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Chemical Properties of Coconut Sap Obtained at Different Tapping Time and Addition of Preservatives

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Abstract:
Coconut sap is the sweet translucent substance that is derived by tapping coconut flowers commonly used as raw material of palm sugar. This tapping process is commonly conduc[5]t twice a day, i.e. during nighttime and daytime for about 15 and 9 hours, respectively. In this paper, chemical properties of coconut sap obtained during nighttime (CSN) and daytime (CSD) were measured. The preservatives used were lime and mangosteen peel powder added at concentration 0; 0.28; 0.56 and 0.84 g/L. The observed chemical properties were pH value, total soluble solids, water content, reducing sugar, sucrose, and total free amino acids content. The results of the study showed that the pH value, total soluble solids and sucrose content of CSN were lower than those of CSD. On the other hand, the water content and reducing sugar were found[7]gnificantly higher amount in CSN than in CSD. The difference of tapping time and preservative significantly affected the chemical properties of coconut sap and the palm sugar yielded. Due to the higher sucrose content and lower reducing sugar CSD was suitable as raw material than CSN to produce granulated coconut sugar. The optimum concentration of the preservatives added was 0.56 g/L.

Keywords: *coconut sap, tapping process, pH value, reducing sugar, sucrose content, palm sugar.*

1. Introduction

Coconut sap is sweet, oysterwhite and translucent exudates [12]obtained by tapping the unopened inflorescence of coconut palm (Xia et al., 2011). Coconut sap is rich in nutritious components such as 15-18% sugar mainly in the form of sucrose (Atputharajah, Widanapathirana, & Samarajeewa, 1986), proteins, vitamins, minerals, etc. However, it can be spontaneously fermented by several microorganisms. Lime is commonly used by traditional coconut farmers as a preservative of sap. As reported by Hariharan, Singaravadivel, & Alagusundaram, (2014); Samarajeewa & Wijeratna, (1983); Samarajeewa & Wijeratne, (1979), lime is used to control the rapid ferm[2]tation of sap during tapping process.

Lime milk ($Ca(OH)_2$) provides hydroxyl ion that will alkalize the sap[2]and prevent the growth of microorganisms. The antimicrobial action of lime is influenced by the speed of its dissociation into calcium ions and hydrox[2]ions. The hydroxyl ions will increase pH levels that can destroy or inactivate microorganisms (Silveira et al., 2011). In addition, several natural and synthetic preservatives are also usually used as preservatives of the sap. Sodium metabisulfite is mostly used as a preservative agent

because of its antimicrobial activity (Hariharan et al., 2014; Purnomo, 2007). Unfortunately, sodium metabisulfite found as a residue in sugar product is not good for health, especially for people with asthma.

Coconut farmers in Indonesia commonly use mangosteen peel and jackfruit wood as preservatives of sap during tapping. Scientific research has revealed that mangosteen peel, contains polyphenol compound known as xanthonoids like α-mangostin and γ-mangostin (Nivetha and Roy, 2015). The total phenolic content of peel was 5027.7 ± 188.0 mg per of dry matter of sap, and identified by GC-FID the phenolics compound were protocatechuic acid and m-Hydroxybenzoic acid as the major phenolic acid in the peel (Zadernowski, Czaplicki, & Naczk, 2009). Xanthones and their derivatives, which could be isolated from the peel, have been shown to have several health benefits such as antioxidant activity. Xanthones have remarkable antibacterial activities (Palakawong, Sophanodora, Pisuchpen, & Phongpaichit, 2010; Pedraza-chaverri, Cárdenas-rodríguez, Orozco-ibarra, & Pérez-rojas, 2008). In this present paper, the utilization of mixture of lime milk and mangosteen peel powder as a preservative to prevent the deterioration of the coconut sap during tapping process was investigated. The mixture of lime and mangosteen peel is expected to provide abetter preservation effect with single application as a comparison.

In South East Asia, coconut sap can either be consumed as fresh drink or be used as a raw material of coconut sugar. Commonly, coconut sap as the raw material of coconut sugar is slightly alkaline with pH of 7.5-8 and has around 15% of sugar (Hebbar et al., 2015). Furthermore, according to Samarajeewa and Wijeratne, (1979), sucrose content of coconut sap varies from 15.30 to 17.70 g/100 mL of sap in Sri Lanka.Banyumas Regency is one of the coconut sugar processing region in Central Java Province, Indonesia. In this area, tapping process is typically conducted twice a day, *i.e.* in the daytime for about 9 hours and during night for about 15 hours. The different tapping time might influence the chemical composition of the coconut sap.

A research in relation to the chemical properties of coconut sap have been conducted. Barh & Mazumdar, (2008) reported that the biochemical and microbiological characteristics as well as nutrient composition of coconut sap changed during natural fermentation. Xia et al., (2011) investigated that the characteristics of coconut sap changed during 12 days of fermentation. Ho, Wan Aida, Maskat and Osman (2008) also found that the characteristics of palm sap changed during heating process. According to literature survey, there is no specific information regarding the chemical properties of coconut sap derived at different tapping time with addition of mangosteen peel powder as preservatives. The aims of the study were to evaluate the chemical properties of coconut sap tapped during night (CSN) and in the daytime (CSD) and to examine the effect of addition of mangosteen peel powder on chemical properties of both CSN and CSD. The information of the chemical properties of coconut sap is important to ascertain the quality of coconut sugar produced through subsequent processing steps.

2.Materials and Methods

2.1. Materials

The coconut sap was collected by tapping inflorescence of coconut palm cultivar *Dalam* grown in experimental farm of Sumbang District, Banyumas Regency, Central Java, Indonesia with altitude of 500-1000 meters above sea level. The tapping process was conducted in fine weather with temperature at night 23-24.5 °Cand in the daytime, was 24-27 °C. The relative humidity in the night ranged 91-95% and in the daytime 91-92%. Mangosteen peel was dried, ground to make a powder and subsequently applicated as a preservative on coconut sap.

Several chemicals, *i.e.* potassium hydrogen tartrate (PubChem CID: 23681127), phenol (PubChem CID: 996), sodium sulfite (PubChem CID: 24437), sodium hydroxide (PubChem CID: 14798), hydrochloric acid (PubChem CID: 313), D-glucose (PubChem CID: 5793), ninhydrin (PubChem CID: 10236), dipotassium hydrogen phosphate (PubChem CID: 24450), potassium dihydrogen phosphate (PubChem CID: 516951), stannous chloride (PubChem CID: 24479), L-glutamic acid (PubChem CID: 33032) and ethanol (PubChem CID: 702) were purchased from Merck (Darmstadt, Germany) while 3,5-Dinitrosalicylic acid (PubChem CID: 11873) was obtained from Sigma-Aldrich (St. Louis, USA).

2.2. Collection of Coconut Sap

The coconut sap was obtained from tapped inflorescense of 15 coconut trees. The sap was collected into plastic containers which have been washed using hot water to minimize microbial contamination. The preservatives added were 1.7 g/L lime with addition of 0, 0.28, 0.56, and 0.84 g/L of mangosteen peel powder. The control treatment was preservatives that used commonly by local farmers *i.e.* mixture of 1.7 g/L of lime, 0.28 g/L of chopped jackfruit wood and 0.28 g/L of sliced mangosteen peel. The tapping procedure, the tip of coconut inflorescence was cut by sterilized stainless knife, and then the plastic container that have been added the preservatives was set up on to inflorescence with the cut filled in the container. The tapping process was conducted twice a day in the daytime and at nighttime for 9 hours (06.00 pm – 03.00 am) and 15 hours (03.00 pm - 06.00 am), respectively. The temperature of the collected coconut sap was maintained at 4 °C in a cool box.

2.3. Chemical analysis

The chemical properties of the coconut sap analyzed were pH value, total soluble solids, water content, reducing sugar, sucrose content and free amino acid content.

2.3.1. pH

The acidity (pH) of the sap was measured by means of a digital pH meter (Ohaus ST10-USA), which has been calibrated by using buffer solution with a pH of 6.86 at 25 °C.

2.3.2. Total Soluble Solids

The total soluble solids were measured by a portable refractometer (Atago, Japan).

2.3.3. Water Content

The water content of the coconut sap was measured using thermogravimetric-based procedure (AOAC, 1990).

2.3.4. Reducing Sugar and Sucrose Content

The reducing sugar content was determined by procedure of Miller (1959) with slightly modifications. A 1.0 g of coconut sap sample was dissolved in 5.0 mL distilled water and then a volume of 3.0 mL of the mixture was taken into a test tube. A volume of 3 mL of 1% 3,5-Dinitrosalicylic acid reagent was then added to the mixture in the test tube. Subsequently the mixture was heated in a waterbath at a temperature of 100 °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 at room temperature for 5 minutes and measured its absorbance at a wavelength of 540 nm. A standard glucose solution was used 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 25% at a temperature of 70 °C for 10 minutes. The mixture was then cooled at room temperature and neutralized with NaOH 45%. A volume of 3.0 mL sample then prepared for reducing sugar determination. The sucrose content was calculated by subtracting reducing sugar from total sugar.

2.3.5. Total Free Amino Acid Content

The amino acid was measured by method of Yao et al., (2006). A 1.0 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 minutes. Then the flask cooled to room temperature for 5 minutes and the solution in the flask was filled up to 25 mL with distilled water. The absorbance of the diluted solution was measured using Spectronic 200 (Thermoscientific) at 570 nm. Glutamic acid was used to prepare the standard curve to quantify the samples.

2.3.6. Processing Granulated Coconut Sugar

A 2.0 L of coconut sap was filtered with filter cloth, then filled in aluminium pan, and heated by gas stove for 2 hours. Agitation was done during heating process until the temperature of sap reached 118 °C. The viscous sap was then let at room temperature and cristallization process occurred by agitation to form granulated sugar. The granulated sugar was then dried under sunlight to reduce the water content. The sugar product was then observed visually for the formation of sugar granule.

2.4. Statistical Analysis

The data were statistically analyzed using IBM SPSS Statistic 20 and reported as mean ± standard deviation (SD). The data of samples were divided into two groups of tapping time, *i.e.* the nighttime and the daytime. The differences among the experimental groups were identified by paired-samples T test. The statistical significance was considered at $P = 0.05$. The effect of preservatives concentrations was analyzed by one-way analysis of variance (ANOVA) using Duncan's multiple range test with a significance level of $P = 0.05$.

2.Result and Discussion

3.1. pH Value

The pH of coconut sap obtained during nighttime (CSN) and coconut sap obtained during the daytime (CSD) with different concentration of mangosteen peel powder as preservative is depicted in Figure 1.

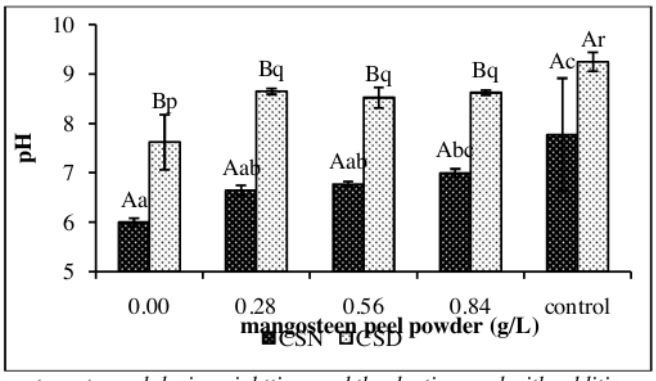

Figure 1: pH value of coconut sap tapped during nighttime and the daytime and with addition mangosteen peel powder as preservatives at different concentration.

Note: Letters A and B indicate the significant difference between tapping time (night and daytime) while letters a, b and c indicate the significant differences among the preservative concentration in CSN and letters p, q and r indicate the significant differences ($P<0.05$) among the preservative concentration in CSD.

As seen in Figure 1, the pH of CSN is lower (6.00 - 7.78) than that of CSD (7.63 - 9.25). As mentioned in the previous section, the coconut sap was collected by tapping coconut inflorescense in the daytime and at night for 9 and 15 hours, respectively. The longer the period of the tapping process, the higher the organic acid content would be. Coconut sap is subjected to a natural fermentation caused by lactic acid bacteria (Atputharajah et al., 1986; Manel et al., 2011). Naknean, Meenune and Roudaut (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.

As shown in Figure 1, the addition of mangosteen peel powder as preservative had a significant effect on the pH of coconut sap. The increment of mangosteen peel powder concentration tended to increase the pH value of both CSN and CSD. The higher pH is indicated less organic acid produce by the microorganism. The mangosteen peel powder show inhibitory activity against several microorganisms such as bacteria (Teh, Chan, Kamal, Shahidan, & Wahid, 2014). Xanthone and its derivatives are the bioactive compound which have responsibility on antibacterial activity (Palakawong et al., 2010; Pedraza-chaverri et al., 2008). According to Geetha, Roy and Lakshmi (2011) the aqueous extract of mangosteen peel powder can be employed as a good source of antimicrobial agent against bacterial pathogens.

3.2. Total Soluble Solids and Water Content

The total soluble solids (° brix) and water content of the CSN and the CSD are shown in Figure 2. In the Figure 2, it can be seen that the differences of the total soluble solids and water content of coconut sap collected from both tapping times are not significantly different (P>0.05). The different concentration of mangosteen peel powder as preservatives did not significantly affect total soluble solids of CSN, but significantly affected total soluble solids of CSD. However, the different concentration of preservatives influenced water content of both the CSN and the CSD.

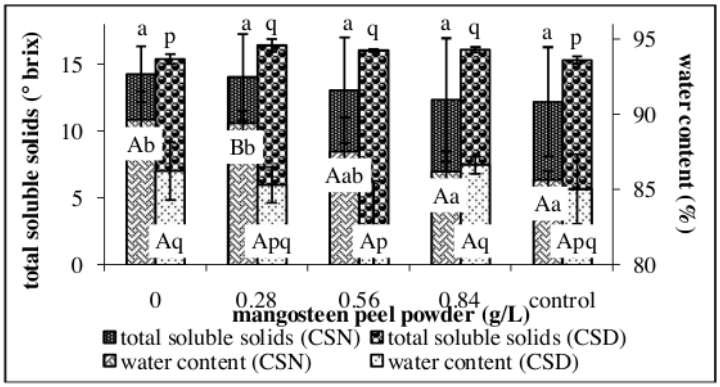

Figure 2: Total soluble solids and water content of coconut sap tapped during nighttime and the daytime and with addition mangosteen peel powder as preservatives at different concentration

Note: Letters A and B indicate the significant difference between tapping time (night and daytime) while letters a, b and c indicate the significant differences among the preservative concentration in CSN and letters p, q and r indicate the significant differences (P<0.05) among the preservative concentration in CSD.

As seen in Figure 2, the total soluble solids of CSN and CSD are 12.2 - 14.3 and 15.3 – 16.4 °brix, respectively, this values correspond to sugar content. During the daytime, sucrose is synthesized by photosynthesis with the consequence the total soluble solids of CSD is relatively higher than that of CSN. According to Lantemona, Abadi, Rachmansyah, & Pontoh (2013), the impact of tapping period in sugar productions among several plants is caused by abiotic components such as temperature, sun radiation and humidity which play an important role in plant metabolism. The addition of mangosteen peel powder as the preservative prevents the sucrose content from invertase activity during tapping process in the daytime. A plethora of studies in regard to antimicrobial activity of pericarp (peel or rind) extract of mangosteen due to its xanthones, polyphenols and flavonoids content have been reported (Fernando & Dasanayake, 2006; Nivetha & Roy, 2015; Pedraza-chaverri et al., 2008; Priya, Jainu, Mohan, Saraswathi, & Gopan, 2010). As shown in Figure 2, the water content of CSN and CSD are 85.66 – 89.64% and 82.26 – 86.64%, respectively. The water content of CSN was not significantly different from that of CSD.

3.3. Reducing Sugar and Sucrose Content

As shown in Figure 3, the reducing sugar content of the CSN is higher than that of the CSD. The reducing sugar contents of CSN and CSD are 0.16 - 0.99 g/100 g and 0.05 - 0.43 g/100 g respectively. The high amount of reducing sugar in the CSN was caused probably by invertase from yeast because of the longer tapping time. The application of mangosteen peel powder as the preservative can effectively lower the reducing sugar both the CSN and the CSD. Mangosteen peel powder has been demonstrated as an effective antibacterial agent (Geetha et al., 2011) and show inhibitory effect of invertase from yeast (Naknean et al., 2010).

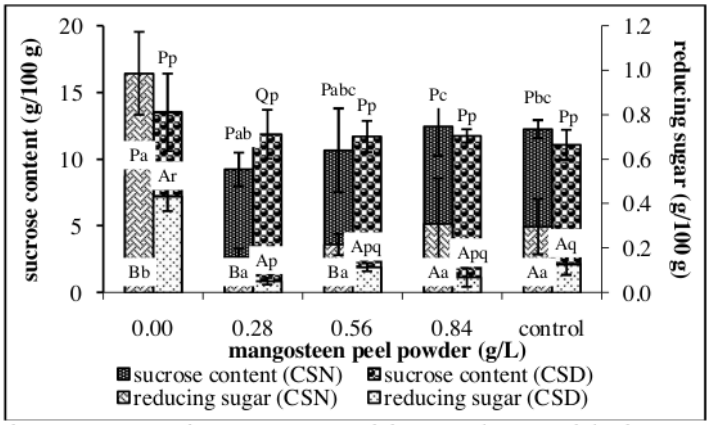

Figure 3: Reducing sugar and sucrose content of coconut sap tapped during nighttime and the daytime and with addition mangosteen peel powder as preservatives at different concentration.

Note: Letters A and B or P and Q indicate the significant difference between tapping time (night and daytime) while letters a, b and c indicate the significant differences among the preservative concentration in CSN and letters p, q and r indicate the significant differences (P<0.05) among the preservative concentration in CSD.

The invertase in coconut sap occurring naturally or produced by microorganisms. The invertase converts sucrose to glucose and fructose and subsequently transform to organic acids and alcohols (Borse, Rao, Ramalakshmi, & Raghavan, 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., 2010). On the other hand, decrease in pH are also responsible for the inversion reaction. The inversion reaction occurs when the glycosidic linkage of disaccharide is hydrolyzed, releasing the monosaccharide units *i.e.* glucose and fructose are formed (Wienen & Shallenberger, 1988).

The sucrose contents of CSN and CSD were 8.74 – 12.48 g/100 g and 11.10 – 13.54 g/100 g respectively. The sucrose content of CSN lower than that of the CSD due to the inversion reaction by invertase activity as well as acid condition during night tapping process. Spontaneous fermentation occurs during tapping process. Sucrose is synthesized in the day during photosynthesis (El-Naggar & Swedan, 2009). Furthermore, the shading reduced total non-structural carbohydrate content. Sugars especially sucrose is the product of photosynthesis activity in palm tree (Obahiagbon & Osagie, 2007).

The preservative used in this research, *i.e.* mangosteen peel powder, can maintain the sucrose content of the sap during tapping process due to its antibacterial content. According to Pedraza-chaverri et al (2008), the pericarp of mangosteen (peel, rind, hull or ripe) is a source of xanthones and other bioactive substances which have antibacterial activity. It can be seen in Figure 3 that the optimum mangosteen peel powder of 0.56 g/L could maintain the sucrose content of the sap.

3.4. Total Free Amino Acids Content

Total free amino acid content of CSN and CSD samples were found in a range of 0.1364-0.2121 and 0.1315-0.2099 g glutamic acid equivalent/100 g respectively (Figure 4).

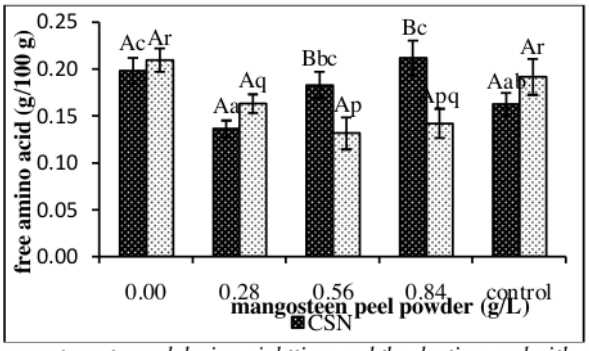

Figure 4: Total free amino acid of coconut sap tapped during nighttime and the daytime and with addition mangosteen peel powder as preservatives at different concentration.

Note: Letters A and B indicate the significant difference between tapping time (night and daytime) while letters a, b and c indicate the significant differences among the preservative concentration in CSN and letters p, q and r indicate the significant differences (P<0.05) among the preservative concentration in CSD.

As seen in Figure 4., the free amino acids content in CSN is higher than that in CSD. The difference of tapping time causes different temperature condition, and therefore leads to different effect on the rate of metabolism. These would contribute a significant effect to plant's amino acids content such as in coconut flower. According to El-Naggar & Swedan (2009) the amino acids were used in protein biosynthesis or as the precursor of other essential nitrogen containing molecules in these tissues. Mostly, amino acids transported in the plants vascular system becomes the nutritional needs of other organs. Those tissues, which include developing leaves, meristems and reproductive organs, must import amino acids to support growth and development (Ortiz-Lopez, Chang, & Bush, 2000).
The higher concentration of mangosteen peel powder added increased the total free amino acid content, especially in CSN, but not in CSD. The variation of free amino acid content in coconut sap might be affected by the different coconut tree. The total and free amino acid composition in coconut sap influence in Maillard reaction during sugar processing.

3.5. Coconut Sap as a Raw Material of Granulated Coconut Sugar Processing

As described in the previous description, coconut sap obtained during different tapping times has different chemical properties. The addition of different concentration of mangosteen peel powder as preservative affected the chemical composition of the coconut sap. The chemical properties of coconut sap determine the granulated coconut sugar formation, as seen in Table 1.

Tapping time	Mangosteen peel powder added (g/L)	Batch	Chemical properties of coconut sap					Granulated sugar formation
			pH	Total soluble solids (°Brix)	Water content (%)	Reducing sugar (g/100 g)	Sucrose content (g/100 g)	
Daytime	0	1	8.1	15.1	87.65	0.47	11.04	(+)
		2	7.2	15.7	84.84	0.39	16.05	(+)
	0.28	1	8.6	16.9	84.64	0.05	13.38	(+)
		2	8.7	16.0	86.00	0.06	10.39	(+)
	0.56	1	8.4	16.1	84.95	0.12	12.07	(+)
		2	8.7	16.0	79.57	0.11	11.37	(-)
	0.84	1	8.7	16.0	86.40	0.11	12.15	(+)
		2	8.6	16.2	86.88	0.03	11.39	(+)
Nighttime	0	1	6.0	16.1	87.99	1.15	9.94	(-)
		2	6.1	12.5	91.30	0.83	7.54	(-)
	0.28	1	6.6	16.9	88.84	0.15	10.17	(+)
		2	6.7	11.3	90.01	0.17	8.33	(-)
	0.56	1	6.8	16.5	85.76	0.18	13.29	(+)
		2	6.8	9.7	89.35	0.26	8.10	(-)
	0.84	1	7.1	16.4	86.00	0.48	13.20	(+)
		2	7.0	8.4	86.43	0.14	11.76	(-)

Table 1: Effect of chemical properties of coconut sap tapped at different times and the different concentration of mangosteen peel powder as a preservative on the formation of granulated coconut sugar
Note: (+) granulated coconut sugar formed, (-) granulated coconut sugar did not formed.

In this research, eight sap samples of CSN and CSD was processed into granulated coconut sugar form. Table 1 shows that not all of the sap sample could be processed to become granulated form as indicated by (-) symbol. It can be seen that from eight samples of CSD, seven sap samples (87.5%) formed granulated sugar (+), while eight samples of CSN, three samples (37.5%) formed. The sucrose inversion to glucose and fructose during tapping contributed in inhibiting the sugar crystallization during processing (Samarajeewa & Wijeratne, 1979). The hydrolytic activity of invertase from *Saccharomyces cerevisiae* was found to be influenced by temperature and pH of the medium, and the optimum temperature and pH were 55 °C and 4.5, respectively (Mahmood, 2010). The pH value, total soluble solids, water content, reducing sugar and sucrose content were the important parameters of coconut sap to produce coconut sugar. The coconut sap that can be processed to produce granulated coconut sugar required chemical properties, *i.e.* pH higher than 7.2, total soluble solids higher than 15.1 °brix, water content lower than 87.65%, reducing sugar lower than 0.48 g/100 g and the sucrose content higher than 10.39 g/100 g.

Conclusion

The pH and the total soluble solids and sucrose of the coconut sap tapped during night time (CSN) were lower than that of the sap tapped in the daytime (CSD). On the other hand, the water content, reducing sugar and total free amino acids was found in higher amount in CSN than CSD. The difference of tapping time, *i.e.* during night and in the daytime affected chemical properties of coconut sap and palm sugar yielded. The coconut sap collected during the daytime commonly can be used as the raw material of granulated coconut sugar production. The pH value, as well as total soluble solids, water content, reducing sugar and sucrose content of sap were the important properties to produce granulated coconut sugar. In the present research, coconut sap that can be processed to produce granulated coconut sugar required properties, *i.e.* pH bigger than 7.2, total soluble solids higher than 15.1 °brix, water content not

higher than 87.65%, reducing sugar lower than 0.48 g/100 g and the sucrose content higher 10.39 g/100 g. Further research is needed to determine the effect chemical properties of coconut sap on physical, chemical and sensory properties of granulated coconut sugar.

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