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The Chemical Composition of Coconut Sap at Different Tapping Condition

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Abstract. Coconut sap is a sweet, transparent, oyster-white liquid derived by tapping coconut inflorescent. This tapping procedure is usually carried out twice a day, at night and during the day, for around 15 and 9 hours, respectively. Coconut sap is especially sensitive to spontaneous fermentation because it contains sugar with a pH close to neutral. Coconut farmers generally use preservatives to keep the sap's quality throughout the tapping process. In this paper, a proximate analysis of coconut sap obtained during night (CTN) and in the daytime (CTD) were measured. The reducing sugar and amino acid profiles of coconut sap were also measured using High Performance Liquid Chromatography. This research aimed to determine the chemical composition of coconut sap at different tapping condition and variations of mangosteen peel powder concentration. The best preservative concentration of mangosteen peel powder was 0.84 g/L of coconut sap tapped during the daytime, according to the findings. This treatment yielded sap with chemical characteristic i.e. water content of 84.21%; ash content of 3.51% (db); protein content of 2.69% (db); total lipid of 0.10% (db) and total carbohydrate content of 94,62% (db). The CTN contained glucose and fructose of 0.85 and 1.04 g/100 g, respectively, higher than the CTD were 0.52 and 0.58 g/100 g. The CTN contained 13 amino acids which was lower than CTD.

INTRODUCTION

Coconut sap is obtained by tapping unopened coconut buds for a specific period of time [1][2]. Coconut sap is high in nutritious components, such as 15-18% sugar (mostly sucrose), protein, vitamins, minerals, and so on [3]. Some microorganisms can spontaneously ferment sap. Coconut farmers commonly use lime as a preservative. Lime is utilized to inhibit nira fermentation during the tapping process, according to [4][5][6]. Lime milk with the chemical formula $Ca(OH)_2$ will provide hydroxyl ions to provide alkaline properties on the sap and inhibit the growth of microorganisms. The mechanism of antimicrobial action is determined by the rate of separation into calcium and hydroxyl ions. The hydroxyl ion will increase the pH which is enough to inactivate microorganisms [7]. According to [8] bivalent metal ion such as Ca^{2+} significantly inhibited the activity of invertase. Furthermore, a variety of natural and synthetic preservatives are widely employed as preservatives. Because of its antibacterial properties, sodium metabisulfite is commonly employed as a preservative [4][9]. However, sodium metabisulfite, which is found as a residue in sugar products, is harmful to one's health, especially for those who suffer from asthma.

When tapping coconut sap in Indonesia, farmers use mangosteen peel and jackfruit wood as preservatives. Mangosteen peel contains polyphenolic substances known as xanthonoids, such as α-mangostin and β-mangostin, according to scientific studies [10]. The total phenolic acid content of the skin identified by GC-FID was 5027.7 ± 188.0 mg per kg of dry matter sample. Protocatechuic acid and m-Hydroxybenzoic acid are the main phenolic acids in the skin [11]. It is possible to separate xanthones and their derivatives from the skin, which has various advantages.

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Several studies have shown that xanthones obtained from mangosteen have remarkable biological activities such as antioxidant, and antibacterial activity [12][13]. In this article, a study will be undertaken whether a mixture of lime milk and mangosteen peel powder may be used as a preservative to prevent coconut sap from being degraded during the tapping process. When compared to a single application of lime and mangosteen peel, a mixture is predicted to have a greater preservation effect.

Coconut sap is used as a fresh or ready-to-drink beverage in Southeast Asia, as well as a raw material for fermented beverages and coconut sugar. Reducing sugar and coconut sap amino acids play an important role in the formation of the brown color and the distinctive aroma of the brown sugar. When sugars and proteins in most foods are heated together, Maillard reactions occur, which are associated to the creation of color [14]. According to [15] sap undergoes a Maillard browning reaction during heating. In Indonesia's Central Java Province, Banyumas Regency is one of the processing centers for coconut sugar. The tapping technique is commonly done twice a day in this location, once during the day for about 9 hours and again at night for around 15 hours. This variation of the tapping period may affect the chemical content of the coconut sap produced.

The biochemical and microbiological characteristics, as well as the nutritional composition of coconut sap, change during spontaneous fermentation [16]. Furthermore, during the 12 days of fermentation, the properties of coconut sap changed [1]. According to our review of the literature, there is no particular information on the proximate composition of coconut sap obtained at various tapping periods with different mangosteen peel powder concentration as a preservative. As a result, the objective of this research is to find out the proximate composition of coconut sap with the addition of variations of mangosteen rind powder tapped at night (CTN) and during the day (CTD), and to determine the composition of reducing sugars and amino acids of coconut sap CTN and CTD. To ensure the quality of coconut sugar, information on the chemical composition of coconut sap is required.

MATERIAL AND METHODS

The coconut sap was collected by tapping spathes of the 'Dalam' coconut palm cultivar, which was grown on an experimental farm in Sikapat Village, Sumbang District, Banyumas Regency, Indonesia. Coconut trees were grown at elevations of 500-1000 meters above sea level. The tapping operation took place in clear weather, with temperatures of 23-24.5 and 24-27 °C during night and day, respectively, and relative humidity of 91-95 and 91-92 percent. Mangosteen peel was dried and processed into a powder, which was then used as a preservative in coconut sap.

Merck (Darmstadt, Germany) provided sodium hydroxide, hydrochloric acid, boric acid, sulfuric acid, hydrogen peroxide, copper sulfate, sodium thiosulfate, methyl red, HPLC grade methanol, and acetonitrile, while Sigma-Aldrich provided amino acid mix solution standard and o-phthalaldehyde (OPA) (St. Louis, USA).

Collection of Coconut sap

The coconut sap used in this study was collected from 15 local coconut trees' tapped flower buds. To avoid microbial contamination, the sap was collected into plastic containers containing preservatives that had been cleaned in advance with hot water. The preservation agents utilized were 1.7 g/L milk of lime with a mangosteen peel powder addition of 0, 0.28, 0.56, and 0.84 g/L. The control treatment was a mixture of 1.7 g/L milk of lime, 0.28 g/L chopped jackfruit wood, and 0.28 g/L sliced mangosteen peel, which is typically employed by traditional coconut sugar farmers. The tapping was done twice a day for 9 and 15 hours, respectively, throughout the day and at night. In a cool box, the collected coconut sap was kept at 4^{o}C.

Chemical Analysis

Water content

The water content of the samples were measured using thermogravimetric-based procedure [17].

Ash content

The samples' ash content was determined using a thermogravimetric method [17].

Protein content

The protein content of the samples was determined using the Kjeldahl method, which involves calculating the nitrogen concentration and then utilizing that value to compute protein content [17].

Lipid content

The lipid content of the samples were determined using soxhlet method. The solvent from the boiling flask was volatilized and condensed to build up in the extraction chamber that contained the sample [17].

Carbohydrates

After establishing the other components of proximate composition, the total carbohydrate content is estimated by difference according to Equation (1):

$$\text{Total carbohydrate (\%)} = 100\% - (\%\text{moisture} + \%\text{ash} + \%\text{lipid} + \%\text{protein}) \quad (1)$$

Reducing sugar

Reducing sugar component was identified by high-performance liquid chromatography (HPLC) Knauer Smartline Pump 1000. A test tube was filled with fifty milligrams of dried coconut sap. A 2 mL H_2O was used to dissolve the sap. The mixture was then mixed for 1 minute before being sonicated for 10 minutes. In a volumetric flask, the sap was diluted with H_2O until the volume reached 5 mL, then centrifuged at 8,000 g for 5 minutes (Thermo Scientific; Carlsbad, CA, USA). The supernatant was filtered with millex 0.45 µm after it was taken from a test tube. The following were the HPLC optimal conditions: 5 mL of diluted sample was injected into a Metacharb 87C column (300 x 6.5 mm) and eluted with H_2O at 0.6 mL/minute flow with an oven temperature of 85 °C. Later that, the samples were detected utilizing a G 1362 A Refractive index (RI) detector. Sugar concentrations of 50, 100, 200, 500, 1000, and 2000 ppm for fructose and glucose were employed as standards, and 20µL of each were injected. An external standard was eventually used to calculate the sugar concentration in the sap.

Amino acid composition

The supernatant was filtered using a 0.45 µm nylon filter membrane after 10 mL of sap were centrifuged at 2054 g. The composition of free amino acid in coconut sap was determined using the method proposed by [18]. A mixture of 140 liters of sample infusion (or standard amino acid) and 20µL of OPA solution was incubated at 25 °C for 2 min, according to the method. This mixture was utilized for HPLC analysis right away. The OPA-based derivatization was carried out according to [19]'s modified technique. The Shimadzu LC 10AD HPLC system with UV detector was used to determine the free amino acids in the sap (Shimadzu Technologies, Kyoto, Japan). The analysis was performed at a flow rate of 1.0 mL/min on a Licrospher Lichrocart C18 column (125x 4.6 mm, 5 m, Merck, Darmstadt, Germany). The injection volume was 100 µL, and the free amino acids were measured at λ 338 nm. The calibration curve of the amino acids mix solutions was used to identify and compute each amino acid. The amount of each amino acid in each sample was measured in mg per kg.

RESULT AND DISCUSSION

The coconut sap that was tapped at night had a lower ash content than the ash content of the coconut sap that was tapped during the day. The water, protein, lipid and carbohydrate content of coconut sap from night tapping was not significantly different from that of coconut sap from night tapping. The results of the proximate analysis of coconut sap from tapping during the night and day showed in Table 1.

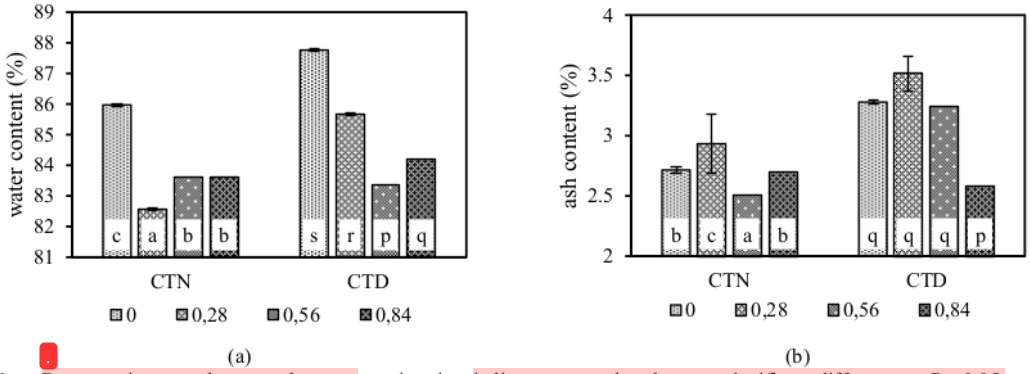
TABLE 1. Proximate composition of coconut sap during different tapping condition.

Proximate composition	CTN	CTD
Water (%)	83.95	85.26
Ash (%db)	2.71[a]	3.16[b]
Protein (%db)	3.09	4.05
Lipid (%db)	0.14	0.14
Carbohydrate (%db)	94.05	92.66

Note: Numbers were followed by different letters mean significantly different at $p<0.05$.

The ash content of coconut sap describes the levels of inorganic compounds, namely minerals, including Ca, P, Na, Fe, K, Mg [20][15]. The high ash content in coconut sap resulting from daytime tapping is caused by absorption of soil minerals by plants that occurs more quickly during the day. According to [21] day and night temperatures affect the absorption of K, Ca and Mg in the test plants.

The water content and ash content of the tapped coconut sap with the addition of variations of mangosteen peel powder are presented in Fig. 1.



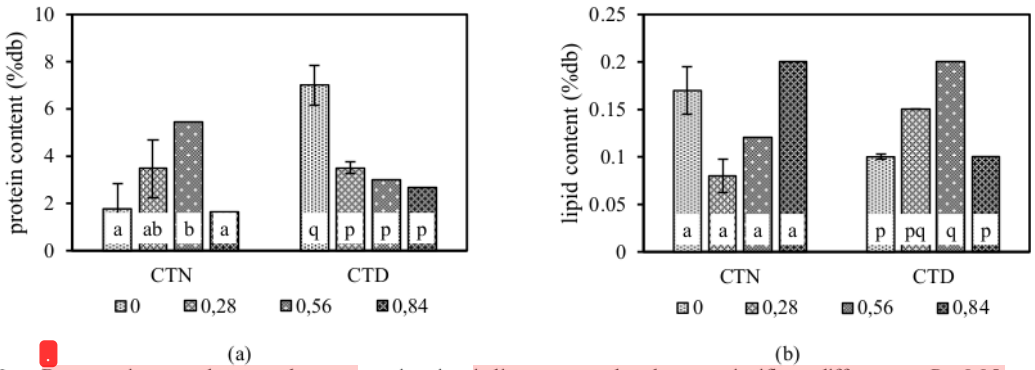
Note: Bars carrying same letters at the same tapping time indicate mean values have no significant difference at $P > 0.05$.

FIGURE 1. Water (a) and ash content (b) of coconut sap tapped at night (CTN) and during the day (CTD), with varied concentrations of mangosteen peel powder added as preservatives.

Fig. 1 shows that the addition of mangosteen peel powder reduces the water content of the sap. The addition of solids will reduce the water content. The sap powder will trap water so that the water content decreased. The addition of mangosteen peel powder with a higher concentration reduced the ash content of coconut sap. This was due to the interaction between the active compounds in the mangosteen peel powder and the minerals present in the sap.

The protein content and lipid content of the tapped coconut sap with the addition of variations of mangosteen peel powder are presented in Fig. 2. Fig. 2 shows the addition of mangosteen peel powder until to 0.56 mg/L increased the protein content of CTD. However, the addition of more mangosteen peel powder resulted decreased in protein content. The rise of the mangosteen peel powder concentration decrease in the ash content of coconut sap resulting from daytime tapping (CTD). This is due to the interaction between the active compounds in the mangosteen peel powder and the protein in coconut sap. According to [22], phenolic compounds can bind proteins effectively at neutral pH.

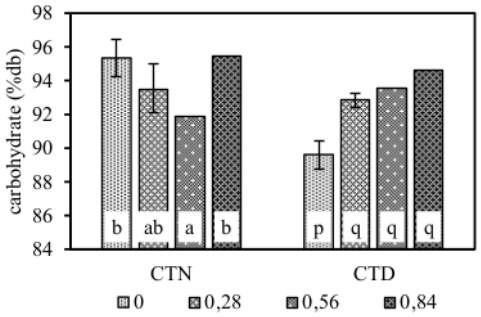
Lipid levels of coconut sap from night tapping (CTN) were not affected by the addition of variations in the concentration of mangosteen peel powder. Meanwhile, the addition of mangosteen rind powder up to 0.56 mg/L to the coconut sap from the day tapping (CTD) showed an increase in lipid levels but decreased with the addition of more mangosteen peel powder. The lipid component in coconut sap is thought to be vitamin A. Coconut sap contains 43 IU of vitamin A [16]. The decrease in lipid levels in sap is thought to be caused by the interaction between the active components in mangosteen peel powder and vitamin A. According to [22] phenolic compounds can interact with hydrophobic components.



Note: Bars carrying same letters at the same tapping time indicate mean values have no significant difference at $P > 0.05$.

FIGURE 2. Protein (a) and lipid content (b) of coconut sap tapped at night (CTN) and during the day (CTD), with varied concentrations of mangosteen peel powder added as preservatives.

Carbohydrate content by difference of tapped coconut sap with the addition of variations of mangosteen peel powder is presented in Fig. 3.

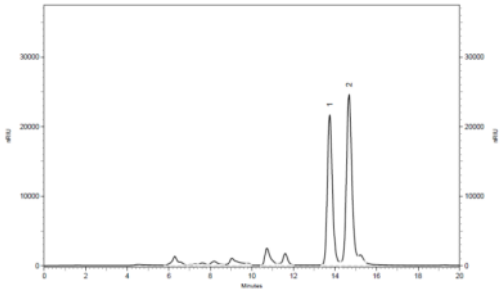


Note: Bars carrying same letters at the same tapping time indicate mean values have no significant difference at $P > 0.05$.

FIGURE 3. Carbohydrate content of coconut sap tapped during nighttime (CTN) and the daytime (CTD) and with addition mangosteen peel powder as preservatives at different concentration

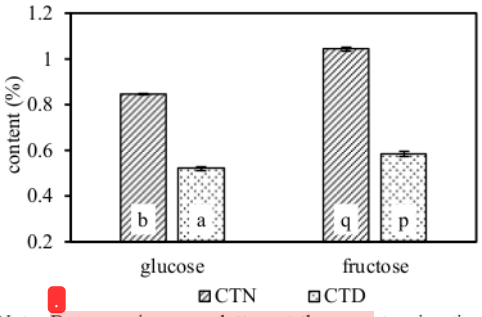
The carbohydrates in coconut sap are dominated by sucrose, glucose and fructose. Coconut sap from night tapping (CTN) and daytime (CTD) contains higher carbohydrates with the addition of mangosteen peel powder. This is due to the ability of mangosteen peel powder to inhibit the conversion of carbohydrates into organic acids. Damage to coconut sap begins with an increase in organic acids resulting from carbohydrate degradation. According to [1] coconut sap can undergo fermentation which is indicated by the presence of organic acids produced from the breakdown of glucose.

The composition of reducing sugar of coconut sap from tapping at night and during the day is presented in Fig. 4.



Note: 1. glucose; 2. fructose

(a)



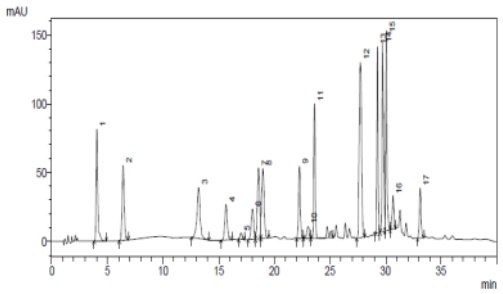
Note: Bars carrying same letters at the same tapping time indicate mean values have no significant difference at $P > 0.05$.

(b)

FIGURE 4. HPLC chromatograms of 30 µl reducing sugar standard mix solution (a) and reducing sugar of coconut sap tapped during nighttime (CTN) and the daytime (CTD) measured by HPLC method (b)

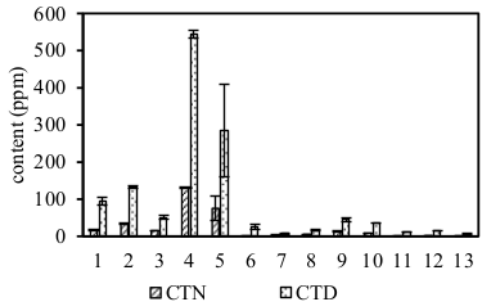
Carbohydrates are the most dominant component of coconut sap. Types of carbohydrates in coconut sap, especially sucrose and reducing sugar. Reducing sugars such as glucose and fructose play an important role in the Maillard browning reaction when the sap is processed into coconut sugar. Maillard reaction was a non-enzymatic reaction between reducing sugars and amino acids to generate the Maillard reaction products (MRPs) [23]. Browning will develop during the heating process. The heating treatment might reduce remarkably the luminosity (L*) because of the formation of brown color product [24]. Fig. 4(b) shows that night-tapping coconut sap (CTN) contains higher glucose and fructose than CTD. This is due to the conversion of sucrose to reducing sugar occurs during night tapping. Tapping sap during the night lasts longer than tapping during the day. According to [25], invertase changes sucrose to glucose and fructose and then oxidized to organic and alcohol.

The amino acid composition of coconut sap from night and day tapping is presented in Fig. 5.



Note: 1. L-asp acid; 2. L-glu acid; 3. L-pro; 4. L-ser; 5. L-his; 6. L-arg; 7. L-gly; 8. L-thr; 9. L-ala; 10. L-val; 11. L-tyr; 12. L-met; 13. L-phe; 14. L-ile; 15. L-leu; 16. Cys; 17. L-lys

(a)



Note: 1. L-asp acid; 2. L-glu acid; 3. L-pro; 4. L-ser; 5. L-his; 6. L-gly; 7. L-thr; 8. L-ala; 9. L-val; 10. L-tyr; 11. L-met; 12. L-phe; 13. L-lys

(b)

FIGURE 5. HPLC chromatograms of 88.16 ppm of amino acid standard mix solution (a) and amino acid of coconut sap tapped during nighttime (CTN) and the daytime (CTD) (b)

Based on the tested CTD and CTN free amino acid chromatograms using HPLC, 13 peaks out of 14 dominant peaks that appear in both CTD and CTN can be identified. Based on Fig, 5(b)., the composition of free amino acids of CTD as much as 1268.02 ppm consists of aspartic acid (94.63 ppm), glutamic acid (133.09 ppm), proline (51.61 ppm), serine (544.05 ppm), histidine (284.45 ppm), glycine (24.51 ppm), threonine (6.93 ppm), alanine (16.36 ppm),

valine (44.67 ppm), tyrosine (35.76 ppm), methionine (10.42 ppm),phenylalanine (14.87 ppm) and lysine (6.67 ppm). The composition of CTN free amino acids is lower at 309.61 ppm consisting of from aspartic acid (17.92 ppm), glutamic acid (33.83 ppm), proline (15.02 ppm), serine (130.55 ppm), histidine (75.62 ppm), glycine (1.15 ppm), threonine (4.28 ppm), alanine (16.36 ppm), valine (5.34 ppm), tyrosine (12.95 ppm), methionine (1.30 ppm), phenylalanine (1.63 ppm) and lysine (1.37 ppm). Total levels of free amino acids sap influenced by the levels of free amino acids in coconut flowers. According to [26], most of the amino acids transported in the vascular system plants become the nutritional needs of other plant organs that do not play a role directly in nitrogen assimilation, e.g. leaves, meristems and reproductive organs, require amino acids to support growth and development. The dominant amino acids in coconut sap are aspartic acid, glutamic acid, serine and histidine.

CONCLUSION

Based on the proximate analysis of coconut sap, the ash content of the CTN was higher than that of the CTD, while the other proximate compositions between the CTN and the CTD were not different. The addition of mangosteen peel powder resulted in a decrease of the water content of coconut sap. The more addition of the mangosteen peel powder, the coconut sap contained less ash content. The addition of 0.56 g/mL of mangosteen peel powder on the CTN resulted in the highest protein and the lowest of carbohydrate, as well as high lipid content in the CTD. Glucose and fructose levels on the CTN were higher than the CTD sap. The CTN and the CTD sap contained 13 types of amino acids with the CTD tend to be higher than the CTN sap. The optimum preservative concentration of mangosteen peel powder was 0.84 g/L of coconut sap which was tapped during daytime.

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