(a) and 2(b), respectively. The pH values and titratable acidity of all palm sap samples were ranged from 4.5 to 8.7 and from 0.02 to 0.07 %, respectively. The pH value of the palm sap at the beginning of harvesting was almost neutral (Davis and Johnson, 1987; Jagannadha *et al.*, 2009; Naknean, 2010). It gradually decreased during harvesting taken for several hours. The longer the harvesting time, the lower was the pH value, which was due to acid formation from fermentation of yeast and extracted substances derived from Kiam wood added in collected containers during harvesting to prevent microbial growth (Ho *et al*., 2008; Naknean, 2010). The pH value of the non-adulterated palm sap samples (S6 to S8) was lower than 7.0 but that of the adulterated ones was either lower or higher than 7.0. It should be noted that the non-adulterated palm sap samples showed higher titratable acidity than the adulterated ones. On the contrary, the adulterated samples coded S4, S11, S12, and S14 had higher pH value than 7.0. The reason would be explained that lime substance containing calcium hydroxide was added to the palm sap samples for clarification. Haze formation in the sap occurred due to cell fragment, interactions between protein and polyphenol, and between sugars or metal ions and proteins (Siebert *et al.*, 1996; Naknean *et al*., 2014).

*Color values*

Lightness ($L^*$ value) of the non-adulterated and adulterated palm sap was not much different from each other and ranged from around 53 to 63 as shown in Figure 3(a). Each chroma value of all the 14 palm sap samples was ranged from around 13 to 43 (Figure 3(b)). It was calculated according to the equation (1) which were derived from the $a^*$ values varied from -2 to 3.8 and from the $b^*$ values varied from 13 to 42. The higher the chroma and $a^*$ value, the paler is the redness and the higher the chroma and $b^*$ value, the paler is the yellowness (Peungradsamee and Ikeda, 2008). The non-adulterated palm sap samples had a significantly lower chroma value than the adulterated ones. The visual color of the palm sap samples was varied from little yellow to brown color depended on the samples' compositions and heating period, which

Table 1. Classification of four additional palm saps using canonical discriminant function and their physical and chemical properties

| Source | Total soluble solid [°Brix] | Reducing sugar content [mg/mL] | Titratable acidity [% ] | pH value | $L^*$ | $b^*$ | Discriminant score | Predicted classification |
|---|---|---|---|---|---|---|---|---|
| $S_A$ | 15.0 | 35.2 | 0.060 | 5.40 | 56.34 | 18.4 | -13.7 | Non-adulterated |
| $S_B$ | 21.3 | 17.3 | 0.024 | 8.67 | 59.12 | 42.5 | 10.2 | Adulterated |
| $S_C$ | 24.0 | 33.5 | 0.048 | 7.62 | 56.33 | 35.5 | 4.10 | Adulterated |
| $S_D$ | 16.0 | 32.8 | 0.058 | 5.60 | 52.54 | 18.8 | -14.8 | Non-adulterated |

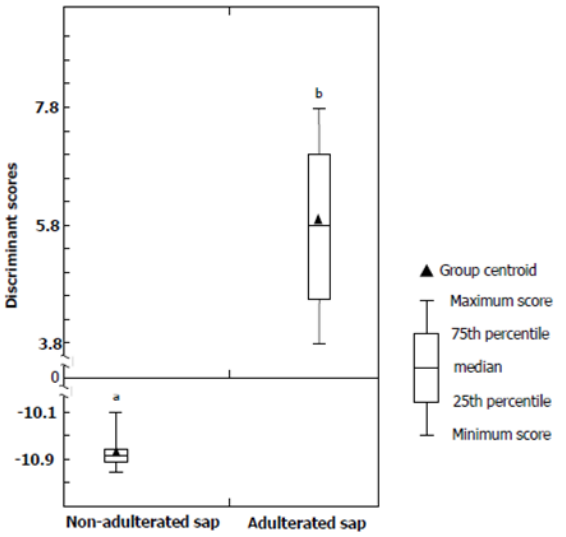


Figure 4. Box-and-whisker plot illustrating the distribution of discriminant scores of all fourteen adulterated and non-adulterated palm sap samples. Means with the different letters on the boxes are significantly different ($p < 0.05$)

were in agreement with the other researchers' reports (Ho *et al.*, 2008; Naknean, 2010; Phaichamnam *et al.*, 2010;). The fresh palm sap has oyster white color (Ho *et al.*, 2008; Phaichamnam *et al.*, 2010). After heating to pasteurize the palm sap, the brown color was developed. The longer the heating periods and the higher the sugar content, the higher was the chroma value.

*Discrimination of the palm sap samples into the adulterated and non-adulterated groups*

Canonical discriminant function analysis is a task of multiple linear regressions and used to categorize the qualitatively different food products based on a set of their known properties. All 14 palm sap samples were classified into the adulterated and non-adulterated ones according to various variables evaluated in this study related to all nine physical and chemical properties of the palm sap samples including $L^*$, $a^*$, $b^*$ and chroma values, total sugar content, reducing sugar content, total soluble solid, pH value, and titratable acidity. As a result all properties are not appropriate variables used to categorize the palm sap samples, the suitable properties were selected by the stepwise linear discriminant method using the statistical software to reduce data over-fitting. Each of all nine variables was individually entered into the discriminant function and selected only if it provided the largest positive or negative correlation that significantly improved the prediction outcome and satisfied the stringent criterion at the confident interval of 95% ($p < 0.05$) (Huberty, 1994). A canonical discriminant function was resulted from entering all nine variables and only six of them were selected as the predictor variables including the total soluble solid (with a canonical function coefficient of 2.351), reducing sugar content (-1.609), titratable acidity (-1.064), pH value (-0.435), and the color values of $L^*$ (2.517) and $b^*$(2.355). The magnitude of the six canonical function coefficients indicates the relative contribution of each predictor variable to the discriminant function controlling for all other variables. The higher the magnitude of the coefficient, the higher was an influence of the predictor variable to control the function. In addition, a positive or negative sign of the coefficients indicates either the positive or negative contribution, respectively (Field, 2000). The variables of total soluble solid, $L^*$ and $b^*$ values reflected more positively contribution to the function than those of the reducing sugar, titratable acidity, and pH value, which reflected the negative one. The variable of $L^*$ value was the most important to control the function following by the variables of $b^*$ value and total soluble solid. The variable of pH value was of less importance than the others. Figure 4 shows box-and-whisker plot of the discriminant scores of all the non-adulterated and adulterated palm sap samples. The distribution of the scores was calculated from the coefficients and allowed 100% of the grouped samples to be correctly classified into the non-adulterated and adulterated ones. The group means so called centroids of the predictor variables were used to interpret results of discriminant scores. Means of the non-adulterated and adulterated palm

sap samples were -10.8 and 5.9, respectively, which were significantly different from each other ($p < 0.001$). Any palm sap sample with the score near a centroid is predicted as belonging to that group. The larger spread of the discriminant scores from 3.8 to 7.7 was observed with the adulterated palm sap, while the smaller distribution of the scores from -10.1 to -10.9 belonged to the non-adulterated one. Wilks' lambda value of the function was 0.015, which was quite small. The Wilks' lambda value indicates the significance of the discriminant function on the box plot. The closer the values to 0, the more different and suitable are the group means to discriminate the palm sap samples between both groups. On the contrary if the Wilks' lambda value is closer to 1, the group means are no significant difference between the groups (Field, 2000).

*Validation of the discriminant function*

To examine the predictive discrimination power of the canonical function model established, additional palm sap samples obtained from four different manufacturers (SA, SB, SC, and SD) were discriminated using the coefficients as described above. Table 1 shows classification of the four additional palm sap samples to examine validation of the canonical discriminant function. The SB sample was adulterated with some sucrose, lime substance, and water. The SC sample was added with some coconut sugar and water to increase the production quantities. Thus, the discriminant model could correctly categorize the palm sap samples if the palm sap samples were adulterated with other substances.

## Conclusions

Some physical and chemical properties including total soluble solid, reducing sugar, titratable acidity, pH, $L^*$, and $b^*$ values combined with the canonical discriminant analysis could be used to differentiate the 14 palm sap samples into two groups of adulterated and non-adulterated ones. While the properties of the reducing sugar, titratable acidity and pH value were the important contribution variables to control the canonical function for the non-adulterated group, the properties of $L^*$ value, $b^*$ value and total soluble solid were important to control the function for the adulterated group.

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