COMPARISON OF CHEMICAL PROPERTIES OF COCONUT INFLORESCENSE SAP TAPPED AT NIGHT AND IN DAYTIME

Pepita Haryanti[1)], Umar Santoso[2)], Djagal W. Marseno[2)] and Supriyadi[2)]

[1)] Student of Food Science Doctoral Program Gadjah Mada University, Yogyakarta; Faculty of Agriculture Jenderal Soedirman University, Purwokerto
[2)] Faculty of Agricultural Technology Gadjah Mada University, Yogyakarta

ABSTRACT

Coconut inflorescense sap is the sweet translucent substance that is derived by tapping coconut flowers. This tapping process is commonly conducted twice a day, i.e. at night for about 15 hours and in daytime for about 9 hours. In this research, a number of chemical properties of coconut sap, which were obtained at night and day, were measured. The observed properties included pH, total dissolved solids, reducing sugar, total sugar, sucrose content, and free amino acids. Based on the experiments, the pH, total dissolved solids, total sugar, and sucrose content of the coconut sap tapped at night time were lower than that of the sap tapped in daytime. On the other hand, the reducing sugar was found in higher amount in the coconut sap obtained at night rather than that acquired in daytime. Free amino acids content in the sap collected at both times, however, was not significantly different. The difference of tapping period at night and in daytime will lead to variation of chemical properties of the produced coconut sap and sugar. In order to produce granulated coconut sugar, the coconut sap must meet several requirements, i.e. pH is bigger than 8, reducing sugar is between 0.05±0.01 to 0.70±0.02 %, and sucrose content ranging from 12.08±0.88 to 15.75±0.54%.

Keywords: coconut inflorescence sap, tapping, reducing sugar, sucrose content.

Introduction

Coconut inflorescence sap is the sugary exudate that is obtained from the unopened spathe at a certain stage through a tapping process for 10-12 hours (Soekardi, 2012). Coconut sap is nutritious and can be used to cure nephritis and bladder infections in Madagascar (Barh and Mazumdar, 2008). Several microorganisms can grow well in coconut sap since it is high in healthy nutrients. The utilization of preservatives, for instance mix of milk of lime and mangosteen peel, can prevent the deterioration of the coconut sap during the tapping process. Lime in water with the chemical formula $Ca(OH)_2$ will provide OH^- ion to alkalize the sap as well as prevent

the growth of the microorganisms (Naufalin et al, 2012). In addition, Poeloengan dan Praptiwi (2010) reported that mangosteen peel contains some antibacterial chemical compounds, such as saponin, tannin, and flavonoid.

In South East Asia, coconut sap can either be consumed as fresh drink or be used as a raw material of fermented drinks and coconut sugar. Banyumas Regency is one of the coconut sugar processing centers in Central Java Province, Indonesia. In this center, coconut tapping process is usually conducted twice a day, i.e. in the daytime for about 9 hours and at night for about 15 hours. The variations of these tapping periods might influence the chemical characteristics of the produced coconut sap.

A research in relation to the characteristics of coconut sap which was conducted by Atputharajah et al. (1986) showed that the biochemical and microbiological characteristics as well as nutrient composition of coconut sap changed during natural fermentation (Barh and Mazumdar, 2008). Furthermore, Xia et al. (2011) reported that the characteristics of coconut sap changed during 12 days of fermentation. Ho et al. (2008) also found that the characteristics of palm sap changed during heating process. There is no specific information regarding the chemical characteristics of coconut sap derived from different tapping periods. This research aims to compare the characteristics of coconut sap tapped in the daytime and at night. The information of the characteristics of coconut sap is considerably important to find out the quality of the produced coconut sugar.

Materials and methods

Material

Dinitrosalicylic acid, Rochelle salts, phenol, sodium sulfit, sodium hydroxide, hydrochloric acid, glutamic acid, glucose anhidrous, dipotassium phosphate,

potassium dihydrogen phosphate, ninhydrin, ethanol, stannous chloride, distilled water.

Collection of coconut inflorescense sap

The coconut sap used in this research was obtained from tapped flower buds of 15 local coconut trees grown in the Experimental Farm, Sikapat Village, Sumbang, Banyumas Regency. The sap was collected into plastic containers which were washed in advance using hot water to minimize microbial contamination. The substance used as the preservative was 1.7 g/L of milk of lime with addition of mangosteen peel powder 0.28, 0.56, and 0.84 g/L and without mixture of the powder. Moreover, a mixture of 1.7 g/L of milk of lime, 0.28 g/L of chopped jackfruit wood and 0.28 g/L of sliced mangosteen peel, which is commonly utilized by the coconut sugar farmers, was used as the control treatment. The tapping process was conducted twice a day in the daytime and at night for 9 and 15 hours, respectively. The temperature of the collected coconut sap was maintained at 4 ^{o}C in a cool box. The chemical characteristics of the sap, namely pH, total dissolved solids, reducing sugar, total sugar, sucrose content, and free amino acid, were then measured.

Determination of acidity (pH) and total dissolved solids

The acidity (pH) of the sap was measured using a digital pH meter (ATC-China), which has been calibrated by using buffer solution with a pH of 6.86 and a temperature of 25 ^{o}C. Meanwhile, the total dissolved solids was determined by using a portable refractometer (ATC-China).

Determination of reducing sugar, total sugar and sucrose content

In this step, the values of reducing sugar, total sugar and sucrose content were determined by using the analysis procedure of Miller (1959) with several modifications. A coconut sap sample weight of approximately 0.1 g was dissolved in distilled water

until the mixture's volume reached 50 mL and then a volume of 3 mL of the mixture was taken into a test tube. A volume of 3 mL of 1% DNS reagent was then added to the mixture in the test tube. Subsequently, the mixture was heated in a waterbath at a temperature of 90 ^{o}C for 15 minutes.To stabilize the color of the mixture, an amount of 1 mL of potassium tartrat 40% was added. The mixture was cooled afterward at room temperature and measured its absorbance at a wavelength of 575 nm. A standard glucose solution is used to calibration curve and get the straight line equation to quantify samples. The total sugar of the coconut sap was determined by hydrolyzing the sample. The hydrolysis process was done by incubating the sample with 3 mL of HCl at a temperature of 70 ^{o}C for 10 minutes. The mixture was then cooled at room temperature and neutralized with NaOH 45%. Sucrose content was calculated by subtracting reducing sugar from total sugar.

Determination of amino acid content

The amino acid was measured by using the procedure proposed by Yao et al. (2006). 1 g coconut sap, 0.5 mL buffer solution and 0.5 mL ninhydrin solution were placed in a 25 ml volumetric flask and the flask was heated in a boiling water bath for 15 min. Then the flask cooled to room temperature and the solution in the flask was diluted to 25 ml with distilled water. The absorbance of the diluted solution was measured using Spectronic 200 spectrophotometer (Thermoscientific) at 570 nm. This measurement was analysed in duplicate. Glutamic acid was used to prepare the standard curve.

Statistical analysis

The data were statistically analyzed using IBM SPSS Statistic 20 and reported as mean ± SD. The differences among the experimental groups were identified by one-way

analysis of variance (ANOVA) using Duncan's multiple range test. The statistical significance was considered at $P < 0.05$. All experiments were repeated at least twice.

Result and discussion

Comparison Acidity (pH value) of coconut sap tapped at day and night time

The pH of the coconut sap tapped in the daytime and at night is depicted in Figure 1.

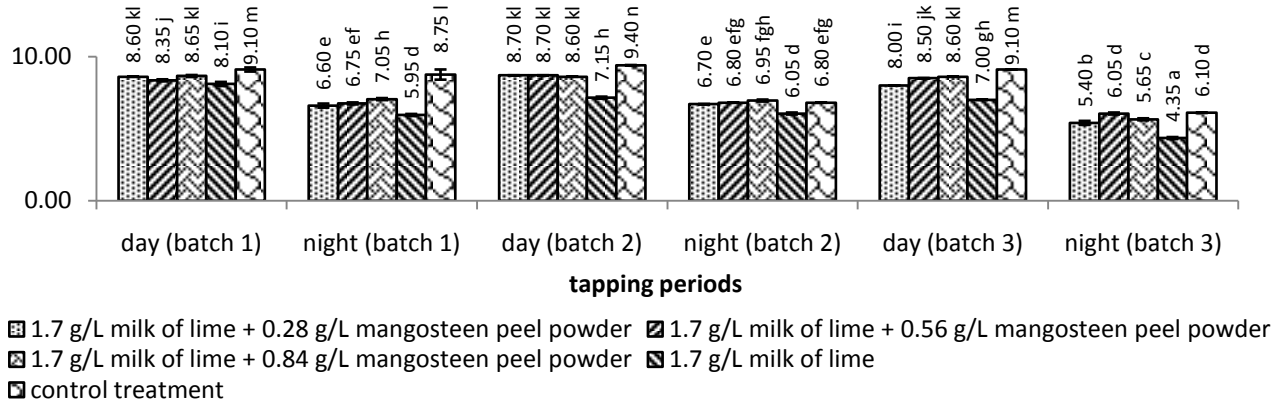

Figure 1. Acidity (pH value) of coconut sap. The results are representative of two independent experiments and values are expressed as mean ± SD from two experiments.

As seen in Figure 1, the acidity of the coconut sap tapped at night is lower (4.35±0.07 - 8.75±0.35) than that of the sap tapped in the daytime (7.00±0.00 - 9.40±0.00). The longer the period of the tapping process, the higher the organic acid content will be. Sap will be subject to a natural fermentation caused by lactic acid bacteria (Atputharajah et al, 1986; Manel et al, 2011). Furthermore, lactic acid can reduce the sap acidity. Naknean et al. (2010) reported that yeasts can convert sucrose to glucose and fructose by invertase and finally to organic acids and alcohols which will decrease the pH of the sap.

Comparison total dissolved solids of coconut sap tapped at day and night time

Total dissolved solids of coconut sap samples tapped at daytime and night varied from 8.35±0.07 °Brix to 16.95±0.07 °Brix (Figure 2). The variation of total dissolved

solids of palm sap depends on different source of palm sap and fermentation of sugar caused by microorganisms. According to Naknean et al. (2010) and Singaravadival et al. (2012), total dissolved solids of sap will decline during fermentation process since it is transformed into alcohol by microorganisms.

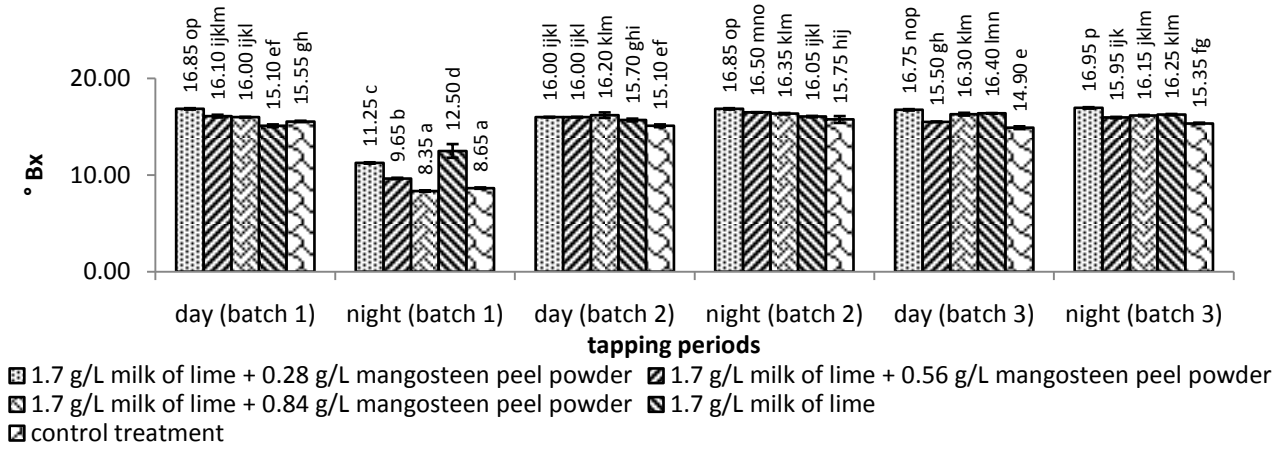

Figure 2. Total dissolved solution of coconut sap. The results are representative of two independent experiments and values are expressed as mean ± SD from two experiments.

Comparison reducing sugar of coconut sap tapped at day and night time

As shown in Figure 3, the reducing sugar content of the sap tapped at night is higher than that of the sap tapped in the daytime.

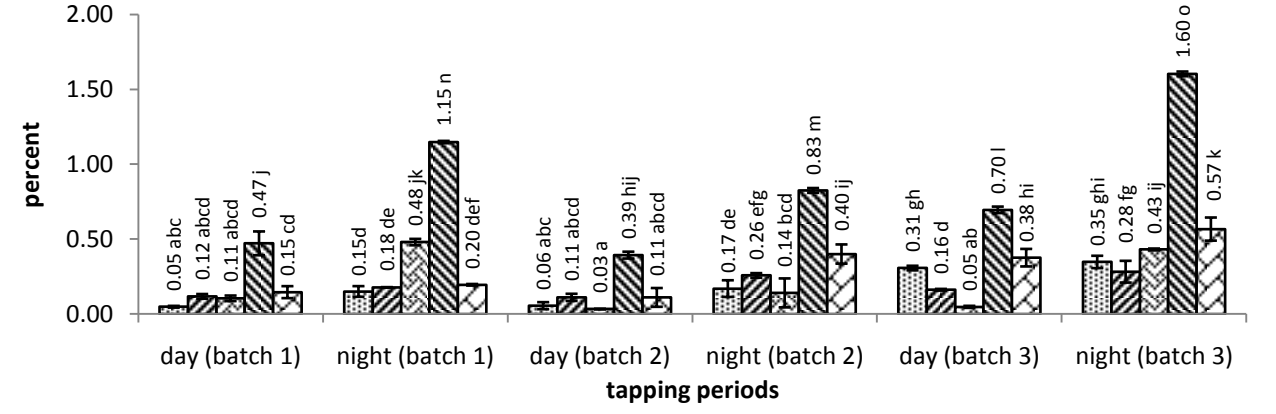

Figure 3. Reducing sugar of coconut sap. The results are representative of two independent experiments and values are expressed as mean ± SD from two experiments.

The reducing sugar contents of coconut sap tapped at night and in the day are 0.15±0.04 - 1.60±0.02% and 0.05±0.01 - 0.07±0.02% respectively. The high amount of reducing sugar in the coconut sap was caused by invertase enzyme. The occurrence of invertase in coconut 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 coconut sap (Borse et al, 2007). 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* (Naknean et al, 2011). Moreover, 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 coconut sugar. High reducing sugars content presented in coconut sap also influence on the browning colour of coconut sugar afterward, due to Maillard reaction.

Comparison total sugar of coconut sap tapped at day and night time

Total sugars were analyzed in this study, showed significant different across samples ($P<0.05$) (Figure 4). The total sugar contents of coconut sap tapped at night and in the day are 6.05±1.02 – 14.28±2.65% and 5.94±1.49 – 17.19±0.24% respectively. In addition, the variations of the total sugars were influenced by physiological characteristics of the coconut trees. Sugars especially sucrose is the product of photosynthesis activity in palm tree (Obahiagbon and Osagie, 2007).

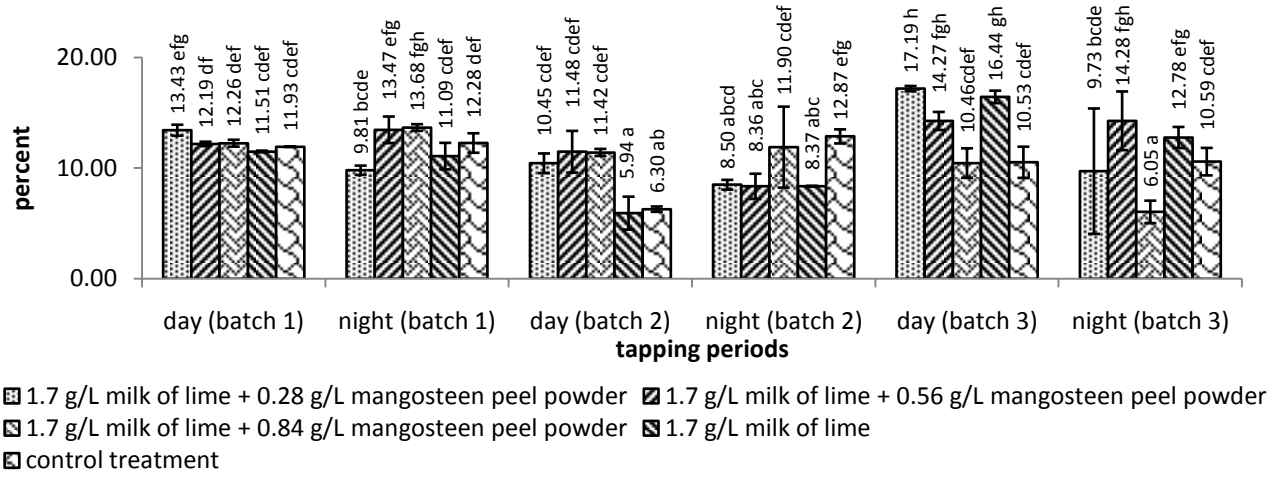

Figure 4. Total sugar of coconut sap. The results are representative of two independent experiments and values are expressed as mean ± SD from two experiments.

Comparison sucrose content of coconut sap tapped at day and night time

The sucrose content of coconut sap tapped at night and in the day are 5.62±1.02 – 14.00±2.58% and 5.54±1.46 – 16.88±0.25% respectively (Figure 5). This might be due to the inversion reaction caused by invertase activity and acid condition.

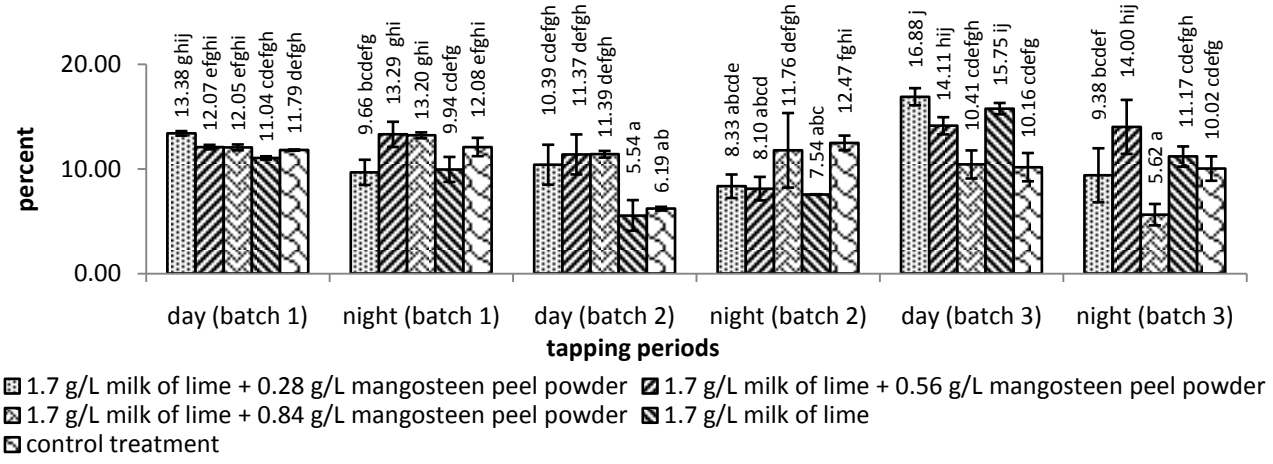

Figure 5. Sucrose content of coconut sap. The results are representative of two independent experiments and values are expressed as mean ± SD from two experiments.

Spontaneous fermentation occurs during tapping process due to microorganisms' activity. Yeasts such as *Saccharomyces cerevisiae, Saccharomyces carlsbergensis* and fungi such as *Aspergillus oryzae* and *Aspergillus niger* produce invertase to hydrolyze

sucrose into glucose and fructose. Tapping process at night is usually conducted longer than that in the daytime. The sucrose content, thus, is less in amount as it is hydrolyzed much more at night than in the daytime. Moreover, at night, plants undergo respiration which needs to break down sucrose. On the other hand, sucrose will be synthesized in the day during photosynthesis process. Therefore, the sucrose content in the coconut sap tapped in the daytime is relatively higher than that tapped at night.

Comparison free amino acid of coconut sap tapped at day and night time

Free amino acid content of all coconut sap samples was found in a range of 0.1041±0.0236 g eq glutamic acid/100 g - 0.5669±0.0024 g eq glutamic acid/100 g (Figure 6). The variation of free amino acid content in coconut sap may also due to the different sources of coconut sap. In addition, microorganisms may use free amino acid as a carbon source or as a nitrogen source for their metabolism and genetic material.

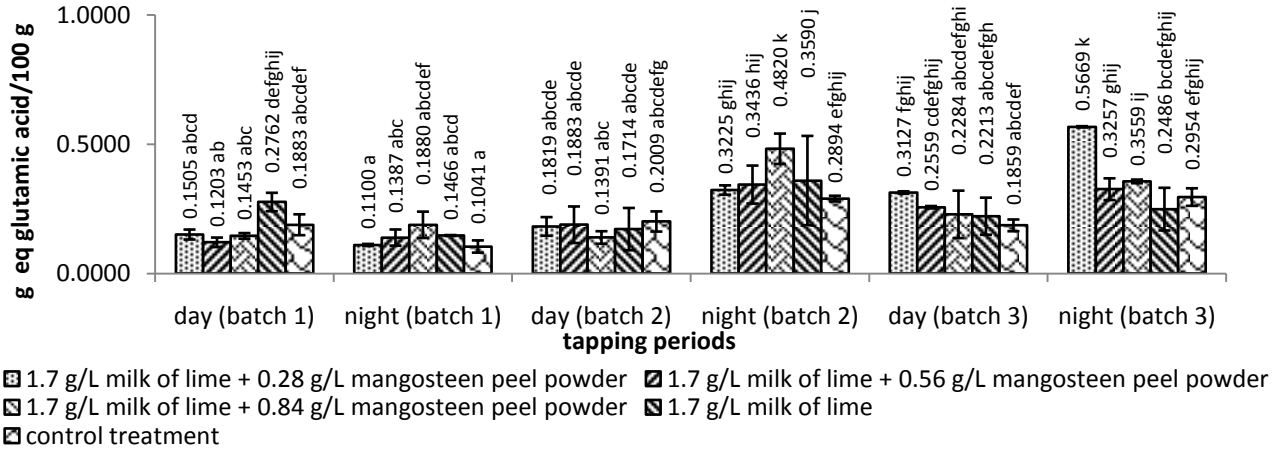

Figure 6. Free amino acid of coconut sap. The results are representative of two independent experiments and values are expressed as mean ± SD from two experiments.

Characteristics of coconut sap as raw material of granulated coconut sugar

Coconut sap which is tapped at night and in the day has different characteristics. The variations of preservative addition and sample collection time will

also lead to different characteristics. The characteristics of coconut sap will affect coconut sugar produced, as seen in Table 1.

Table 1. Effect of characteristics of coconut sap tapped at different times, variations of preservatives and collection batch to granulated coconut sugar formation

Tapping time	Weather conditions in the farm			Variation of preservatives				
	Dry bulb temperature (°C)	Wet bulb temperature (°C)	RH (%)	P1	P2	P3	P4	P5
Batch 1								
Day	25	24	91	+	+	+	+	+
Night	24.5	24	95	-	+	+	-	+
Batch 2								
Day	27	26	92	+	+	-	+	+
Night	24	23	91	-	-	-	+	+
Batch 3								
Day	22	21	90	-	-	-	+	+
Night	22.5	22	95	-	-	-	-	-

Note: (+) granulated coconut sugar formation occurs, (-) granulated coconut sugar formation does not occur
P1: 1.7 g/L milk of lime + 0.28 g/L mangosteen peel powder
P2: 1.7 g/L milk of lime + 0.56 g/L mangosteen peel powder
P3: 1.7 g/L milk of lime + 0.84 g/L mangosteen peel powder
P4: 1.7 g/L milk of lime + 0.28 g/L mangosteen peel powder
P5: control treatment

Table 1 shows that 36.67% and only 16.67% of coconut sap tapped in the day and at night, respectively, can be processed further to produce granulated coconut sugar. In order to produce granulated coconut sugar, the coconut sap must meet several requirements, i.e. pH is bigger than 8, reducing sugar is between 0.05±0.01 to 0.70±0.02 %, and sucrose content ranging from 12.08±0.88 to 15.75±0.54%.

Conclusion

The pH, total dissolved solids, total sugar, and sucrose content of the coconut sap tapped at night time were lower than that of the sap tapped in daytime. On the other hand, the reducing sugar was found in higher amount in the coconut sap obtained at night rather than that acquired in daytime. Free amino acids content in the sap collected at both times, however, was not significantly different. The difference of tapping period at night and in daytime will lead to variation of chemical properties of the produced coconut sap

and sugar. As much as 36.67% and 16.67% of the coconut sap collected at night and in the daytime, respectively, can be used as the raw material of granulated coconut sugar production. In order to produce granulated coconut sugar, the coconut sap must meet several requirements, i.e. pH is bigger than 8, reducing sugar is between 0.05±0.01 to 0.70±0.02 %, and sucrose content ranging from 12.08±0.88 to 15.75±0.54%.

References

Atputharajah J. D., Widanapathirana, S. and Samarajeewa, U. (1986). Microbiology and biochemistry of natural fermentation of coconut palm sap. *Food Microbiology* **3**: 273-280.

Barh, D. and Mazumdar, B.C. (2008). Comparative nutritive values of palm saps before and after their partial fermentation and effective use of wild date *(Phoenix sylvestris* Roxb.*)* sap in treatment of anemia. *Research Journal of Medicine and Medical Sciences* **3** (2): 173-176.

Borse, B.B., Rao, L.J.M, Ramalakshmi, K. and Raghavan. B. (2007). Chemical composition of volatiles from coconut sap (neera) and effect of processing. *Food Chemistry* **101**: 877–880.

Ho, C.W., Wan Aida, W.M., Maskat, M.Y. and Osman, H. (2008). Effect of thermal processing of palm sap on the physico-chemical composition of traditional palm sugar. *Pakistan Journal of Biological Sciences* **11** (7): 989 – 995.

Manel, Z., Sana, M. Nedia, K. Moktar, H and Ali, F. (2011). Microbiological analysis and screening of lactic acid bacteria from Tunisian date palm sap. *African Journal of Microbiology Research* **5** (19): 2929-2935.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* **31** (3): 426-428.

Naknean, P., Meenune, M. and Roudaut, G. (2010). Characterization of palm sap harvested in Songkhla province, Southern Thailand. *International Food Research Journal* **17**: 977-986.

Naufalin, R., Yanto, T. and Binardjo, A.G. (2012). Penambahan Konsentrasi $Ca(OH)_2$ dan Bahan Pengawet Alami untuk Peningkatan Kualitas Nira Kelapa. *Jurnal Pembangunan Pedesaan* **12** (2): 86- 96.

Obahiagbon, F. I. and Osagie, A. U. (2007). Sugar and macrominerals composition of sap produced by *Raphia hookeri* palms. *African Journal of Biotechnology* **6** (6): 744-750.

Poeloengan,M. and Praptiwi. (2010). Antibacterial activity test of mangosteen (*Gardnia mangostana* Linn) peel. *Media Litbang Kesehatan* **20** (2):65-69.

Singaravadivel, K., Alagusundaram, K. and Hariharan, B. (2012). Physicochemical properties of fresh and stored coconut palm toddy. *Open Access Scientific Reports* **1** (8): 1-2.

Soekardi, Y. (2012). *Pemanfaatan dan Pengolahan Kelapa Menjadi Berbagai Bahan Makanan dan Obat Berbagai Penyakit*. Yrama Widya, Bandung.

Wienen, W. J. and Shallenberger, R. S. 1988. Influence of acid and temperature on the rate of inversion of sucrose. *Food Chemistry* **29**: 51-55.

Xia, Q., Li, R., Zhao, S., Chen, W., Chen, H., Xin, B., Huang, Y. dan Tang, M. (2011). Chemical composition changes of post-harvest coconut inflorescence sap during natural fermentation. *African Journal of Biotechnology* **10** (66): 14999 – 15005.

Yao, L., Liu, X., Jiang, Y., Caffin, N., D'Arcy, B., Singanusong, R., Datta, N. and Xu, Y. (2006). Compositional analysis of teas from Australian supermarkets. *Food Chemistry* **94**: 115 – 122.