The Chemical Properties and Antioxidant Activity of Coconut Sap at Different Tapping Condition

Sifat-sifat kimia dan aktivitas antioksidan nira kelapa pada kondisi penyadapan yang berbeda

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Abstract

Coconut sap is sweet, oyster white and translucent, obtained by tapping coconut flowers for 8-12 hours. It contains sugar with a nearly neutral pH which are highly susceptible to spontaneous fermentation. To maintain the quality of the sap during tapping process, coconut farmers usually add preservative substances, either natural preservatives, such as mix of lime with mangosteen peel and jackfruit wood, or synthetic preservatives, such as sodium metabisulphite. The chemical properties of coconut sap also considerably affected by weather conditions during the tapping process. This research aims to determine the effect of weather condition during tapping process and variations of mangosteen peel powder concentration on the chemical properties and antioxidant activity of the coconut sap. The results showed that the optimum preservative concentration of mangosteen peel powder was 0.84 g/L from coconut sap which was tapped in sunny weather condition. This treatment yielded sap with chemical properties as follows: pH value 6.7, total soluble solids 16.23 °brix, reducing sugar 0.04 g/100 g, sucrose content 13.64 g/100g, total polyphenol 0.32gGAE/100 g, vitamin C 26.06 mg/100 g. On the other hand, the antioxidant activity of this treatment was similar to that of the addition of and 0.28 and 0.56g/L mangosteen peel powder i.e. 34.39 % RSA evaluated by DPPH method.

Keywords: antioxidant activity, coconut sap, mangosteen peel powder

1. INTRODUCTION

Coconut sap as the raw material of coconut sugar is a sugary exudates that is obtained from young inflorescence of a coconut tree (Cocos nucifera L.) with pH value between 6.0 to 7.0 (Marsigit, 2005). Moreover, this material is rich in nutritious components such as sugar, proteins, vitamins, minerals, etc. Coconut sap is usually collected in a bamboo tube, which is placed at the top of the palm for tapping process at least 8-12 hours (Hebbar et al., 2015). In general, coconut sap will suffer spontaneous fermentation and become alcoholic and acidic due

to microbial activity (Hariharan et al., 2014). Chemical properties of coconut sap usually change during the tapping period including decreasing pH value and sucrose content.

To maintain the quality of the sap during the tapping process, coconut farmers usually add a preservative substance such as lime which is commonly used, to inhibit fermentation. According to Mohammadi and Dummer (2011), lime addition will increase the pH value of coconut sap. On the other hand, it is not effective to be utilized as a single preservative. A combination of several preservatives, thus, is needed to produce more effective inhibitory action. There are natural preservatives, such as mangosteen peel and jackfruit wood, or synthetic preservatives, such as sodium metabisulphite which are usually combined with lime as an antifermented agent. Methyl p-hydroxybenzoate is one of the common synthetic preservatives utilized to inhibit the growing of yeasts, bacteria and fungi.

Hence, this research was carried out in order to inhibit the spontaneous fermentation in freshly-collected coconut sap using a combination of lime and methyl p-hydroxybenzoate during the tapping process. Due to the high pK values, methyl p-hydroxybenzoate is effective as an antimicrobial agent against bacteria, yeasts, and molds. Microorganisms such as Saccharomyces cerevesiae, Candida tropicalis, Enterobacter, Bacillus, and Staphylococcus species might be developed during fermentation (Hariharan et al., 2014). Methyl p-hydroxybenzoate has potential as a suitable preservative to prevent fermentation in coconut sap. Methyl p-hydroxybenzoate has been applied in beverage products at 200 ppm to1000 ppm or 0.1% (Davidson et al., 2005). It is allowed as a food additive at up to 0.1% by weight, according to Addition of Food Preservatives Regulation No. 36 published by BPOM Indonesia (2013).

In general, as a tropical country, weather in Indonesia can be divided into two season i.e. wet and dry. The chemical properties of coconut sap are also influenced by the weather condition. Naknean et al (2010) reported that the composition and quality of sap vary depending on place, time, season and duration of tapping. During summer season, sap will favor the rapid growth of microbial loads. In this research, the chemical properties were pH value, water content, reducing sugar, sucrose content, total polyphenol, vitamin C and antioxidant activity were evaluated at three different weather conditions, namely sunny day, drizzling rain, and heavy rain.

2. MATERIALS AND METHODS

2.1. Materials

The coconut sap was tapped from spathes of coconut cultivar Dalam Bojong Bulat cultivated in Klepu, Hargowilis village, Kokap districts, Kulon Progo regency, Yogyakarta, Indonesia. Coconut sap was collected at various weather conditions, namely sunny day, drizzling rain and heavy rain with relative humidity of 91.5, 91.5 and 93.5%, respectively. Coconut sap used in this research was obtained from 30 March-27 April 2016.

2.2. Chemicals

Rochelle salts, phenol, sodium sulphite, sodium hydroxide, hydrochloric acid, glucose anhydrous, Folin-Ciocalteu reagent, ethanol, gallic acid, natrium carbonate, dipotassium phosphate, potassium dihydrogen phosphate, phosphoric acid, 2,2`-dipyridyl, trichloroacetic acid, ferric trichloride and methanol were purchased from Merck (Darmstadt, Germany). 3,5-Dinitrosalicylic acid, and 2,2-diphenyl-1-picrylhydrazyl were obtained from Sigma-Aldrich (St. Louis, USA).

2.3. Collecting of coconut sap

Spandix of coconut tree was cut and the sap was subsequently tapped and collected in a bamboo tube. The variations of methyl p-hydroxybenzoate concentration are 0, 80, 120, and 160 ppm. This preservative was combined with lime at concentration 0.004 g/mL. At the next step, 20 mL of this mixed preservative was added in the bamboo tube. Tapping was ended after 12 hours and the sap was transferred to the laboratory in a cool box at temperature of 5 °C-8 °C for further chemical analysis.

2.4. Chemical analysis of coconut sap

The analyzed chemical properties of the coconut sap were pH, water content, reducing sugar, sucrose content, total polyphenol, vitamin C and antioxidant activity.

2.4.1. pH value

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

2.4.2. Water content

The water content of coconut sap was measured using thermogravimetric procedure according to AOAC (1995).

2.4.3. Reducing sugar and sucrose content

Reducing sugar and sucrose content were determined as described by modified method of Miller (1959). A volume of 3 mL of coconut sap sample was mixed with 3 mL of 1% 3,5-Dinitrosalicylic acid (DNS). The mixture was heated at 90 ^{O}C in water for 10 minutes and then cooled at room temperature. To stabilize the color of the mixture, an amount of 1 mL of potassium tartrate 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 was used to obtain standard 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.

2.4.5. Total polyphenol

The estimation of the total polyphenol content of the coconut sap was performed according to the Folin-Ciocalteu method with slight modifications (Payet et al., 2005). Five hundred microliters of sample was added with 2.5 mL of 10% Folin-Ciocalteu reagent in a test tube. After incubated at 8 minutes, an amount of 2 mL of 7.5% in water Na_2CO_3 must be added. The sample was then incubated for 1 hours at 30 °C, and the absorbance at 765 nm was measured. For the blank measurement, the sample was replaced by an appropriate solvent which was subtracted from the absorbance at 765 nm. The measurement result was then obtained by reporting the absorbance in the standard curve of gallic acid used as the standard phenol. The results were expressed in milligrams of gallic acid equivalent per milligram of sample (GAE/100 mL of sample).

2.4.6. Vitamin C

One milliliters of coconut sap was put in the Eppendorf tubes, then centrifuged at 5 °C for 20 minutes. Vitamin C content of coconut sap was determined spectrophotometrically according to Samarajeewa et al (1985) procedure. A volume of 0.2 mL of sample was mixed with 0.8 ml of 0.2 mol/L phosphate buffer, 0.8 ml of 43% phosphoric acid, 0.8 ml of 4% 2, 2`-dipyridyl, 1.0 ml of 10% trichloroacetic acid, and 0.4 ml of 3% ferric trichloride. The mixture was then incubated at 42 °C in a water bath for 40 minutes and the absorbance was read at 525 nm using spectrophotometer UV 200.

2.4.7. Antioxidant activity

Antioxidant activity of coconut sap was measured using radical DPPH scavenging activity method (Payet et al., 2005). Two hundred and eighteen milliliters of 0.1 mM DPPH• methanolic solution was pipetted into each tube test followed by 200 µL of sample, or solvent for the blank. The mixture was incubated at 30 °C for 1 hours, and the absorbance at 515 nm was measured with a spectrophotometer. The antioxidant activity was evaluated as percentage of the radical scavenging activity (RSA) calculated using the equation:

$$RSA\ (\%) = \frac{(A0-AS)}{A0} \times 100 \qquad (1)$$

where A0 is absorbance of the blank and AS is absorbance of the sample at 515 nm.

2.5. 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 $P < 0.05$.

3. RESULT AND DISCUSSION

3.1. Reducing sugar, sucrose and total sugar content

Reducing sugar and sucrose content are the properties that influenced the form of coconut sugar. The variety of reducing sugar and sucrose content of coconut sap tapped at different weather conditions and preservative concentrations is tabulated in Table 1.

The sap that used as the coconut sugar raw material had reducing sugar content <0.7 g/100 mL of sap. The existence of reducing sugar content was due to the invertase enzyme presented in the sap. Furthermore, the invertase enzyme catalyzed sucrose hydrolysis to produce invert sugar. Invertase was produced by microorganism in coconut sap, likes from Saccharomyces cerevisiae (Mahmood et al., 2010). Hence, there is a strong correlation between the number of microorganisms and the invertase enzyme. Sap tapped at a sunny day condition would have higher microorganism number that produced invertase rather than that at a rainy day (Naknean et al., 2010).

Coconut sap tapped at sunny day condition with addition of 120 and 160 ppm methyl p-hydroxybenzoate had reducing sugar content of 0.16 and 0.30 g/100 mL. They were then proper to be used as raw material of coconut sugar. However, as seen in Table 1., the reducing sugar fluctuated at different weather conditions (sunny day, drizzling rain, and heavy rain) and preservative concentrations (80 ppm, 120 ppm, 160 ppm). The high value of the reducing sugar indicates a more intensive activity of invert sugar produced by the coconut tree. The fluctuation might occur since the invert sugar was naturally synthesized by the plant during the photosynthesis which produce carbon C3 up to C6 (Xia et al., 2011).

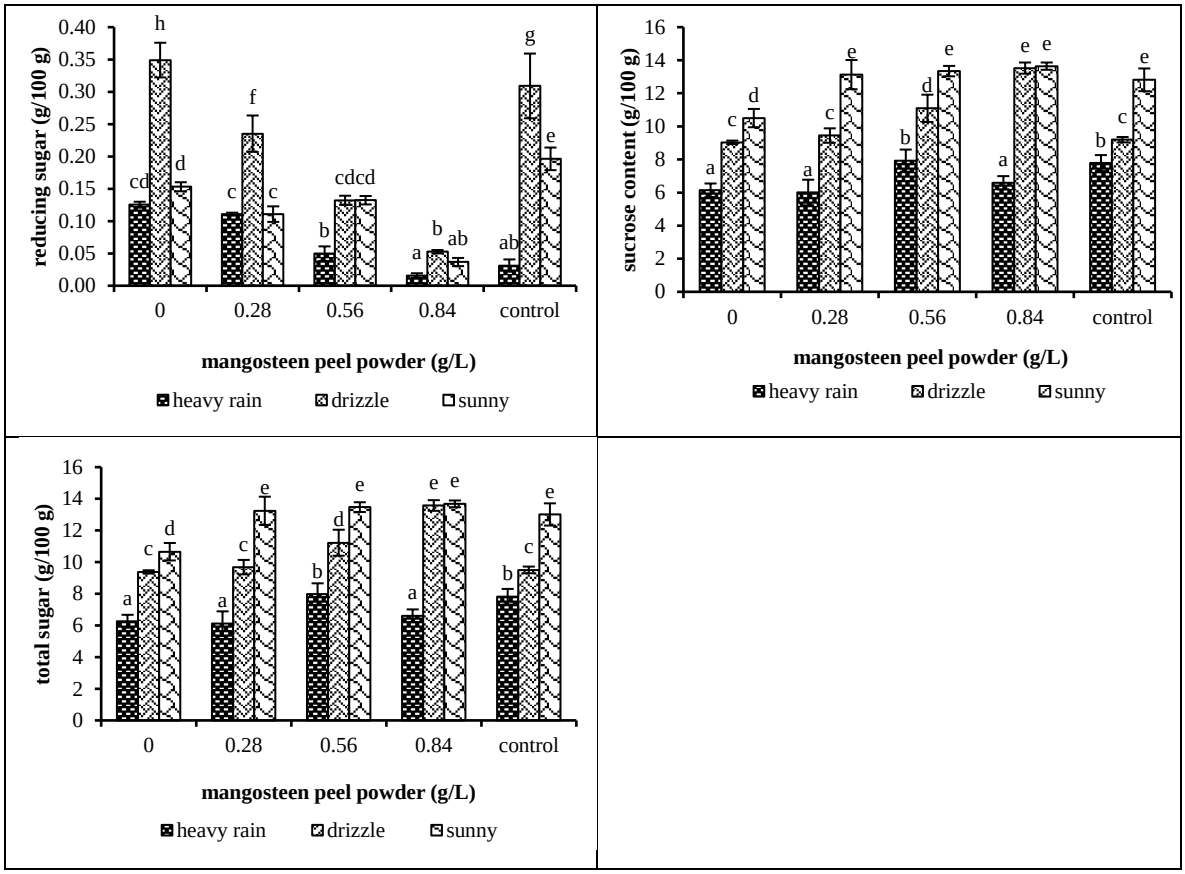

Figure 2. Reducing sugar, sucrose and total sugar content of coconut sap tapped at different weather conditions and variation of mangosteen peel powder concentrations

Sucrose content is one of the critical properties that influenced the coconut sugar appearances. Sucrose is naturally synthesized by the plant. The metabolism of plant was influenced by abiotic component such as temperature, sun radiation, precipitation, and humidity (Zheng et al., 2012 in Lantemona et al., 2013). Sucrose content of coconut sap as the raw material of coconut sugar varies from 15.30 to 17.70 g/100 mL of sap (Samarajeewa, 1979). Moreover, precipitation and humidity give impact to sucrose value of sap. At rainy day, there is lack of sunlight that provides energy for photosynthesis. The sucrose value of sap tapped at sunny day is higher compared to that at rainy day (Lantemona et al., 2013).

As seen in Table 1., at sunny day, coconut sap added with 120 and 160 ppm methyl p-hydroxybenzoate had sucrose content of 15.08 and 18.62 g/100 mL respectively. Such sap are suitable as raw material of coconut sugar. On the other hand, the sucrose of sap tapped at different weather conditions and preservative concentrations had fluctuated number. It might be influenced by the different rate of metabolism activity of each plant. The high value of the sugar content denotes a more intensive activity of sugar synthesis produced by the coconut tree. In addition, Ysidor et al (2014) reported that each coconut cultivar has different capacity of mineral absorption and photosynthesis.

3.2. Total polyphenol, vitamin C and antioxidant activity

The difference of the total polyphenol in coconut sap is caused by either the amount of phenolic compound synthesized during spontaneous fermentation process or microorganism activity. The variety of total polyphenol, vitamin C and antioxidant activity of coconut sap tapped at different weather conditions and preservative concentrations was given in Fig. 2.

A research conducted by Hebbar (2015) shows that total polyphenol in coconut sap varies between 4.80 to 5.40 mg/100 ml GAE. In addition, phenolic compound in coconut sap consists of gallic acid, protocatechuic acid, caffeic acid, p-coumaric acid, and galangin (Xia et al., 2011). The increase of total polyphenol can be caused by a bonding reaction between polyphenol in plants and sugar group, protein, cellulose and starch which will produce glycoside compound. In the spontaneous fermentation during tapping process, the glycoside compound will be degraded into phenolic compound. Moreover, total polyphenol in coconut sap can be affected by the metabolism activity of microorganisms.

The variation of vitamin C in coconut sap can be caused by several reasons, such as, weather conditions, soils and cultivar types of coconut tree. At rainy day, vitamin C of coconut sap tends to be higher than that sap tapped at sunny day. The small value of vitamin C in coconut sap tapped at rainy day might occur, due to high water content. Hebbar (2015) reported that vitamin C content in coconut sap is about 17.5 mg/100 mL.

Chemical compounds which play as antioxidant are polyphenol and vitamin C (Xia et al., 2011). Antioxidant activity in coconut sap varies depending on the condition of weather and soil of the coconut tree plantation area. The antioxidants can prevent biomacromolecule in coconut sap from deterioration due to free radicals. The activity of microorganisms is also able to influence the antioxidant activity since the synthesis of phenolic compound and vitamin C can be affected by metabolism activity of microorganisms.

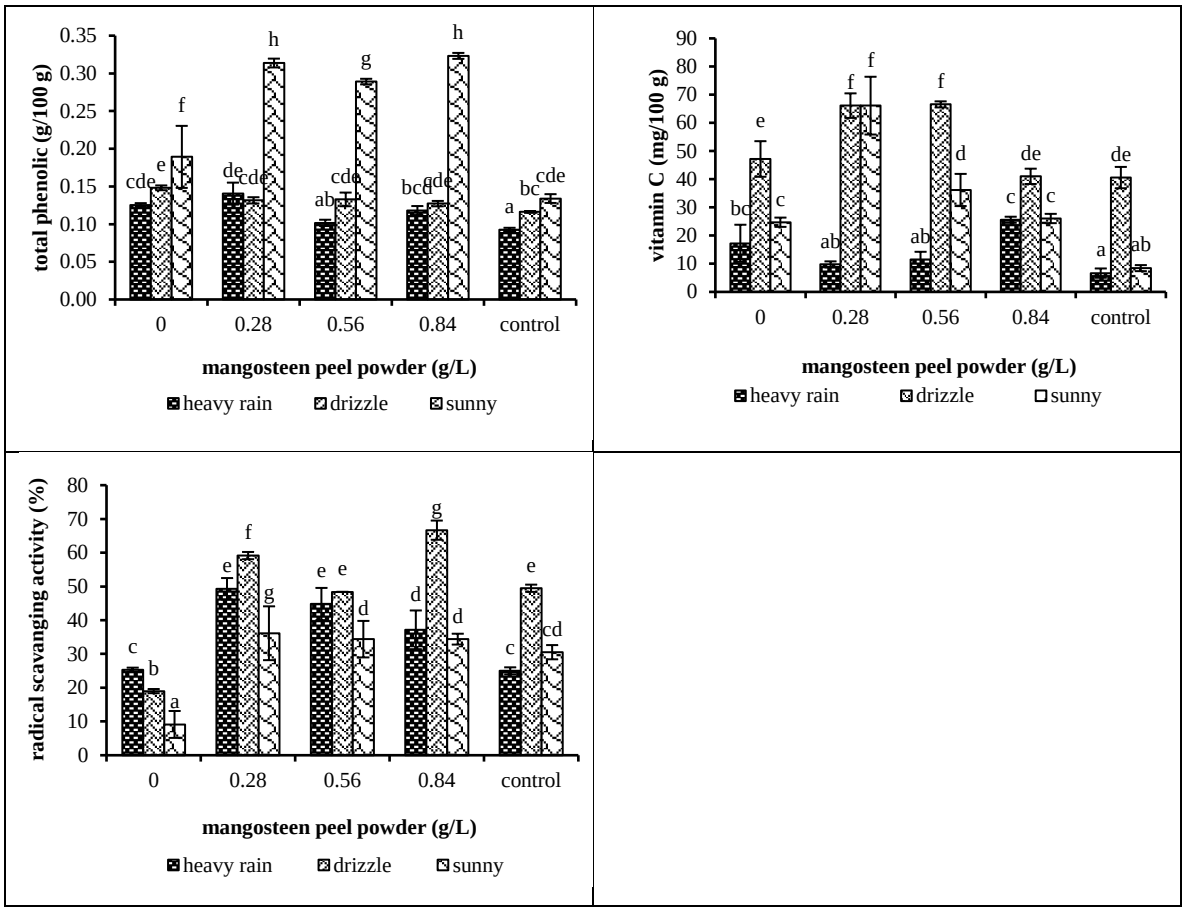

Figure 3. Total polyphenol, vitamin C and antioxidant activity of coconut sap tapped at different weather condition and preservative concentration

Table 1. Pearson Correlation matrix of chemical properties of coconut sap

Chemical properties	pH value	Water content	Total soluble solids	Reducing sugar	Sucrose content	Total free amino acids
pH value	1					
Water content	0.209	1				
Total soluble solids	-0.345*	-0.605**	1			
Reducing sugar	-0.352*	-0.425**	0.527**	1		
Sucrose content	0.036	-0.403**	0.749**	0.004	1	
Total free amino acids	-0.374*	-0.656**	0.778**	0.302*	0.691**	1

Note: **Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

Table 1. Antioxidant properties of coconut sap and the sugar produced

Mangosteen peel powder (g/L)	Weather conditions	Coconut sap			Coconut sugar	
		Total phenolic (g/100 g)	Vitamin C (mg/100g)	Antioxidant activity (%RSA)	Total phenolic (g/100 g)	Antioxidant activity (%RSA)
0	Heavy rain					
	Drizzle					
	Sunny					
0.28	Heavy rain					
	Drizzle					
	Sunny					
0.56	Heavy rain					
	Drizzle					
	Sunny					
0.84	Heavy rain					
	Drizzle					
	Sunny					
control	Heavy rain					
	Drizzle					
	Sunny					

CONCLUSION

The addition of methyl p-hydroxybenzoate can reduce pH, however has no significant effect on water content, reducing sugar and sucrose content. The tapping process at sunny day yields coconut sap with reducing sugar lower than sap tapped at rainy conditions. However, the sucrose content, total polyphenol, vitamin C and antioxidant activity of the sap collected at sunny day are superior to that of the sap obtained at both drizzling and heavy rain. Tapping at sunny day condition with addition of 160 ppm of methyl p-hydroxybenzoate yielded sap with chemical properties as follows: pH value 6.15, reducing sugar 0.16 g/100 mL, sucrose content 18.62 g/100 mL, total polyphenol 211.70 mgGAE/100 mL and vitamin C 27.73 mg/100 mL. On the other hand, the antioxidant activity of this treatment was similar to that of addition of 120 ppm methyl p-hydroxybenzoate i.e. 44.33 % RSA evaluated by DPPH method. Its chemical properties proper as raw material for coconut sugar.

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REFERENCES

AOAC. 1990. *Official Methods of Analysis*, 15 th edn. Association of Official Analytical Chemists Inc., Virginia.

BPOM Indonesia. 2013. *Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia.* Nomor 36 Tahun 2013 tentang Batas Maksimum Penggunaan Bahan Tambahan Pangan Pengawet. Badan Pengawas Obat dan Makanan Republik Indonesia.

Davidson, P.M., J.N. Sofos, A.L. Branen. 2005. *Antimicrobials in Food*. Third Edition. Boca Raton: CRC Press, Taylor and Francis Group.

Hariharan B, K. Singaravadivel, K. Alagusundaram. 2014. Effect of food grade preservatives on the physicochemical and microbiological properties of coconut toddy during fermentation. *Journal Nutritional Food Science,* 4 (5): 1-5.

Hebbar, K.B., M. Arivalagan, M.R. Manikantan, A.C. Mathew, C. Thamban, G.V. Thomas, P. Chowdappa. 2015. Coconut inflorescence sap and its value addition as sugar collection techniques, yield, properties and market prespective. *Current Science*, 109 (8): 1411-1417.

Lantemona H, A.L. Abadi, A. Rachmansyah, J. Pontoh. 2013. Impact of Altitude and Seasons to Volume, Brix Content, and Chemical Composisition of Aren Sap in North Sukawesi. *IOSR Journal of Enviromental Science, Toxicology and Food Technology*, 4 (2): 42-48.

Mahmood, W.A. 2010. Hidrolysis of Sucrose by Immobilized Sacchharomyces cerevisiae Invertase. *Mesopotamia Journal of Agricultural*, 38 (1): 1-10.

Marsigit, W. 2005. Penggunaan bahan tambahan pada nira dan mutu gula aren yang dihasilkan dibeberapa Sentra Produksi di Bengkulu. *Jurnal Penelitian UNIB*, 11(1): 42-48.

Miller, G.L. 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*. 31(3): 426–428.

Mohammadi, Z., Dummer P.M. 2011. Properties and applications of calcium hydroxide in endodentic and dental traumatology. *International Endodentic Journal*, 44(8): 697-730.

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

Payet, B., A.S.C. Sin, J. Smadja. 2005. Aassessment of antioxidant activity of cane brown sugars by ABTS and DPPH radical scavenging assays: Determination of their polyphenolic and volatile constituents. *Journal of Agricultural of Food Chemistry*, 53: 10074-10079.

Samarajeewa U, M.C.P. Wijeratne. 1979. Methods for determining suitability of coconut sap for preparation of jaggery, sugar and golden syrup. *Ceylon cocon. Q*, 30: 72-80.

Samarajeewa, U., D.T. Mathes, M.C.P. Wijeratne T. Warnakula. 1985. Effect of sodium metabisulphite on ethanol production in coconut inflorescence sap. *Food Microbiology*, 2: 11-17.

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

Ysidor, K.N., A.R. Rachel, K.K. J. Louis, O. D. Muriel, A. Prades, A. Kouassi, B.G.H. Marius. 2014. Glucide factors of the inflorescence sap of four coconut (Cocos nucifera L.) cultivars from Cote D'ivoire. *International Journal Biochemistry Reaserch and Review*, 4(2): 116-127.