Chemical and antioxidant properties of Maillard reaction products (MRPs) and melanoidin of coconut sap induced with arginine and histidine generated during the heating process and on the granulated sugar produced

Sifat-sifat kimia dan antioksidan produk reaksi Maillard (MRPs) dan melanoidin nira kelapa dengan penambahan arginin dan histidin yang terbentuk selama pemanasan nira dan pada gula semut yang dihasilkan

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 conducted twice a day, *i.e.* during nighttime and daytime for about 15 and 9 hours, respectively. In order to maintain the quality of the sap during the tapping process, coconut farmers usually add preservative substances. The preservatives used were lime and mangosteen peel powder added at concentration 0; 0.28; 0.56 and 0.84 g/L. The optimum of tapping condition was important to obtain coconut sap which meet the requirements as raw material of coconut sugar. A 2.5 L of coconut sap obtained was then added for each with arginine and histidine, heated with an open process until the end of process *i.e.* the temperature of 118 ºC of sap was reached. The change of chemical and antioxidant properties of coconut sap during heating process and the sugar produced were measured.

This work was divided into three separate experiments. The first experiment was performed to determine the optimum condition of tapping process including the time of tapping and preservatives for obtaining the suitable coconut sap characteristic as raw material of coconut sugar. The second experiment was aimed to evaluate the effects of arginine and histidine on chemical and antioxidant properties of Maillard reaction products (MRPs) formed during heating process of coconut sap. The third experiment was done in order to evaluate the efficacy melanoidin fractions of granulated coconut sugar as antioxidant compounds evaluated using different antioxidant activity methods.

The results showed that 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 coconut sap obtained during daytime was suitable as raw material than coconut sap obtained during nighttime to produce granulated coconut sugar. The optimum concentration of the preservatives added was 0.56 g/L.

The addition of arginine to sap produced granulated coconut sugar with the radical scavenging activity and chelating activity, which were higher than sugar produced from sap without the addition of amino acids. The increase of radical scavenging and chelating activity of sugar produced from sap with arginine addition were contributed by melanoidin fraction 14-50 kDa. This fraction also had the highest phenolic total (45.36 ppm). The addition of histidine to sap produced sugar which had the radical scavenging activity tended to be higher (36.24%) compared to sugar from sap without the addition of amino acid, while the chelating activity of sugar from sap with histidine addition was not significantly different from that without the addition of amino acid. The ability of radical scavenging and chelating activity of sugar from sap with histidine addition was contributed by fraction melanoidin> 50 kDa. This fraction also had the highest phenolic total (31.86 ppm).

Keywords: antioxidant activity; coconut sap; Maillard reaction; mangosteen peel powder; melanoidin

1. Introduction

Coconut sap is sugary exudates, sweet, oyster-white color and translucent, with nearly neutral pH that is obtained from young inflorescence of a coconut tree (*Cocos nucifera* L.) (Borse, Rao, Ramalakshmi, & Raghavan, 2007). Coconut sap can be processed to beverage or as a raw material of coconut sugar. Because of its nutritious, coconut sap will suffer spontaneous fermentation and become alcoholic and acidic due to microbial activity (Hariharan, Singaravadivel, & Alagusundaram, 2014).

Chemical properties of coconut sap usually change during the tapping period *i.e.* decreasing pH value and sucrose content. In order 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. A combination of several preservatives, thus, can produce inhibitory action more effective. Mangosteen peel contains a class of naturally-occurring polyphenol compounds known as xanthonoids like α-mangostin and γ-mangostin. According to Nivetha & Roy (2015), ethanolic extract of mangosteen peel contains high amount of polyphenols (3.717 μg/ml) and flavonoids (2.98 μg/ml) which have capabilities as antioxidant and antimicrobial agents. The whole form of mangosteen peel which was usually used by coconut farmer may cause the liberation of the antibacterial compounds from mangosteen peel into lime milk not optimum. The use of powder form of mangosteen peel, however might liberate the antibacterial compounds more easily than the whole form.

The chemical properties of coconut sap were also influenced by the duration of tapping process. Naknean, Meenune, & Roudaut (2010) reported that the composition and quality of sap vary depending on place, time, season and duration of tapping. In this research, the chemical properties of coconut sap, were evaluated at two different tapping time, *i.e.* daytime and nighttime. The chemical properties of coconut sap will, indeed, affect the granulated form of sugar produced.

Granulated coconut sugar is produced from coconut sap through a lengthy heating process. The typical attribute of coconut sugar quality is the brown color that occurs during the heating process of the coconut sap. The brown color occured during the heating of the sap is caused by Maillard reaction. This reaction is important in the production of sugar *i.e.* its impact to the flavor, color, and aroma (Asikin et al., 2014).

Maillard reaction intensity is commonly influenced by the composition of reactants, *i.e.* reducing sugar and amino acids contained in sap (Nagai, Kai, Tanoue, & Suzuki, 2018), as well as the temperature of heating process (Carciochi, Dimitrov, & Galván D´Alessandro, 2016). According to Ho, Wan Aida, Maskat, & Osman (2008), reactant such as free amino

acids are important as source of amino groups, free ammonia or nitrogen atoms through both deamination and retro-aldol reactions, while monosaccharides such as glucose and fructose play a role in the initial Maillard reaction by forming an abundant pool of high reactive C2, C2 and C4 dicarbonyl compounds.

The addition of arginine and histidine provided more basic amino groups, so the Maillard reaction occured at the alkaline pH created the products via the 2.3-enolization track via 1-deoxy-2,3-dicarbonyl. This route will produce reductone compound that have antioxidant activity. Therefore, it is an important to know the change of chemical and antioxidant properties of coconut sap induced with arginine and histidine which generated Maillard reaction products and melanoidin during heating process and also were found the sugar product. The Maillard reaction products and melanoidin were contributed to antioxidant activity of granulated coconut sugar. Isolation and fractionation of melanoidin from coconut sugar was necessary to find out the efficacy of melanoidin fractions as antioxidant.

Wijewickreme, Krejpcio, & Kitts (1999) reported that the amino acid and reducing sugar in Maillard reaction were contributed on food color, flavor and antioxidant. *In vitro* studies, showed that MRPs may have antioxidant activity as they can role as metal chelators, radical scavengers (J. Kim, 2013a; JS. Kim, 2013b), and inhibition on lipid peroxidation in a linoleic acid emulsion (Jung, Park, Ahn, & Je, 2014). Yan, Yu, & Jing (2018) reported that MRPs derived from chitooligosaccharide and glycine model system exhibited strong antioxidant activity. The addition of this MRPs to fruit juices also increased the antioxidant capacity of these beverages. Karseno, Erminawati, Tri Yanto, Setyawati, & Haryanti (2018) stated that there was a significant correlation between the browning intensity and DPPH radical scavenging activity with a correlation coefficient (r) of 0.93.

The objectives of this research were (1) to determine the effects of tapping condition and preservatives on chemical properties of coconut sap, (2) to explain the chemical properties and antioxidant activity of coconut sap induced arginine and histidine during heating process and the sugar produced and (3) to evaluate the efficacy melanoidin fractions of granulated coconut sugar as antioxidant compounds evaluated using different antioxidant activity methods.

2. Materials and methods

2.1. Materials

The coconut sap was tapped from spathes of tall variety of coconut trees (*Cocos nucifera*, L.) cultivated in Sikapat Village, Sumbang District, Banyumas Regency, Central

Java, Indonesia with altitude of 500-1000 meters above sea level. The tapping process was conducted during the daytime for 9 h (06.00 am-15.00 pm) in fine weather with temperature of 24-27 °C. The relative humidity ranged about 91-92%.

2.2. Chemicals

Several chemicals, *i.e.* potassium hydrogen tartrate, phenol, sodium sulfite, sodium hydroxide, hydrochloric acid, D-glucose, ninhydrin, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, stannous chloride, L-glutamic acid, ethanol, Folin Ciocalteu, sodium carbonate, ammonium thiocyanate, ferrous chloride, hydrochloric acid and trichloroacetic acid were purchased from Merck (Darmstadt, Germany). Ferrozine was procured from Fluka Chemical. Co. (Buchs, Switzerland) while 3,5-dinitrosalicylic acid, 2,2-diphenyl-1-picrylhydrazyl, linoleic acid and thiobarbituric acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. 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 procedure of the tapping process was as follows. The tip of the coconut inflorescence was cut by a sterilized stainless steel knife. A plastic container with the preservatives substance inside was placed covered up the cut inflorescence to collect the coconut sap. The coconut sap collected was measured the pH value and total soluble solid by a portable refractometer (Atago, Japan) immediately. The sap samples which their chemical properties meet the requirement for granulated coconut sugar production were then processed.

2.4. Collecting sap samples during heating and the granulated sugar produced

A 7.5 L of coconut sap was filtered with a filter cloth, then divided into three parts. The first part was sap sample without addition of amino acid, the second and third part were added with 0.8 mM arginine and 0.8 mM histidine, respectively. A 2.5 L of coconut sap from farmers was also used as a control. Each of sap samples were then filled in an aluminium pan, heated by a gas stove, then continuously agitated during the heating process until the temperature of the sap reached 118 °C (for about 50 min). The viscous sap was still agitated at room temperature until the sap crystallized and granulated sugar was formed. The granulated sugar was then dried under sunlight to reduce its water content. Fifty grams of sap

samples was collected when the sap temperature reached 80, 100 and 118 °C during heating treatment. The same weight of granulated sugar produced was also collected subsequently. The sugar product was observed visually afterward for the formation of sugar granule. The sap samples were filled in a glass vial then cooled immediately to discontinue the chemical reaction in the coconut sap. The chemical and antioxidant properties of the sap samples and the sugar produced were then analyzed.

2.5. Isolation and fractionation of melanoidin from granulated coconut sugar

A dialysis membrane separation system with a molecular weight cut off (MWCO) 14 kDa was used as described by Vignoli, Bassoli, & Benassi (2011) with modifications. A soluble sugar solution (100 mL) with a concentration of 30% was prepared. The solution was transferred to the membrane and placed in a beaker with 300 mL distilled water under agitation at room temperature for about 8 h. The material free was lyophilized and used to estimate the mass of the material with molecular weight lower 14 kDa. While the retained on the membrane was then transferred to membrane with MWCO 50 kDa and dialyzed at the same condition with the previous step. The material both free and retained were collected then lyophilized to obtain the material with molecular weight ranged from 14 to 50 and over 50 kDa respectively. The antioxidant activity of all fractions obtained were evaluated using different methods.

2.6. Chemical analysis

Both of the unheated of coconut sap and collected during heating process, and the granulated coconut sugar produced were analyzed its chemical and antioxidant properties, *i.e.* pH value, water content, reducing sugar, sucrose content, total free amino acid, total protein, browning intensity, total phenolic, DPPH radical scavenging activity, Fe2+ chelating activity and inhibition of lipid peroxidation evaluated both by Ferric thiocyanate (FTC) and Thiobarbituric acid reactive substances (TBARs) methods.

2.6.1. pH value

The pH value 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 °C.

2.6.2. Water content

The water content of coconut sap was measured using thermogravimetric procedure according to AOAC (1990).

2.6.3. Reducing sugar, total sugar and sucrose content

Reducing sugar, total 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 then heated at 90 ^{O}C in water for 10 minutes and subsequently 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 by incubating with 3 mL of 25 % 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.

2.6.4. Total free amino acid content

The amino acid was measured by applying the method of Yao et al., (2006). A weight of 1.0 g of coconut sap, 0.5 mL of buffer solution and 0.5 mL of ninhydrin solution were placed in a 25-mL volumetric flask and the flask was subsequently heated in a boiling water bath for 15 minutes. The flask was then cooled at room temperature for 5 minutes and the solution in the flask was diluted by 25 mL of distilled water. The absorbance of the diluted solution was measured using a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific; Carlsbad, CA, USA) at 570 nm. Glutamic acid was used to prepare the standard curve to quantify the samples.

2.6.5. Total phenolic

The estimation of the total phenolics content of the coconut sap was performed according to the Folin-Ciocalteu method with slight modifications (Payet, Sing, & Smadja, 2005). Thirty microliters of sample was added with 150 µL of 10% Folin-Ciocalteu reagent in a test tube. After incubated for 8 minutes, an amount of 120 µL of 7.5% Na_2CO_3 dissolved in distilled water must be added. The sample was then incubated for 1 hour 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 phenolic compound. The results were expressed in milligrams of gallic acid equivalent per 100 gram of sample (mgGAE/100 g of sample).

2.6.6. Browning intensity

The browning color of coconut sap samples during heating process were measured according to the method of Ajandouz, Tchiakpe, Ore, Benajiba, & Puigserver (2001) with

slight modification. The sap samples were adjusted with distilled water (1:25 w/v), then centrifuged at 1006 g for 15 min. The absorbance of browning was measured using a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific; Carlsbad, CA, USA) at 420 nm, as an index of the brown polymers formed in more advanced stages.

2.6.7. DPPH radical scavenging activity

DPPH radical scavenging activity of coconut sap was measured using the method of Payet et al. (2005). A volume of 280 µL of 0.1 mM DPPH• methanolic solution was pipetted into each tube test followed by 20 µL of sample, or solvent for the blank. The mixture was subsequently incubated at room temperature for 30 minutes, and the absorbance at 515 nm was then measured with a spectrophotometer. The antioxidant activity can be evaluated as a percentage of the radical scavenging activity (RSA) using the following equation:

$$\text{RSA (\%)} = \frac{Ao-As}{Ao} \text{ x } 100 \quad \text{..... (1)}$$

where Ao is absorbance of the blank and As is absorbance of the sample at 515 nm after 30 minutes.

2.6.8. Fe^{2+} chelating activity

The ability of sap samples to chelate the metal ions Fe^{2+} was investigated according to Kim (2013). One gram of sap samples with 5-fold dilution was prepared, then filtered with filter paper to obtain sap solution. One hundred microliters of coconut sap solution was added with 600 µL of distilled water and 100 µL of 0.2 mM $FeCl_2 \cdot 4H_2O$. The mixture was allowed to rest at room temperature for 30 s. The reaction mixture, which contained 100 µL of distilled water instead of sample, was served as the control. The reaction mixture was then added with 200 µL of 1 mM ferrozine and its changes in color were monitored at 562 nm with a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific; Carlsbad, CA, USA) after 10 min of resting time at room temperature. The Fe^{2+} chelating activities were calculated using the following equation:

$$Chelation\ activity\ (\%) = \frac{Ao-As}{Ao}\ x\ 100 \quad \text{..... (2)}$$

where, Ao is the absorbance of the control and As is the absorbance of the sample after 10 min incubation.

2.6.9. Inhibition of lipid peroxidation using linoleic acid model system

a. Ferric thiocyanate (FTC) method

The methods of Kikuzaki & Nakatani (1993) was used. A mixture of 4 mL of a weighed sample in 99.5% ethanol, 4.1 mL of 2.51% linoleic acid in 99.5% ethanol, 8 mL of 0.05M phosphate buffer (pH 7.0) and 3.9 mL of water was placed in a vial 60 mL with a screw cap and then placed in an oven at 40°C in the dark. To 0.1 mL of this solution was added 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate. Precisely 3 min after addition of 0.1 mL of 0.02 M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of red color was measured at 500 nm every 24 h until one day after absorbance of the control reached maximum.

b. Thiobarbituric acid reactive substances (TBA) method

The method of Kikuzaki & Nakatani (1993) was used. To 1 mL of sample solution, prepared and incubated as described, was added 2 mL of 20% trichloroacetic acid aq. and 2 mL of TBA in 95% acetic acid (glacial) solutions. This mixture was placed in a boiling water bath for 10 min and, after cooling, was centrifugated at 3000 rpm for 20 min. Absorbance of supernatant was measured at 532 nm. Antioxidant activity based on absorbance on the final day.

2.7. Statistical Analysis

The data of chemical properties and antioxidant activity were statistically analyzed using IBM SPSS Statistic 20 and reported as mean ± standard deviation (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. Chemical properties of coconut sap as raw material of coconut sugar

In the previous research by Haryanti, Supriyadi, Marseno, & Santoso (2017), eight sap samples of coconut sap tapped during nighttime (CSN) and coconut sap tapped during daytime (CSD) added with mangosteen peel powder as preservative were processed into granulated coconut sugar form. The value of pH, total soluble solids, water content, reducing sugar and sucrose content of coconut sap tapped at different tapping condition as well as the possibility of granulated sugar formation are given in Table 1.

Table 1. Chemical properties of coconut sap tapped at different tapping condition on the formation of granulated coconut sugar

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	(-)

Note: (+) and (-) signs indicate that granulated coconut sugar was formed and was not formed, respectively.

As seen in Table 1., not all of the sap samples can be processed further into granulated form as indicated by (-) symbol. It can be seen that seven of eight CSD samples (87.5%) and three of eight CSN samples (37.5%) can be processed into granulated sugar.

The pH of CSD is higher (7.2-8.7) than the CSN (6.0-7.1). The longer the period of the tapping process, the higher the organic acid content would be. 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. The increment of mangosteen peel powder concentration tends to increase the pH value of both CSN and CSD. The mangosteen peel powder shows 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, Sophanodora, Pisuchpen, & Phongpaichit, 2010; Pedraza-chaverri, Cárdenas-rodríguez, Orozco-ibarra, & Pérez-rojas, 2008).

Total soluble solid of CSD is 15.1-16.9 °brix, which is superior to that of CSN (8.4-16.9). It is well known that the total soluble solid of coconut sap is dominated by sugars component especially sucrose which is synthesized by photosynthesis. During night, the photosynthesis process cannot be completed due to less sunlight intensity. As for the effect of the preservatives, the variations of mangosteen peel powder concentrations could prevent the

inversion of sucrose during daytime. Because of the total soluble solid of CSD was higher than that of CSN, the water content of CSD was lower (79.57-87.65%) than that of CSN (85.76–91.30%).

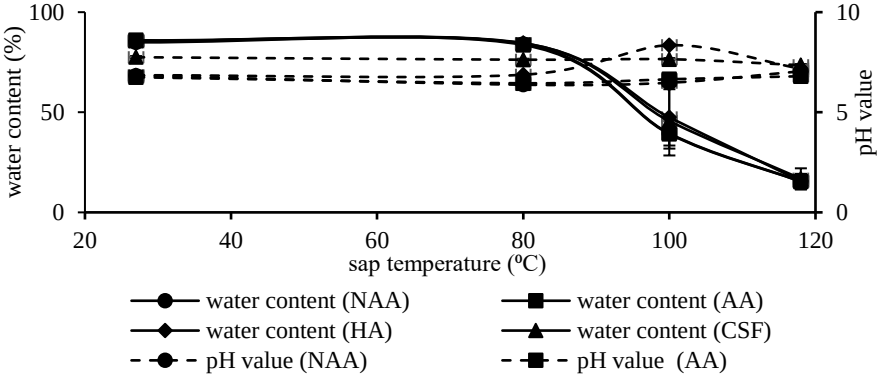
As seen in Table 1, the reducing sugar of CSD ranged from 0.03 to 0.47 g/100 g, which was lower than that of CSN (0.14-1.15 g/100 g). The reducing sugar of both CSD and CSN also decreased if the use of mangosteen peel powder increased. Using the more preservative will inhibit the invertase reaction that converts sucrose to reducing sugar. The sucrose content of CSD and CSN varied from 10.39–16.05 and 7.54-13.29 g/100 g, respectively. The sucrose content of CSN was lower than that of CSD since the less intensity of sunlight at night may cause the photosynthesis is not optimum. According to El-Naggar & Swedan (2009), the less intensity of sunlight reduced total nonstructural carbohydrate content, especially sucrose. Sucrose content has significant effects on coconut sugar production.

The sucrose inversion to glucose and fructose during tapping contributed in inhibiting the sugar crystallization during processing (Samarajeewa & Wijeratne, 1979). 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 6.6, 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.

3.2. The change of chemical properties of coconut sap during the granulated coconut sugar processing

3.2.1. The pH value and water content

The pH value and water content of coconut sap without addition of amino acids (NAA), added with arginine (AA), histidine (HA) and coconut sap from farmers (CSF) as control are shown in Fig.1.



Note: NAA=no amino acid addition; AA=arginine addition, HA=histidine addition and CSF=coconut sugar farmers

Fig. 1. The pH value and water content of coconut sap during heating process

According to Fig. 1, the initial pH of coconut sap ranged from 6.8 to 7.8. The pH of NAA, AA and HA tended to increase during heating process. The addition of arginine and histidine in sap resulted in a great increase of the pH during heating (slope= 0.0086), in contrast to the increment of pH value during heating of NAA (slope=0.0005). The pH value of coconut sap from farmers (CSF) however, tends to decrease.

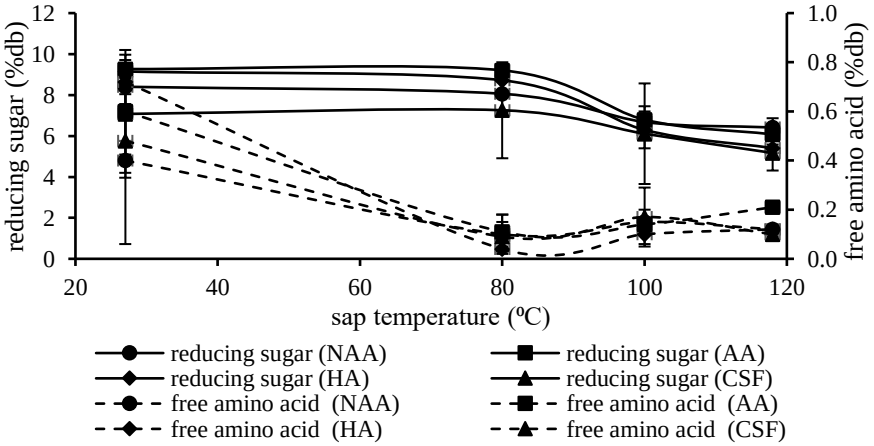
Arginine and histidine are the polar amino acids charged side chains. Addition of arginine 0.8 mM resulted in the increase of arginine content in sap *i.e.* 24.33 ppm from 10.70 ppm of NAA. The addition of histidine 0.8 mM increased the histidine content of sap *i.e.* 22.31 ppm from 18.70 ppm of NAA. According to Ho et al., (2008), the slightly increase of pH value during initial heating was caused by decarboxylation and CO_2 evolution in the reaction system. Furthermore, the longer the heating, the pH decreased due to the loss of alkaline amino groups and the formation of acidic Maillard reaction products namely glyoxal, pyruvaldehyde and furfural. The addition of arginine and histidine provided more basic amino groups, so the Maillard reaction occured at the alkaline pH created the products via the 2.3-enolization track via 1-deoxy-2,3-dicarbonyl. Maillard reaction products formed in this pathway are furanones, C-methylreductone, and α-dicarbonyls (Nursten, 2005).

The initial water content of the sap ranged from 84.95-85.98%. The water content of NAA, AA, HA and CSF decreased during heating to reach water content of 15.33-16.85%. The decrease of water content for all treatments was not significantly different (slopes were not significantly different). The high water content at the initial of heating is a favorable condition for the mobility of the reactants. Moreover, the decrease of water content in further heating is a positive condition for the formation of volatile compounds, namely pyrazine. According to Ho et al. (2008), high water content at the beginning of heating was needed for

the production of free ammonia or nitrogen atoms and dicarbonyl compounds as precursors for Maillard reactions through retro-aldol reactions and deamination.

3.2.2. Reducing sugar and free amino acid content

The reducing sugar and free amino acid content of coconut sap without addition of amino acids (NAA), added with arginine (AA), histidine (HA) and coconut sap from farmers (CSF) as control are shown in Fig.2.



Note: NAA=no amino acid addition; AA=arginine addition, HA=histidine addition and CSF=coconut sugar farmers

Fig. 2. Reducing sugar and free amino acid of coconut sap during heating process

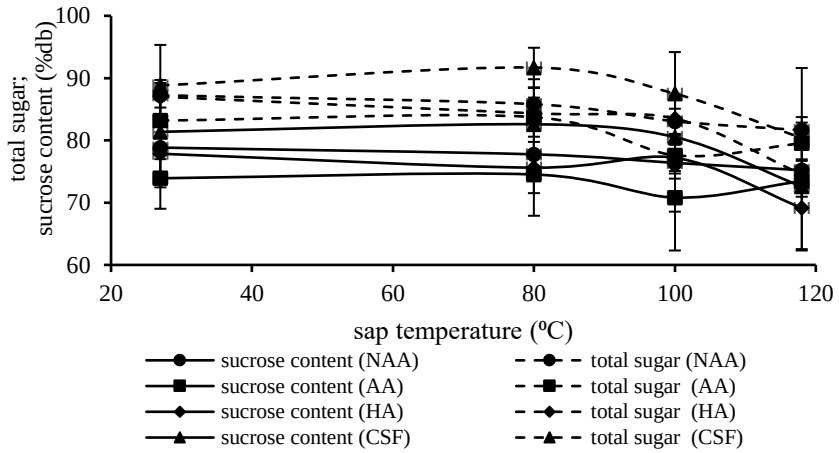
Fig. 2 shows that the reduction of NAA, AA, HA and CSF sugar levels decreased during heating, however, the reduction of reducing sugar levels in all treatments was not significantly different (slope was not significantly different). The reduced sugar content of sap before heating ranged from 7.08-8.40% db and it decreased until the end of heating reached 5.17-6.42% db. The reduction of reducing sugar was caused by the Maillard reaction that occurs during heating of the sap. As previously explained, sap has high moisture content ranging from 84.95 to 85.98%. Reduction sugar becomes more reactive in conditions of high water content. According to Ho et al. (2008), monosaccharides such as glucose and fructose play a role in the initial Maillard reaction by forming an abundant pool of high reactive C2, C2 and C4 dicarbonyl compounds.

The free amino acid levels of NAA, AA, HA and CSF decreased during heating, however, the decrease of free amino acid levels in all treatments was not significantly different (slope was not significantly different). Free amino acids play a role in the Maillard reaction. Free amino acids are a source of amino groups, free ammonia or nitrogen atoms through both deamination and retro-aldol reactions (Ho et al., 2008). This reaction will take

place effectively in the sap at the beginning of the heating because the water content is still high.

3.2.3. Sucrose content and total sugar

The sucrose content and total sugar of coconut sap without addition of amino acids (NAA), added with arginine (AA), histidine (HA) and coconut sap from farmers (CSF) as control are shown in Fig.3.



Note: NAA=no amino acid addition; AA=arginine addition, HA=histidine addition and CSF=coconut sugar farmers

Fig. 3. Sucrose and total sugar of coconut sap during heating process

Fig. 3 shows that the levels of sucrose and total sugar in coconut sap NAA, AA and HA decreased slightly while CSF sap increased slightly but four of the sap samples had a significantly different slope. The sucrose level of coconut juice decreases from 73.92-81.37 to 69.15-75.22% db at the end of heating. Total sugar content of NAA, AA, HA and CSF sap experienced a slight decrease during heating. The decrease in total sugar content of all four samples did not different significantly. The total sugar content of coconut sap decreased from 83.19-88.82 to 74.56-81.63% db at the end of heating.

The total sugar in coconut sap consists of sucrose and reducing sugars, especially glucose and fructose. Reduction sugar is a monosaccharide that is more reactive than sucrose which is a disaccharide especially at the beginning of heating the sap which is still high in water content. The decrease in total sugar at the beginning of the heating of the sap is due to the loss of the reducing sugar used for the amino carbonyl reaction. Longer heating resulted in increased sap temperature. Such condition resulted in the occurrence of hydrolysis of sucrose slowly. According to Ashrafi et al. (2011), sucrose needed a presence of hydrochloric acid or invertase (Mahmood 2010; Siddiqui 2010) as a catalyst to experience hydrolysis. Ho et al.

(2008) reported that sucrose concentration showed an extreme reduction occurred during the first 180 min of heating and almost constant concentration thereafter.

3.3. The chemical properties of granulated coconut sugar

The chemical properties of granulated coconut sugar made from coconut sap without addition of amino acids (NAA), added with arginine (AA), histidine (HA) and coconut sap from farmers (CSF) as control are shown in Table 2.

Table 2. Chemical properties of granulated coconut sugar produced from coconut sap added with arginine and histidine

Granulated coconut sugar	Chemical properties of granulated coconut sugar					
	pH	Water content (%)	Reducing sugar (%db)	Free amino acid (%db)	Sucrose content (%db)	Total sugar (%db)
NAA	7.65	9.48	6.19	0.12	73.77	79.96
AA	7.38	6.63	5.70	0.11	71.73	77.87
HA	7.25	6.22	5.32	0.12	74.08	79.48
CSF	7.35	7.83	4.00	0.12	75.40	80.68

Note: NAA=no amino acid addition; AA=arginine addition, HA=histidine addition and CSF=coconut sugar farmers

As seen in Table 2, the pH values of granulated coconut sugar produced from NAA, AA, HA and CSF were significantly different. Addition of arginine and histidine resulted in sugars with lower pH (7.25-7.38) than without addition of amino acids (7.65) but still in a neutral pH range. The addition of arginine and histidine produced sugar with normal taste that was still in accordance with SNI 01-3743-1995 regarding the quality requirements of palm sugar. The water content of granulated coconut sugar formed from NAA, AA, HA and CSF ranged from 6.22 - 9.48%, however, they were not significantly different. The water content of granulated coconut sugar produced were higher than that required by SNI (maximum 3%). The water content of the sugar can be minimized by artificial or sun drying to prolong its shelf life.

Reducing sugar levels of AA and HA were not different from NAA sugar, which ranged from 5.31-6.19% db, but three of the samples were different from CSF sugar reduction sugar levels (4.00% db). Sugar AA and HA had 5.70 and 5.31% db of reducing sugar which were in accordance with the requirements of SNI 01-3743-1995, which is a maximum of 6%. The levels of free amino acids in sugar produced in NAA, AA, HA and CSF were also not significantly different, ranging from 0.11-0.12% db.

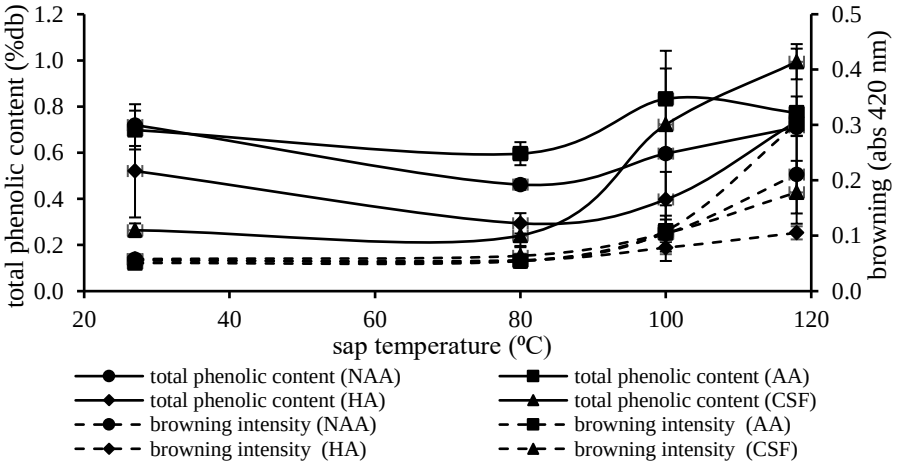
The levels of sucrose and total sugar in sugar produced from NAA, AA, HA and CSF juice were not significantly different. The minimum level of sucrose required for palm

granule sugar is at least 90%. The levels of sucrose of NAA, AA, HA and CSF sugar ranged from 71.73 to 75.40% db and were still under SNI requirements while the total sugar content of sugar produced from NAA, AA, HA and CSF ranged from 77.43 to 80.81% db.

3.4. The change of antioxidant properties of coconut sap during the granulated coconut sugar processing

3.4.1. Total phenolic content and browning intensity

The phenolic content and browning intensity of coconut sap without addition of amino acids (NAA), added with arginine (AA), histidine (HA) and coconut sap from farmers (CSF) as control are shown in Fig.4.



Note: NAA=no amino acid addition; AA=arginine addition, HA=histidine addition and CSF=coconut sugar farmers

Fig. 4. Total phenolic and browning intensity of coconut sap during heating process

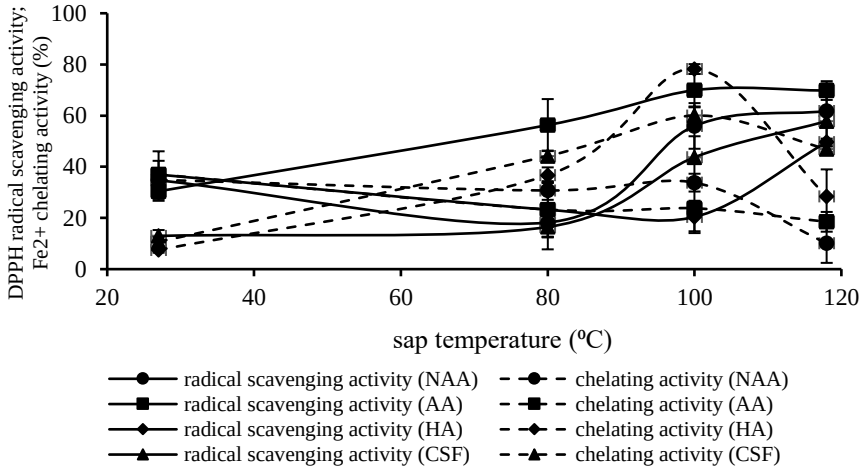
Fig. 4. shows that the addition of arginine (AA) and histidine in coconut sap (HA) before heating process might cause the decrease of total phenolic content to 0.70 and 0.52% db, respectively, compared with NAA (0.72% db). The phenolic compound might interact via the covalent attachment to the protein (Rohn et al. 2004). The total phenolic content at the initial heating of sap (temperature of 80 ºC) decreased and tended to increase at the longer duration of heating. The increment of total phenolic content of AA and HA had a tendency to increase considerably during heating although the increment was not different from that of NAA. Total phenolic content of CFS increased significantly compared to the increase in AA and HA.

Browning intensity of NAA, AA, HA and CSF increased during heating. The addition of arginine might cause a significant increase in browning, however it was not different from

NAA and CFS. The addition of histidine resulted in lower intensity of browning compared to AA. The brown color might be formed through Maillard reaction. The neutral and alkaline pH of coconut sap was suitable condition to accelerate C2 and C3 fragment from sugar degradation which have high reactivity. The sugar degradation products were then polymerized and link by amino compounds built up the brown color product *i.e.* melanoidin (Ho et al., 2008).

3.4.2. DPPH radical scavenging activity and Fe^{2+} chelating activity

The radical scavenging activity and chelating activity of coconut sap without addition of amino acids (NAA), added with arginine (AA), histidine (HA) and coconut sap from farmers (CSF) as control are shown in Fig.5.



Note: NAA=no amino acid addition; AA=arginine addition, HA=histidine addition and CSF=coconut sugar farmers

Fig. 5. DPPH radical scavenging and Fe^{2+} chelating activity of coconut sap during heating process

Fig. 5. shows that the addition of arginine and histidine before heating caused the radical scavenging activity (RSA) of sap not different from NAA which ranged from 30.44-36.95%. The RSA of AA significantly increased during the process (slope 0.4637) compared to that of HA (slope 0.0373). The decrease of RSA in sap sample was caused by phenolic degradation during initial heating. The increased of RSA at the longer duration of heating might be caused by chemical reactions, such as Maillard reaction at early and intermediate stages. There was a high correlation between the DPPH radical scavenging activity on the MRPs after 24 h heating and the contents of total phenolics with r = 0.8833 (Nagai et al. 2018). It suggested that the melanoidins, one of the MRPs contributed to the increase of the activities during heating. The Maillard reaction at intermediate stage produces compounds in

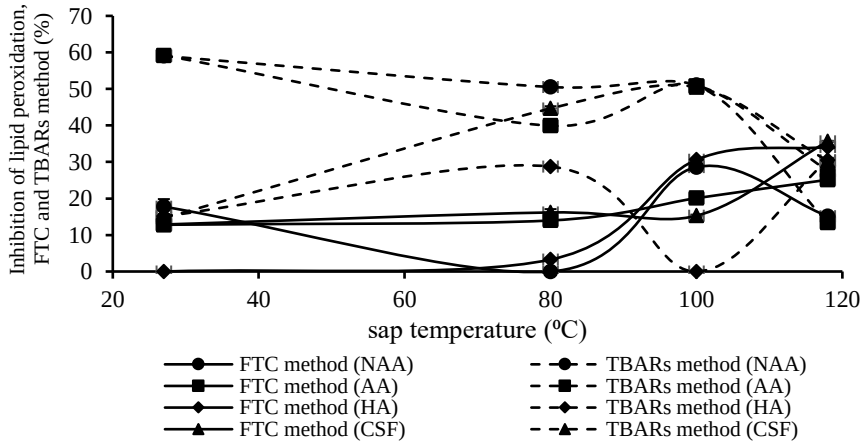
498 which one of them is reductone that has antioxidant properties (Wang et al. 2011). The
499 Maillard reaction products and melanoidin contained phenolic compound that was
500 incorporated during heating treatment (Brudzynski and Miotto 2011). Moreover, the
501 increment of the RSA might be caused by the increment of browning intensity of coconut sap
502 during heating process.

503 Fe^{2+} chelating activity of coconut sap with the addition of arginine and histidine before
504 heating resulted in different chelating abilities *i.e.* 37.05 and 7.49% respectively. The different
505 activity might be caused by different reactivity between arginine or histidine and phenolic
506 compounds in sap. The highest chelating activity can be found in all of the sap samples at a
507 temperature of 100 ºC and decreased when heating was continued. When the sap temperature
508 reached 100 ºC, the intermediate of Maillard reaction may occur and produce MRPs. Until the
509 end of heating (temperature 118 ºC) chelating activity of HA was higher (28.44%) than that of
510 AA (18.50%).

511 **3.4.3. Inhibition of lipid peroxidation using linoleic acid system**

512 The inhibition of lipid peroxidation evaluated using Ferric thiocyanate (FTC) and
513 Thiobarbituric acid reactive substances (TBARs) methods of coconut sap without addition of
514 amino acids (NAA), added with arginine (AA), histidine (HA) and coconut sap from farmers
515 (CSF) as control are shown in Fig. 6.

516



517 Note: NAA=no amino acid addition; AA=arginine addition, HA=histidine addition and CSF=coconut sugar
518 farmers

519 Fig. 6. Inhibition of lipid peroxidation of coconut sap during heating process evaluated with
520 FTC and TBARs methods

521

522 Fig. 6 shows that the increased ability of inhibition of lipid peroxidation of four sap
523 samples were significantly different. The ability of inhibition of lipid peroxidation of HA and
524 NAA decreased during the initial heating of coconut sap, however increased at the longer

duration of heating especially for HA sample. The addition of histidine to the sap increased the ability of sap to inhibit lipid peroxidation at the initiation and propagation reaction (slope 0.3924) evaluated using the FTC method. The addition of histidine to the sap also increased the ability to inhibit lipid peroxidation at the termination reaction (0.2606 slope) evaluated using the TBARs method. Intermediate product of Maillard reaction from HA sap or its polymer which was called melanoidin produced during heating, might be slightly non-polar, it made more soluble in linoleic acid so it is effective for inhibiting oxidation of linoleic acid. According to Borrelli, Visconti, Mennella, Anese, & Fogliano (2002), the inhibition mechanism of linoleic acid peroxidation depends on the polarity of melanoidin and its solubility in the linoleic system because it affects the surface tension of linoleic acid micelles dispersed in the water phase which will affect linoleic acid oxidation.

3.5. The antioxidant properties of granulated coconut sugar

The chemical properties of granulated coconut sugar made from coconut sap without addition of amino acids (NAA), added with arginine (AA), histidine (HA) and coconut sap from farmers (CSF) as control are shown in Table 3.

Table 3. Antioxidant properties of granulated coconut sugar produced from coconut sap added with arginine and histidine

Granulated coconut sugar	Antioxidant properties of granulated coconut sugar					
	Total phenolic (%db)	Browning intensity (abs 420)	Radical scavenging activity (%)	Chelating activity (%)	Inhibition lipid peroxidation (FTC method, %)	Inhibition lipid peroxidation (TBARs method, %)
NAA	0.84	0.25	32.74	50.06	16.13	29.63
AA	0.86	0.44	34.84	53.98	61.97	26.76
HA	0.80	0.27	36.24	44.44	7.01	36.85
CSF	0.72	0.27	29.36	61.23	29.72	42.61
EDTA 20 ppm	Na	Na	Na	61.79	Na	Na
BHT 100 ppm	Na	Na	74.95	Na	90.26	91.21
Vitamin C 800 ppm	Na	Na	85.05	Na	28.22	48.98

Note: NAA=no amino acid addition; AA=arginine addition, HA=histidine addition and CSF=coconut sugar farmers; Na=not analyzed

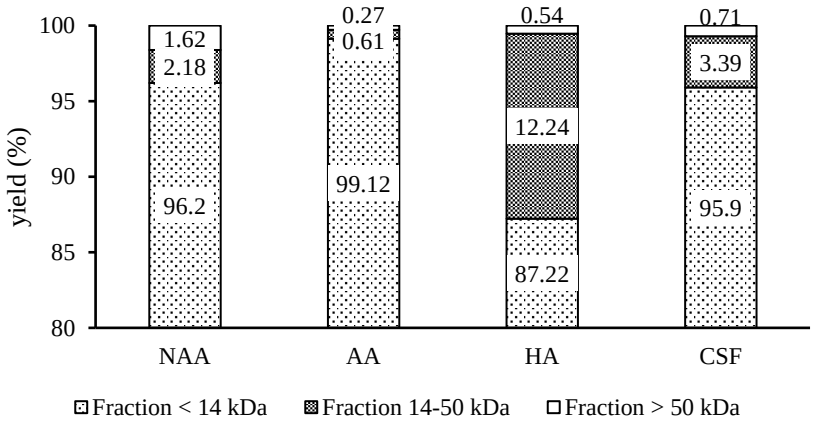
Total phenolic content of granulated coconut sugar from NAA, AA, HA and CSF sap were not different significantly with range 0.72-0.86% db. Browning intensity of sugar produced from NAA, AA, HA and CSF sap were not significantly different, ranged from 0.25-0.44. The RSA of NAA, AA, HA and CSF sugar were not different significantly with ranged of 29.36-36.24%. The addition of arginine and histidine tends to increase the browning

intensity and radical scavenging activity (RSA) of coconut sugar. Chelating activity of sugar produced from NAA, AA and HA, which ranged from 44.44-53.98%, were not significantly different but AA sugar has chelating ability of 53.98% which was not different from CSF sugar (61.23%) and was similar with chelating activity of EDTA 20 ppm (61.79%). The sugar produced from the AA sap had a lipid peroxidation inhibitory capability at the initiation and propagation reaction (61.97%) whereas the sugar produced from HA sap had the ability to inhibit lipid peroxidation at the termination step (36.85%).

3.6. Yield and antioxidant properties of melanoidin fractions from granulated coconut sugar

3.6.1. Melanoidin fractions yield

Melanoidin fractions yield of granulated coconut sugar made from coconut sap without addition of amino acids (NAA), added with arginine (AA), histidine (HA) and coconut sap from farmers (CSF) as control are shown in Fig. 7.



Note: NAA=no amino acid addition; AA=arginine addition, HA=histidine addition and CSF=coconut sugar farmers

Fig. 7. Melanoidin fractions yield of granulated coconut sugar

Fig. 7. shows that the sugar produced from AA sap increased the percentage of melanoidin fraction <14 kDa (99.12%), while the addition of histidine to sap (HA) produced sugar with a percentage of melanoidin fraction greater than 14-50 kDa (12.24%). Melanoidin fraction (MCWO> 14 kDa) obtained from NAA, AA, HA and CSF sugar were 3.44; 0.78; 11.46 and 3.52%, respectively. The fourth sugar samples showed a greater composition of non-melanoidin fractions (MWCO< 14 kDa). This showed that in the processing of sap into coconut sugar, the Maillard reaction occurred was the intermediate stage reaction which

produced MRPs and low molecular weight melanoidin. Granulated coconut sugar was formed by heating the coconut sap up to 118 ºC. At a sap temperature of 118ºC, polymerization of the intermediate products of Maillard reaction were not completed. According to Ames (1990), the brown color product *i.e.* melanoidin was formed by polymerizing the intermediate product of the Maillard reaction depending on temperature and heating time.

3.6.2. Antioxidant properties of melanoidin fractions

Antioxidant properties melanoidin fractions of granulated coconut sugar made from coconut sap without addition of amino acids (NAA), added with arginine (AA), histidine (HA) and coconut sap from farmers (CSF) as control are shown in Table 4.

Table 4. Antioxidant properties of melanoidin fractions

Granulated coconut sugar	Melanoidin Fraction (kDa)	Chemical properties of granulated coconut sugar					
		Total phenolic (ppm)	Browning intensity (abs 420)	Radical scavenging activity (%)	Chelating activity (%)	Inhibition lipid peroxidation (FTC method, %)	Inhibition lipid peroxidation (TBARs method, %)
NAA	< 14	27.31	0.28	46.62	0.18	14.47	29.18
	14 - 50	43.82	0.71	44.95	0.09	5.10	8.96
	>50	66.39	1.82	34.44	0.09	13.71	18.48
AA	< 14	31.82	0.37	45.46	9.54	17.71	41.82
	14 - 50	45.36	0.63	57.92	13.47	9.70	16.58
	>50	41.14	1.31	30.54	3.75	11.08	1.46
HA	< 14	19.36	0.25	25.38	0.76	8.89	32.47
	14 - 50	6.50	0.44	7.59	0.09	13.00	23.32
	>50	31.86	0.62	33.15	4.57	16.47	16.43
CSF	< 14	20.79	0.17	30.69	14.23	6.00	34.27
	14 - 50	30.82	0.41	42.69	3.51	10.62	14.03
	>50	36.07	0.98	15.77	0.09	13.57	19.11
EDTA 20 ppm		Na	Na	Na	61.79	Na	Na
BHT 100 ppm		Na	Na	74.95	Na	90.26	91.21
Vitamin C 800 ppm		Na	Na	85.05	Na	28.22	48.98

Note: NAA=no amino acid addition; AA=arginine addition, HA=histidine addition and CSF=coconut sugar farmers; Na=not analyzed

Table 4. shows that high total phenolic content of NAA, AA and CSF sugars were the melanoidin compound of fraction > 14 kDa, whereas total phenolic HA sugar was high in fraction > 50 kDa. This shows that the high molecular weight of melanoidin in almost all samples had a high total phenolic content. This result is in accordance with that reported by Brudzynski and Miotto (2011), which stated that heat treatment caused an increase in phenolic compounds during the formation of melanoidins in honey. Browning intensity shows that the higher the molecular weight of the fraction, the higher the intensity of browning. This

is consistent with the statement by Ho et al. (2008) that high molecular weight melanoidin is a brown color product formed due to the polymerization of sugar degradation products linked to amino compounds through Maillard reactions.

The highest DPPH radical scavenging activity (RSA) and Fe2 + chelating activity were shown by AA sugar fractions 14-50 kDa *i.e.* 57.92 and 13.47%, respectively while the HA sugar fraction> 50 was the highest RSA and chelating activity compared to the others HA sugar fraction. AA sugar fraction <14 showed the highest inhibition of linoleic acid peroxidation, both tested using FTC and TBA. HA> 50 sugar fraction had the high inhibitory effectiveness in initiation and propagation of lipid peroxidation steps, however the HA sugar fraction <14 kDa was more effective in inhibiting of lipid oxidation in the termination stage.

Conclusions

The coconut sap collected during the daytime commonly can be used as the raw material of granulated coconut sugar production. The optimum addition of mangosteen peel powder as preservative was 0.56 g/L. The chemical properties of coconut sap that can be processed further to produce granulated coconut sugar were as follows: pH bigger than 6.6, 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.

The addition of arginine to sap (AA) produced granulated coconut sugar with the radical scavenging activity (RSA) and chelating activity to be higher 34.84% and 53.98% respectively, compared with sugar produced from sap without the addition of amino acids (NAA). The increase of RSA and chelating activity of AA sugar were contributed by melanoidin fraction 14-50 kDa. This fraction also had the highest phenolic total (45.36 ppm). The addition of histidine to sap (HA) produced sugar caused the radical scavenging (RSA) tended to be higher (36.24%) compared to NAA sugar, while the chelating activity of HA sugar was not significantly different from NAA sugar. The ability of RSA and chelating of HA sugar was contributed by fraction melanoidin> 50 kDa. This fraction also had the highest phenolic total (31.86 ppm).

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