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The effect of arginine addition on chemical and antioxidant properties of coconut sap during heating

The effect of arginine addition on chemical and antioxidant properties of coconut sap during heating

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Abstract. Arginine contains positive charged ends that are suitable to bind molecules with negative charge like carbonyl groups of reducing sugar in coconut sap. This research aimed to determine the effect of various arginine concentrations on chemical and antioxidant properties of coconut sap during the heating process. A 2.5 L of coconut sap was added with 0.4; 0.8 and 1.2 mM arginine and heated with an open process until the temperature of sap reached 118 ºC. Fifty grams of sap samples were collected in the temperature of 80, 100 and 118 ºC during heating treatment. The results showed that the variation of arginine concentration did not significantly affect water content, reducing sugar, total sugar, and sucrose of coconut sap samples. Coconut sap added with 0.4 mM showed the highest pH during heating. The highest of free amino acid content was shown on coconut sap added with 0.8 mM arginine at the end of heating temperature. The 0.8 mM of arginine concentration and the sap temperature of 100 ºC were the optimum condition to obtain the highest DPPH radical scavenging activity *i.e.* 69.93%, while the chelating activity of coconut sap added with 0.4 mM was significantly higher than other treatments (34.42%).

1. Introduction

Coconut sap is a liquid substance with nearly neutral pH that contains sugars, vitamins, and minerals obtained from tapping coconut inflorescence [1]. Coconut sap tends to undergo spontaneous fermentation due to its high nutritious content. The negative effects of fermentation in sap can be prevented by adding a number of natural and synthetic preservatives. A mixture of mangosteen peel powder and lime commonly used to improve the preservation result. In addition, the optimum concentrations of mangosteen peel powder and lime in the mixture as sap preservative were 0.56 g/L and 1.7 g/L, respectively [2]. Furthermore, the tapping process during sunny days gives better quality of coconut sap than that tapped during a rainy day [3].

Commonly coconut sap is used as a raw material of coconut sugar. Coconut sap will be more viscous and browning that usually occur during the heating process. According to Haryanti et al [4], the color of coconut sap was getting darker along with the increasing heating time. Sugar structure in sap, such as mono, oligo, or polysaccharide form, as well as its reactivity level with amino groups impacted the color creation of the coconut sap. Several studies have revealed that Maillard reaction has a significant contribution in the browning reaction during sap evaporation which resulted in specific characteristics

of coconut sugar, such as its flavor and color. Moreover, the Maillard reaction in sap heating is supported by the alkaline pH and heating temperature of coconut sap [5,6].

The Maillard reaction intensity is largely affected by the reducing sugar and amino acid composition in coconut sap [7] as well as the temperature of heating [8]. Additionally, the production of amino groups as well as free ammonia and nitrogen atoms from free amino acid are through deamination and retro-aldol reactions [5]. The initial Maillard reaction is also influenced by monosaccharides i.e. glucose and fructose by creating an abundant pool of high-reactive C2, C3 and C4 dicarbonyl compounds.

The development of food color, flavor and antioxidant was significantly influenced by amino acid and reducing sugar involved in Maillard reaction [9]. Furthermore, Kim [10] reported that Maillard reaction products (MRPs) showed antioxidant activity due to metal chelators and radical scavengers ability. In addition, a study conducted by Yan et al. [11] showed that antioxidant activity was strongly found in MRPs obtained from the model system of chitooligosaccharide and glycine. An investigation conducted by Karseno et al. [12] found that DPPH radical scavenging activity highly correlated with browning intensity which was shown by high correlation coefficient (r) of 0.93. Moreover, browning intensity as well as aroma compounds are also affected by Maillard reaction time and temperature [13].

Basic amino groups can be more generated by arginine addition. Maillard reaction will produce the intermediate products via the 2.3-enolization pathway during pH more than 7. This track will form a reductone compound which exhibits antioxidant activity. The research conducted by Sulistyo and Haryanti [14] showed that coconut sap added by basic amino acid i.e. lysine with 0.75 mM concentration produced a significant increase of total phenolic content and radical scavenging activity in the sap until the heating temperature reached 118 ºC.

The Maillard reaction may occur during coconut sap heating, generate MRPs (such as reductones) and melanoidin that contain phenolic groups [15]. Melanoidin formed in the final stage of Maillard reaction has specific characteristics, i.e. brown in color, composed by carbohydrate and nitrogen-based polymeric macromolecules. Thus, the quantity of melanoidin can be estimated by measuring browning intensity through spectral absorbance at 405 nm [16]. The mechanism involved in melanoidin formation in granulated coconut sugar processing is still unknown. The measurement of chemical properties and antioxidant activities of coconut sap during heating was the most reasonable technique to understand such Maillard reaction mechanism. Therefore, the present research was conducted to characterize the chemical properties as well as antioxidant activity of coconut sap during the interval period of heating added with various arginine concentrations.

2. Materials and methods

2.1. Materials

The coconut sap was obtained from 15 coconut trees in Sikapat village, Sumbang, Banyumas Regency, Indonesia. The 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 and hydrochloric acid were purchased from Merck (Darmstadt, Germany), ferrozine was obtained from Fluka Chemical. Co. (Buchs, Switzerland), and 3,5-dinitrosalicylic acid and 2,2-diphenyl-1-picrylhydrazyl were from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Tapping of coconut sap

Plastic containers were prepared for sap collection. A mixture of mangosteen peel powder and lime with concentration of 0.56 g/L and 1.7 g/L, respectively, was then poured into the containers as a natural preservative to prevent spontaneous fermentation in sap. Subsequently, coconut sap was tapped during the daytime for about nine hours from 6 am to 3 pm, under fine weather conditions with air temperature of 24-27 °C and relative humidity of 91-92%.

2.3. Heating process of coconut sap

To begin with, ten liters of coconut sap was refined using filter clothes. The filtered sap was then divided into four portions. The first sap portion was not added with amino acid and used as the treatment control whereas the second, third and fourth sap portion were added with arginine in various concentrations, i.e. 0.4, 0.8 and 1.2 mM, respectively. Each sap portion was then poured into an aluminum pan and heated using a gas stove. The sap continuously stirred during heating and when the sap temperature reached 118 °C the heating was stopped. This condition can be reached in about 50 minutes of heating. A 50 g of sap sample was collected at heating temperature of 80, 100 and 118ºC for chemical properties and antioxidant activity analysis. The analysis was performed as described in the following subsection

2.4. Chemical analysis

2.4.1. Water content. Water content was determined according to AOAC method [17]. Thermo-gravimetric method was applied to measure water content of coconut sap.

2.4.2. The pH value. The pH value of all the sap samples were immediately measured by means of pH meter (Ohaus-ST10-USA), which has been calibrated by using buffer solution with a pH of 6.86 at 25 ºC.

2.4.3. Reducing sugar, total sugar and sucrose content. We employed the procedure by Miller [18] to determine reducing sugar, with some small modifications. Firstly, one gram of sap samples was dissolved in 5 mL of distilled water. Three milliliters of the sap was then mixed with three milliliters of 1% 3,5-Dinitrosalicylic acid in a test tube. The mixture was then heated in a waterbath for 15 minutes at a temperature of 90 °C. One milliliter of 40% potassium tartrat was next added to stabilize the mixture color. The mixture was cooled at room temperature for around 5 minutes. The next step was measurement of spectrum absorbance at wavelength of 540 nm. A standard glucose solution was utilized to quantify reducing sugar of samples. In the meanwhile, to determine total sugar content we applied a hydrolysis method. A volume of 3 mL of 25% HCl was used to incubate the sample. The incubation process was completed after 10 minutes at a temperature of 70 ºC. Then, the 45% NaOH was used to neutralize the sap solution at room temperature. Lastly, the content of sucrose was determined by subtracting reducing sugar from total sugar content.

2.4.4. Free amino acid. The free amino acid of coconut sap was determined by Yao's procedure [19]. One gram of coconut sap was firstly poured into a volumetric flask and then buffer solution and ninhydrin solution, with volume of each solution was 0.5 mL, were subsequently added. The mixture in the flask was then heated in a boiling water bath for 15 minutes. Next, the flask was cooled in room temperature for 5 minutes and 25 mL distilled water was filled into the flask. Afterward the next step was measuring spectrum absorbance of the solution at wavelength of 570 nm by means of UV-1900 UV-VIS spectrophotometer (Shimadzu; Kyoto, Japan). To quantify the samples we utilized glutamic acid as the standard.

2.4.5. Total phenolic. We used the Folin-Ciocalteu procedure [20] to determine the total phenolic content of the coconut sap. A volume of 30 µL of sap sample was filled in a test tube and then a volume of 150 µL of Folin-Ciocalteu reagent with a concentration of 10% was added. The admixture was then incubated for 8 minutes and afterward a volume of 120 µL of Na_2CO_3 with a concentration of 7.5% and have been dissolved in distilled water was added. This mixture was then processed for incubation once again for one hour at room temperature and its spectrum absorption at wavelength of 765 nm was subsequently measured. Furthermore, the assessment of the blank was carried out by replacing sap samples with suitable solvent which was subtracted from the spectrum absorbance at the same wavelength. Finally, the measurement result was expressed in milligrams of gallic acid, as the standard phenolic compound, equivalent per 100 gram of sample (mgGAE/100 g of sample).

2.4.6. Browning intensity. The browning color of coconut sap samples was determined by slightly modifying the procedure developed by Ajandouz et al [21]. The sap sample was liquefied with distilled water (1:25 w/v), then centrifuged at 1006 g for 15 minutes. The spectrum absorbance of the sap browning was measured at 420 nm using a UV-1900 UV-VIS spectrophotometer (Shimadzu; Kyoto, Japan).

2.4.7. Radical scavenging activity. To determine the radical scavenging activity (RSA) of coconut sap samples, we employed the procedure from Payet et al [20]. DPPH, a methanolic solution with concentration of 0.1 mM and volume of 280 µL, was put into a test tube and subsequently mixed with a sap sample. In the meanwhile, the solution in the test tube was mixed with solvent for the blank. Both mixtures were then incubated for 30 minutes at room temperature. The spectrum absorbance of the mixtures at wavelength of 515 nm was then measured by means of a spectrophotometer. Lastly, the antioxidant activity of the coconut sap samples was defined as the percentage of radical scavenging activity according to the following formula:

$$RSA(\%) = (Ao - As)/Ao \times 100 \quad (1)$$

where Ao and As are the spectrum absorbance of the blank and the sample, respectively, at 515 nm.

2.4.8. Chelating activity. Chelating activity of coconut sap can be defined as the ability of the sugar to chelate metal ions Fe^{2+}. We used the procedure conducted by Kim [10] to determine chelating activity of sap samples. An amount of one gram sap sample was firstly diluted and filtered with a filter paper. A volume of 100 µL of the sap solution was then mixed with 600 µL of distilled water and 100 µL of 0.2 mM $FeCl_2.4H_2O$. To prepare the control solution, a volume of 100 µL of distilled water was added with 200 µL of 1 mM ferrozine. The color changes of the mixture were observed at spectrum wavelength of 562 nm using a UV-1900 UV-VIS spectrophotometer (Shimadzu; Kyoto, Japan) after 10 minutes of cooling at room temperature. The chelating activity was subsequently determined using the following equation:

$$Chelating\ activity(\%) = (Ao - As)/Ao \times 100 \quad (2)$$

where Ao and As are the spectrum absorbance of the control and the sample solutions, respectively, at 562 nm after 10 minutes of incubation.

2.5. Spectroscopic analysis

The absorption spectra ranging from 200 nm to 700 nm was utilized to analyze the spectroscopic properties of sap. Firstly, 250 mg of coconut sap was dissolved in a volume of 10 mL of demineralized water. The preparation of each solution was undertaken prior to measurement. The record of absorption spectra was performed by means of UV-1900 UV-VIS spectrophotometer (Shimadzu; Kyoto, Japan) [16].

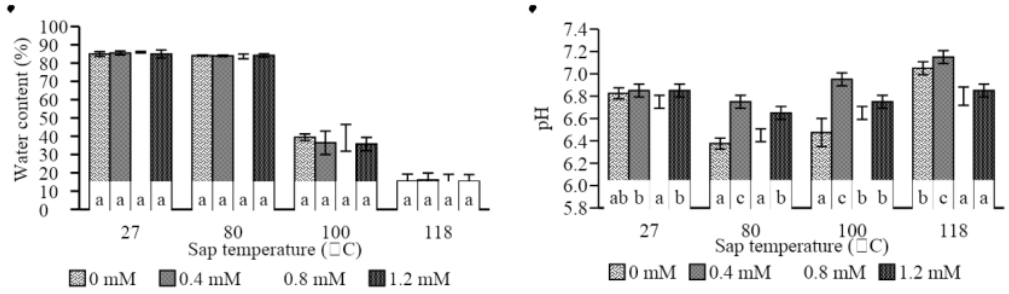
2.6. Statistical analysis

Statistical analysis of all chemical properties and antioxidant activity data were performed using IBM SPSS Statistic 20 and expressed as mean ± standard deviation (SD). The statistical significance was considered at $P < 0.05$. The effect of variation of the added arginine concentration was evaluated by one-way analysis of variance (ANOVA) using Duncan's multiple range test with a significance level of $P < 0.05$.

3. Results and discussion

3.1. Chemical properties of coconut sap during heating

3.1.1. Water content and pH value. Water content of coconut sap added with different concentrations of arginine during heating is revealed in Figure 1(a). The addition of arginine did not provide a significant effect on water content of coconut sap samples. During heating, the evaporation of water reduced the water content of coconut sap from an average of 85.34 to 15.52%. The addition of variations in arginine concentration showed in significant changes in the pH of coconut sap. The decrease of pH of coconut sap at the beginning of heating was due to the loss of the amino-base groups at the initial stage of the Maillard reaction. Moreover, the addition of arginine increased on the pH of the sap, especially on heating to 100 ºC (Figure 1b). The increase of pH of the sap on further heating was due to the decrease in protonation of the nitrogen atom in the amine group [5].



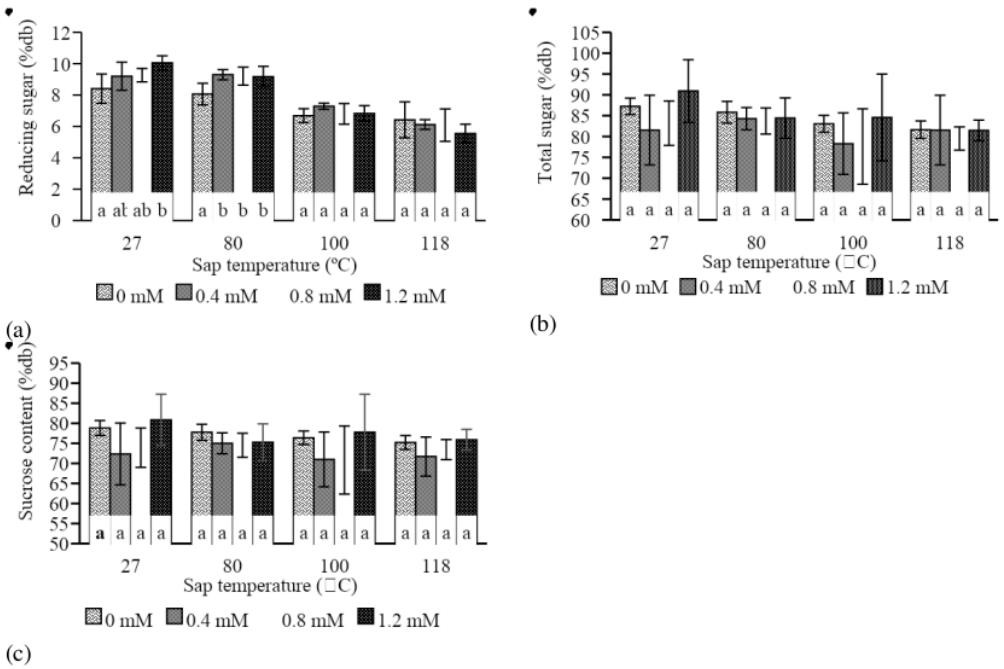
(a) (b)

Note: Different small letters inside the bars in the same temperature indicate the significant difference ($P<0.05$)

Figure 1. Water content (a) and pH value (b) of coconut sap added with arginine during heating treatment.

3.1.2. Reducing sugar, total sugar and sucrose content. Reducing sugar, total sugar and sucrose content of coconut sap added with various arginine concentration on coconut sap are depicted in Figure 2.

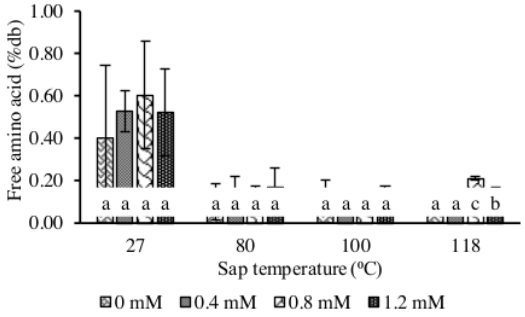
Figure 2 (a) showed that reducing sugar of coconut sap added with arginine was higher than coconut sap with no arginine addition at the initial heating. The increase of reducing sugar might be due to the decrease of pH of coconut sap at the beginning of heating. According to Ho et al [5], the decrease of pH at the earlier of heating was due to the loss of the amino-base groups at the initial stage of the Maillard reaction. Releasing of amino groups can easily generate sugar fragmentation. The total sugar and sucrose content of coconut sap added with various concentrations of arginine did not significantly different.



Note: Different small letters inside the bars in the same temperature indicate the significant difference (P<0.05)

Figure 2. Reducing sugar (a), total sugar (b) and sucrose content (c) of coconut sap added with arginine during heating treatment.

3.1.3. Total free amino acid. The variation of arginine concentration addition in the sap had no significant effect on free amino acid content of coconut sap below heating temperature of 100 ºC (P > 0.05), as seen in Figure 3. However, the addition of 0.8 mM arginine showed the highest of free amino acid content on coconut sap measured at temperature of 118 ºC. The increase of total free amino acid was due to other compounds created during the heating process. This compound was identified as amino acid, such as melanoidin. Furthermore, melanoidin formed during heating of coconut sap is nitrogen-based polymeric macromolecules and might also be detected as amino acid [15].

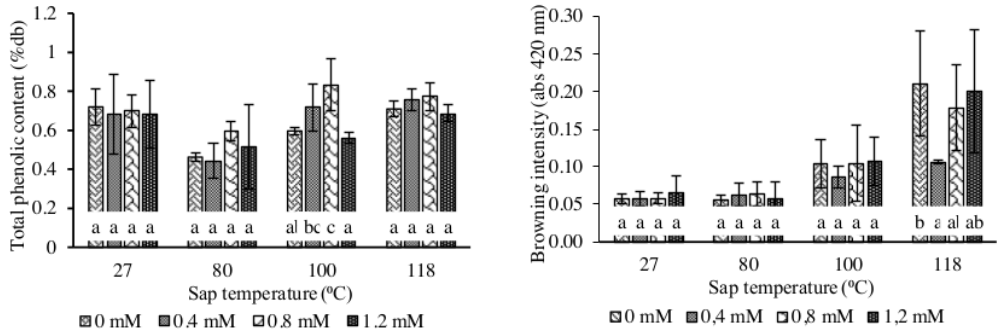


Note: Different small letters inside the bars in the same temperature indicate the significant difference (P<0.05)

Figure 3. Free amino acid of coconut sap added with arginine during heating treatment.

3.1.4. Total phenolic and browning intensity. Total phenolic content and browning intensity of coconut sap added with arginine during heating is revealed in Figure 4.

Total phenolic content of coconut sap added with various concentrations of arginine was not significantly different during heating until temperature of 80 ºC (Figure 4a). However, the addition of 0.8 mM arginine showed the highest total phenolic content of coconut sap at temperature of 100 ºC. The natural phenolic compound in coconut sap decreased after the thermal heating, however, there was an increase of melanoidin in this step. In fact, the increase of melanoidin was the most frequent contributor to the increase of phenolic content in coconut sap after heating treatment. MRPs and melanoidin contain phenolic groups which are created during heating [15,22].



(a) (b)

Note: Different small letters inside the bars in the same temperature indicate the significant difference (P<0.05)

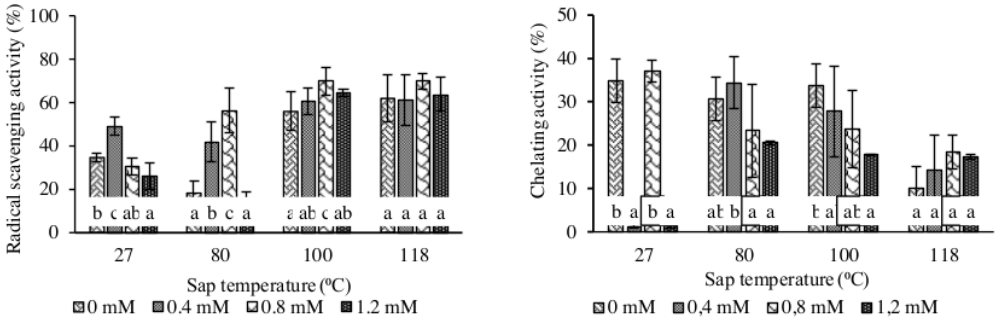
Figure 4. Total phenolic (a) and browning intensity (b) of coconut sap added with arginine during heating treatment.

Browning intensities of coconut sap added with variation of arginine concentration were not significantly different below heating temperature of 100 ºC. The increase of arginine concentration resulted in the increase of brown color on coconut sap at the end of heating (118ºC) (Figure 4b). The addition of 0.8 and 1.2 mM of arginine raised the intensity of brown color on coconut sap. The browning color might relate with the melanoidin formed at the final stage of Maillard reaction. The same result showed by Haryanti et al [4], that the increasing heating time caused the getting darker of coconut sap. According to Sulistyo and Haryanti [14], the changes of browning intensity of sap during heating followed an exponential curve.

3.1.5. Radical scavenging and chelating activity. The radical scavenging activity (RSA) and chelating activity of granulated coconut sugar added with variation concentration of arginine on sap is depicted in Figure 5.

The RSA of coconut sap derived from coconut sap added with 0.8 mM arginine during heating of 80 and 100 ºC were significantly higher than other coconut sap samples i.e. 56.30 and 69.93 %, respectively (Figure 5a). Arginine is the polar charged side chain of amino acid which might play a role in Maillard reaction by providing nitrogen atom sources [5]. The basic properties of arginine have stronger activity during the Maillard reaction due to its two reactive amino groups. According to Delgado-Andrade and Rufian-Henares [23] and Eskin and Shahidi [24], arginine have free amino groups which might participate in Maillard reaction. Maillard reaction products (MRPs) derived from heating of lactose-arginine model systems during temperature of 100 ºC for 8.5 minute have RSA 50% [25]. In addition, Wiriyawattana et al. [26] revealed that thermal heating at 110 ºC was the optimum temperature to produce pregelatinized riceberry flour with the highest antioxidant activity. Another study conducted by Boonmawat et al. [27] reported that heating on superheated steam with extremely high temperature had no effect on antioxidant activity of riceberry bran.

Chelating activity of coconut sap added with 0.4 mM of arginine at temperature of 80 ºC was significantly higher than that from other treatments (34.42%) (Figure 5b). The addition of arginine increased the concentration of amino groups which influenced the production of volatile compounds, i.e. pyrazine. However, the increase of pyrazine formation which is affected by nitrogen atoms in arginine did not lead to the increase of chelating ability of granulated coconut sugar. Therefore, the addition of arginine more than 0.4 mM decreased on chelating activity of coconut sap. Chelating activity of coconut sap might be obtained from the final stage of Maillard reaction i.e. melanoidin. According to Delgado-Andrade et al. [28], MRPs have chelating activity. Moreover, ion chelating affinity of MRPs is the key of antioxidant activity mechanism [29]. Verzelloni et al. [30] also reported that melanoidin could prevent oxidation due to its chelating ability. Melanoidin contained anionic compounds which could chelate metal transition [31,32,33].



(a) (b)

Note: Different small letters inside the bars in the same temperature indicate the significant difference ($P<0.05$)

Figure 5. Radical scavenging (a) and chelating activity (b) of coconut sap added with arginine during heating treatment.

3.2. Spectroscopic analysis

The absorption spectra of coconut sap added with various concentrations of arginine during heating were depicted in Figure 6.

As seen in Figure 6, there are two peaks revealed in the graph, i.e. at 261 – 261.5 and at 401.5 nm. The peak at wavelength of 261 – 261.5 nm might indicate the presence of polyphenols, proteins, sucrose, reducing sugar and MRPs such as reductone. Meanwhile, the peak at wavelength of 401.5 nm indicates the presence of melanoidin. Bekedam et al. [16] reported that the low molecular weight compound from coffee can absorb light at 280 nm while melanoidin showed the absorption spectra at 405 nm. Based on these absorption spectra, the absorption at wavelengths of 261 – 261.5 and 401.5 nm has been proved that it can determine the relative amount of melanoidin and other components in coconut sap. Coconut sap added with 0.8 mM arginine concentration dominated the compound that absorbs light 261 nm (abs value = 2.216), higher than other sap samples. It might prove that coconut sap samples added with 0.8 mM had higher antioxidant activity compared to other sap samples.

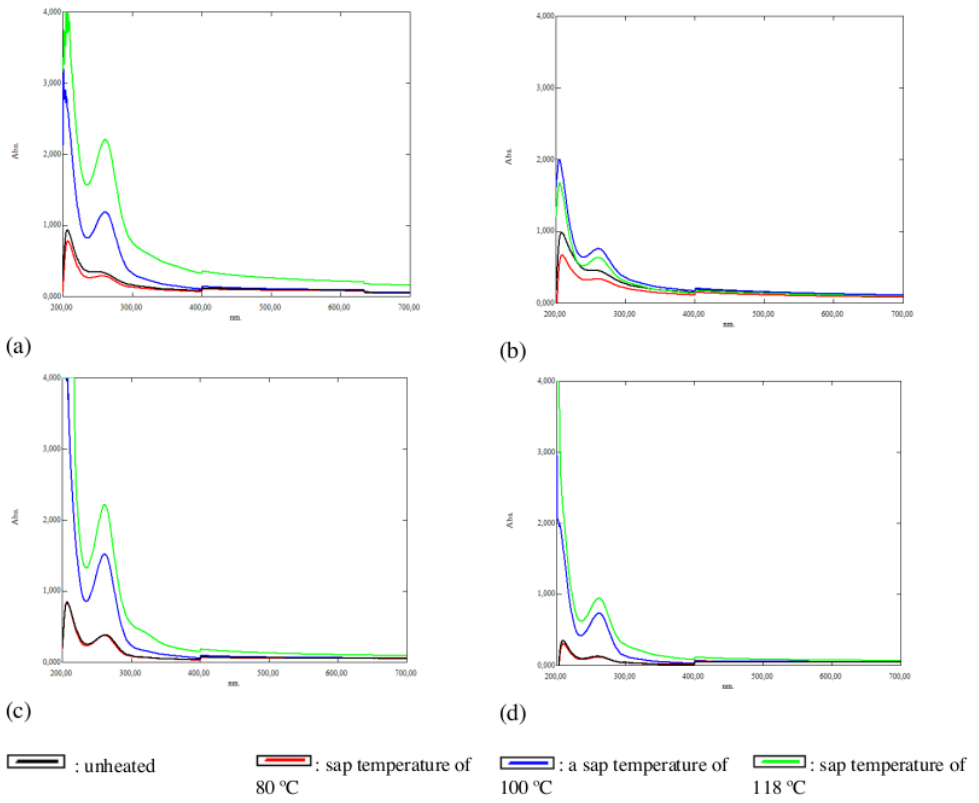


Figure 6. Spectroscopic pattern of coconut sap with no addition of arginine (a), addition of 0.4 mM arginine (b), addition of 0.8 mM arginine (c) and addition of 1.2 mM arginine (d) during heating treatment.

4. Conclusion

The results achieved from the current research confirmed that the increase of arginine concentration had no significant effect on water content, total sugar and sucrose content of coconut sap at the same temperature of heating period. Furthermore, the variation of arginine concentration added on coconut sap has a significant effect on pH, reducing sugar, free amino acid, browning intensity, RSA and chelating activity of coconut sap particularly at the end of the heating process. The findings of this study can be utilized as basic data for future studies of the potential of amino acid addition as an ingredient to form liquid coconut sugar with health benefits due to its antioxidant activity.

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