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FULL PAPER

The effects of adding lysine to sap on chemical characteristics and antioxidant activity of granulated coconut sugar

[1]Haryanti, P. and [2,]*Sulistyo, S.B.

[1]*Food Technology Study Program, Department of Agricultural Technology, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto 53123, Indonesia*
[2]*Agricultural Engineering Study Program, Department of Agricultural Technology, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto 53123, Indonesia*

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Abstract

Reducing sugar and amino acid are chemical features of coconut sap that are critical in the development of the typical colour and flavour of coconut sugar. This study aimed to discover how adding lysine to sap affected the chemical characteristics and antioxidant activity of granulated coconut sugar. The results showed that adding lysine to sap had no considerable influence on water and ash content, total sugar, reducing sugar, sucrose content, free amino acid, or total phenolic content of granulated coconut sugar. The amount of lysine added to coconut sap, on the other hand, had a substantial impact on the browning intensity, radical scavenging, and chelating activities of granulated coconut sugar. The highest radical scavenging activity was obtained in granulated coconut sugar added with 0.5 mM lysine, i.e., 73.96%, while the 0.25 mM of lysine concentration produced sugar with the highest chelating activity i.e., 7.01%.

1. Introduction

Coconut sap is a sweet and lucent liquid with a pH of almost neutral that can be produced by tapping the stem of a coconut flower bloom (Borse *et al*., 2007). Coconut sap contains many sugars as well as vitamins and minerals. Due to the high nutritious content, coconut sap tends to endure spontaneous fermentation. According to Hariharan *et al*. (2014), fermented sap in consequence will contain alcohol and acid liquid due to bacterial activity in the sap. As a result, a variety of natural and synthetic preservatives are routinely used to counteract the detrimental effects of sap fermentation. Haryanti *et al*. (2017) suggested that rather than a single material of the mixture, a blend of mangosteen peel powder and lime could enhance the preservation effect. Furthermore, as a sap preservative, the optimum quantities of mangosteen peel powder and lime in the mixture were 0.56 g/L and 1.7 g/L, respectively. Moreover, the chemical characteristics of coconut sap are altered by the weather. Coconut sap tapped on a sunlit day has superior chemical qualities than sap tapped on a rainy day (Haryanti *et al*, 2018).

Coconut sap is commonly used to make beverages or make coconut sugar. Granulated coconut sugar is one of the products made from coconut sap that has high economic value. Several processes are usually applied to produce granulated coconut sugar. Sap heating is the initial and most critical step of coconut sugar production. The improper procedure of sap heating will lead to the failure of sugar production. Coconut sap becomes thicker during the heating process, and browning commonly exists.

Basically, the browning reaction in food processing might be caused by the Maillard reaction, ascorbic acid oxidation or caramelization. Numerous research has found that during sap heating, the Maillard reaction plays a substantial role in browning, resulting in distinctive coconut sugar characteristics such as flavour, colour, and aroma (Ho *et al*., 2008; Asikin *et al*., 2014). The limy pH and appropriate sap boiling temperature induce the Maillard reaction in sap heating. On the other hand, both ascorbic acid oxidation and caramelization do not exist in coconut sugar processing. These reactions optimally occur in low pH substances. Moreover, caramelization may also occur in alkaline conditions, however, it requires a high heating temperature (higher than 120°C) (Eskin and Shahidi, 2013).

Reducing the sugar and amino acid content of coconut sap affects the strength of the Maillard reaction (Nagai *et al*., 2018). In addition, the Maillard reaction is also influenced by the heating temperature (Carciochi *et*

*Corresponding author.
Email: *susanto.sulistyo@unsoed.ac.id*

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al., 2016). Amino groups, free ammonia, and nitrogen atoms can also be made from free amino acids through deamination and retro-aldol reactions (Ho *et al*., 2008). In the meanwhile, monosaccharides like glucose and fructose influence the initial Maillard process by creating a large pool of high-reactive C2, C3, and C4 dicarbonyl molecules.

In the Maillard reaction, amino acids and reducing sugar play an important role in the production of food colour, flavour, and antioxidants (Wijewickreme *et al*., 1999). Moreover, Kim (2013) found antioxidant activity in Maillard reaction products (MRPs) since MRPs can operate as metal chelators and radical scavengers. MRPs generated from chitooligosaccharide and glycine model systems also demonstrated excellent antioxidant activity, according to Yan *et al*. (2018). Karseno *et al*. (2018) discovered that DPPH radical scavenging activity and browning intensity have a strong correlation as indicated by the coefficient of correlation of 0.93. Furthermore, Maillard reaction duration and temperature affect browning intensity as well as fragrance components (Zhou *et al*., 2016).

The addition of lysine can increase the generation of basic amino groups. The 2.3-enolization track will yield intermediate products from the Maillard reaction, which occurs at a pH greater than 7. As a result of this action, a reductone chemical with antioxidant properties is formed. Additionally, the addition of 0.75 mM lysine to coconut sap and a heating temperature of 118°C not only resulted in a considerable increase in total phenolic content but also in radical scavenging activity in the sap (Sulistyo and Haryanti, 2020).

During the boiling of the sap, the Maillard reaction occurs, producing MRPs (such as reductones) and melanoidin, both of which include phenolic groups (Brudzynski and Miotto, 2011). Melanoidin is a brown polymeric macromolecule made up of carbohydrate and nitrogen-based polymeric macromolecules that are generated in the final step of the Maillard reaction. Thus, quantifying browning intensity via spectral absorbance at 405 nm can be used to quantify melanoidin amount (Bekedam *et al*., 2006). However, the mechanism of melanoidin synthesis in the processing of granulated coconut sugar remains unknown. The most logical method for determining such a mechanism is to measure the chemical characteristics and antioxidant activity of granulated coconut sugar. As a result, the current study delved into the chemical characteristics and antioxidant activity of granulated coconut sugar made from sap with varied lysine concentrations.

2. Materials and methods

2.1 Materials

Materials used in this research, in general, were divided into two categories, i.e. raw material and chemical. Coconut sap was collected from coconut trees in Banyumas Regency, Indonesia, as the raw ingredient. A number of chemical substances were utilized to analyze the chemical properties and antioxidant activities of coconut sap and granulated coconut sugar. The chemicals were obtained from Merck (Darmstadt, Germany), 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. In addition, a chemical namely ferrozine was obtained from Fluka Chemical. Co. (Buchs, Switzerland) while 3,5-dinitrosalicylic acid and 2,2-diphenyl-1-picrylhydrazyl were from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Coconut sap preparation

First, plastic containers for sap collecting were prepared. A blend of mangosteen peel powder and lime with concentrations of 0.56 g/L and 1.7 g/L, respectively, was put as the natural preservative into the containers to avoid spontaneous fermentation in sap. After that, coconut sap was collected by tapping coconut inflorescence from 15 coconut trees into the containers. Sap tapping took place during the day, from 6 a.m. to 3 p.m., in pleasant weather with air temperature and relative humidity ranging from 24-27°C and 91%-92%, respectively.

2.3 Chemical analysis of coconut sap

2.3.1 Total soluble solid and pH value

After sap collection, the total soluble solids and pH value of the collected sap were immediately measured using a portable refractometer and pH meter, respectively.

2.3.2 Reducing sugar

To determine reducing sugar, we employed the procedure introduced by Miller (1959) with some small modifications. Firstly, one gram of sap sample was dissolved in 5 mL of distilled water. The sap (3 mL) were then mixed with 3 mL of 1% 3,5-Dinitrosalicylic acid in a test tube. The mixture was afterwards heated in a water bath for 15 mins and the heating temperature was maintained at 90°C. An aliquot (1 mL) of 40% potassium tartrate was then added to stabilize the mixture colour. After 15 mins of heating, the mixture was cooled at room temperature for around 5 mins. The next step was the measurement of spectrum absorbance. Spectrum absorbance of the mixture was determined at a wavelength of 540 nm and to quantify samples a standard glucose solution was utilized.

2.3.3 Free amino acid

Determination of free amino acid of coconut sap was adopted from Yao's procedure (Yao *et al*., 2006). One gram of coconut sap was firstly poured into a volumetric flask and then buffer solution and ninhydrin solution, with the 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 mins. Subsequently, the flask was cooled at room temperature for 5 mins and 25 mL of distilled water was filled into the flask. Afterwards, the next step was measuring the spectrum absorbance of the solution at a wavelength of 570 nm using a UV-1900 UV-VIS spectrophotometer (Shimadzu; Kyoto, Japan). To quantify the samples we utilized glutamic acid as the standard.

2.3.4 Total phenolic

To evaluate the total phenolic content of the coconut sap, we used the Folin-Ciocalteu technique (Payet *et al*., 2005). The steps were as follows: A volume of 30 µL of sap sample was placed in a test tube, and then 150 µL of Folin-Ciocalteu reagent with a 10% concentration was added. After that, the admixture which was incubated for 8 mins was given a dose of 120 µL Na_2CO_3 dissolved in distilled water at a concentration of 7.5%. This mixture was then treated for a second incubation at 30°C for 1 hr, and the spectrum absorption at 765 nm was then determined. Furthermore, the blank was evaluated by substituting a suitable solvent for the sap sample and subtracting the spectrum absorbance at the same wavelength. Lastly, the result was represented in milligrams of gallic acid equivalent per 100 gram of sample (mg GAE/100 g of sample) as the standard phenolic substance.

2.4 Granulated coconut sugar processing

To begin, 10 L of coconut sap were cleansed by the use of clean filter cloths. After that, the sap was separated into four parts once it was purified. The first sap component was left untreated and served as a treatment control, whilst the second, third, and fourth sap portions were all given lysine at different concentrations, 0.25, 0.5, and 0.75 mM, respectively. After that, each sap part was poured into an aluminium pan and heated on a gas stove. During heating, the sap should be continuously stirred, and when the temperature reaches 118°C, the heating should be turned off. After roughly 50 minutes of heating, this condition can be achieved. The sap was then cooled to room temperature while still being agitated. This process persisted until granulated sugar was formed. In the next step, we performed sun-drying to reduce the water content of the produced granulated sugar. The chemical characteristics and antioxidant activity of 50 g of granulated sugar generated were then tested. The analysis was carried out as detailed in the subsection below.

2.5 Chemical analysis and antioxidant activities of granulated coconut sugar

2.5.1 Water and ash content

Water and ash content were both determined according to the AOAC method (AOAC, 1990). The thermo-gravimetric method was applied to measure the water content of granulated coconut sugar while ash content was measured by oxidizing organic compounds at high temperatures (500-600°C) and than weighing the residual matters after the combustion.

2.5.2 Reducing sugar, total sugar and sucrose content

To determine the reduction sugar of granulated coconut sugar, we employed a similar procedure as in determining reducing sugar of coconut sap. In this step, 1 g of granulated coconut sugar was dissolved in 10 mL, instead of 5 mL, of distilled water. The next steps were then the same as that in reducing sugar analysis of coconut sap. In the meanwhile, hydrolysis method was applied to determine total sugar content. A volume of 3 mL of 25% HCl was utilized to incubate the sample. The incubation process was performed for 10 mins at a temperature of 70°C. Afterwards, the solution was cooled and neutralized at room temperature with 45% NaOH. Finally, the content of sucrose was ascertained by subtracting reducing sugar from total sugar content.

2.5.3 Free amino acid

The free amino acid of granulated coconut sugar was determined by using a similar procedure as in analyzing the free amino acid of coconut sap. Firstly, 1 g of granulated coconut sugar was added with distilled water until the volume reached 10 mL and then filtered with filtered paper. A volume of 0.5 mL of filtrate was put into a volumetric flask and then the next procedure was the same as that described in determining the free amino acid of coconut sap.

2.5.4 Total phenolic

The total phenolic content of granulated coconut sugar was analyzed using the Folin-Ciocalteu procedure as conducted to determine the total phenolic of coconut sap. The first step was the preparation of a 10% sugar solution and then filtering. A volume of 30 μL of the sugar solution was subsequently treated as a sample. The succeeding steps were then the same as described in the aforementioned procedure of total phenolic analysis of coconut sap.

2.5.5 Browning intensity

The browning colour of coconut sugar samples was determined by slightly modifying the procedure developed by Ajandouz *et al*. (2001). The sap sample was liquefied with distilled water (1:25 w/v), and then centrifuged at 1006×*g* for 15 mins. The spectrum absorbance of the sugar browning was measured using a UV-1900 UV-VIS spectrophotometer (Shimadzu; Kyoto, Japan) at 420 nm.

2.5.6 Radical scavenging activity

To determine the radical scavenging activity (RSA) of coconut sugar, we employed the procedure established by Payet *et al*. (2005). DPPH• methanolic solution with a concentration of 0.1 mM and volume of 280 μL was delivered into a test tube using a pipette and subsequently mixed with a sugar sample. In the meanwhile, the solution in the test tube was mixed with solvent for the blank. Both mixtures were then incubated for 30 mins at room temperature. The spectrum absorbance of the mixtures at a wavelength of 515 nm was then analysed employing a spectrophotometer. Lastly, the antioxidant activity of the granulated coconut sugar can be defined as the percentage of radical scavenging activity according to the following equation:

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

where Ao and As are the spectrum absorbance of the blank and the sample, respectively, at 515 nm. The effectiveness of antioxidant activity of the coconut sugar sample according to DPPH radical scavenging was compared with butylated hydroxytoluene (BHT).

2.5.7 Chelating activity

Kim's approach was used to determine the chelating activity of granulated sugar, which is defined as the sugar's ability to chelate metal ions Fe^{2+} (Kim, 2013). A one-gram sample of granulated sugar was diluted before being filtered with filter paper. The sugar solution was then combined with 600 μL of distilled water and 100 μL of 0.2 mM $FeCl_2.4H_2O$ in a volume of 100 μL. The control solution was made by mixing 100 μL of distilled water with 200 μL of 1 mM ferrozine. After 10 mins of cooling at room temperature, the colour changes of the combination were monitored using a UV-1900 UV-VIS spectrophotometer (Shimadzu; Kyoto, Japan) at a spectral wavelength of 562 nm. Following that, the chelating activity was calculated using the following equation:

$$\text{Chelating activity (\%)} = \frac{Ao - As}{Ao} \times 100 \quad (2)$$

where Ao and As are the spectrum absorbance of the control and the sample solutions, respectively, at 562 nm after 10 mins of incubation. The effectiveness of the coconut sugar sample as a chelating agent was compared with ethylenediaminetetraacetic acid (EDTA).

2.5.8 Spectroscopic analysis

To investigate the spectroscopic features of sugar solutions, absorption spectra ranging from 200 nm to 700 nm were used. To begin, 250 mg granulated coconut sugar was dissolved in 10 mL of demineralized water. Prior to taking the measurements, each solution had to be prepared. The UV-1900 UV-VIS spectrophotometer (Shimadzu; Kyoto, Japan) was used to record the absorption spectra (Bekedam *et al*., 2006).

2.6 Statistical analysis

All chemical characteristics and antioxidant activity data were statistically analyzed with IBM SPSS Statistic 20 and represented as mean standard deviation (SD). $P<0.05$ was used to determine statistical significance. One-way analysis of variance (ANOVA) with Duncan's multiple range test with a significance level of $P < 0.05$ was used to assess the influence of variation in the added lysine concentration.

3. Results and discussion

3.1 Chemical properties of coconut sap

The chemical properties of coconut sap measured prior to granulated coconut sugar processing are described in Table 1. Coconut sap should have some requirements to be used for further processing into coconut sugar. The requirements are as following: pH higher than 7.2, total soluble solids around 15.1°Bx and reducing sugar lower than 0.48% (Haryanti *et al*., 2017). As seen in Table 1, the sap sample used in this research was suitable as a raw material of granulated coconut sugar since its chemical properties meet the aforementioned requirements.

Table 1. The chemical properties of coconut sap

No.	Chemical properties	Content
1	Total soluble solid (°Bx)	13.5±1.73
2	The pH value	8.6±0.23
3	Reducing sugar (%)	0.14±0.03
4	Amino acid (%)	0.27±0.14
5	Total phenolic content (mg GAE/100 g)	53.37±5.04

3.2 Chemical properties and antioxidant activities of granulated coconut sugar

The effects of different concentrations of lysine on the water and ash content, reducing sugar, total sugar, sucrose content, free amino acid content, and total phenolic content of granulated coconut sugar were not significant. The addition of lysine, on the other hand, had a substantial impact on browning intensity, radical scavenging, and chelating activity.

3.2.1 Water and ash content

Figure 1 shows the water and ash content of granulated coconut sugar made with various concentrations of lysine added to coconut sap. In addition, the water and ash content of granulated coconut sugar varied between 5.94 and 6.57% and 2.17 and 2.30%, respectively.

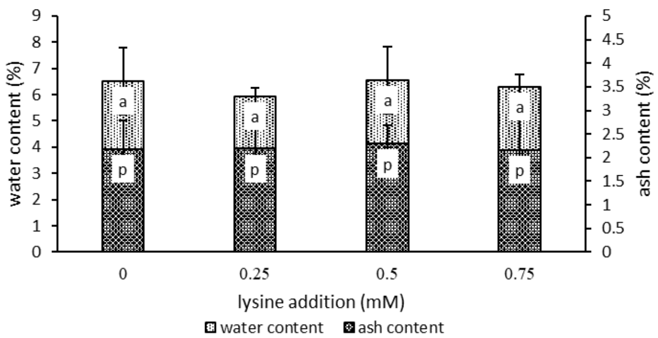

Figure 1. Water content and ash content of granulated coconut sugar. The results are represented in term of mean±SD from six experiments. Bars carrying same letters at the same parameter indicate mean values have no significant difference at P > 0.05.

3.2.2 Reducing sugar, total sugar and sucrose content

The reducing sugar, as well as total sugar and sucrose content of granulated coconut sugar applied with different lysine concentrations on coconut sap, ranged from 2.93 to 3.07% db, 90.21 to 94.37% db, and 87.24 to 91.30% db, respectively. The sugar content of the granulated coconut sugar is depicted in Figure 2.

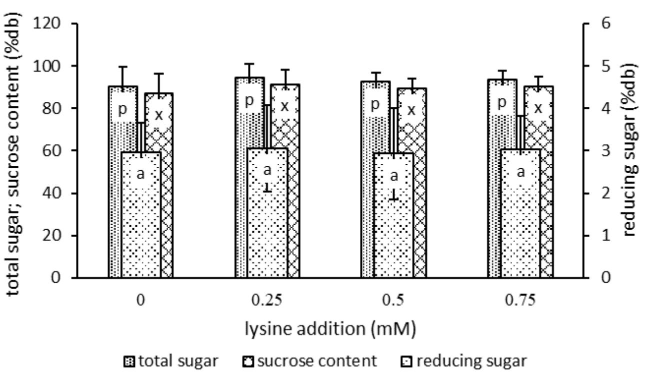

Figure 2. Reducing sugar, total sugar and sucrose content of granulated coconut sugar. The results are represented in term of mean±SD from six experiments. Bars carrying same letters at the same parameter indicate mean values have no significant difference at P > 0.05.

Reducing sugar of coconut sap was lower than that of granulated coconut sugar, i.e. 0.14±0.03% or 1.21±0.19% db. It decreased at the beginning of heating in liquid form, then increased at the end of heating and turned into granulated form. The decrease in reducing sugar of sap at the initial heating was due to the occurrence of the initial Maillard reaction. According to Ho *et al*. (2008), reducing sugar such as glucose and fructose was highly reactive at the initial stage of the heating process. Both glucose and fructose participated in the initial Maillard reaction by creating dicarbonyl compounds. The increment of reducing sugar at the end of heating and in the granulated form was caused by the forming of intermediate products of Maillard reaction bounded ketone or hydroxyl groups, which are highly reactive. Rufian-Henares and De La Cueva (2009) found that intermediate products of the Maillard reaction such as pyranone or pyridone residues contained the ketone or hydroxyl groups.

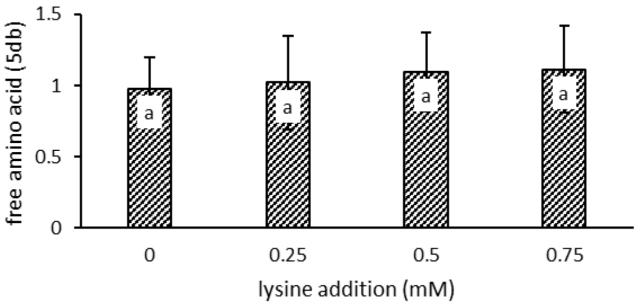

Figure 3. Free amino acid content of granulated coconut sugar. The results are represented in term of mean±SD from six experiments. Bars carrying same letters at the same parameter indicate mean values have no significant difference at P > 0.05.

3.2.3 Total free amino acid

As shown in Figure 3, the amount of lysine added to the sap had no effect on the free amino acid content of granulated coconut sugar (P > 0.05). The free amino acid of granulated coconut sugar measured in this research ranged from 0.98 – 1.11% db. As it possesses two reactive groups, lysine is the most reactive amino acid in the Maillard reaction. In addition, lysine contains nitrogen atoms which affect the pyrazine formation (Ho *et al.*, 2008). Pyrazine is a flavoured compound, so lysine addition may have a significant effect on pyrazine content (Eskin and Shahidi, 2013), but not on the amino acid content of granulated coconut sugar.

The amino acid of granulated sugar was higher than that of coconut sap, i.e. 0.27±0.14% or 2.3±1.12%db. The increase in total free amino acids was attributable to the formation of additional compounds during the heating process. Amino acids, such as ammonia, were identified as this substance. According to Ho *et al.*

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(2008), free ammonia was created at the start of the heat reaction in palm sugar production. Melanoidin, which is generated during the boiling of coconut sap, is a nitrogen-based polymeric macromolecule that may be identified as an amino acid (Brudzynski and Miotto, 2011).

3.2.4 Total phenolic content and browning intensity

With a range of 0.94 - 1.22% db, the total phenolic content of granulated coconut sugar applied with varying concentrations of lysine on sap was not significantly different (Figure 4). Coconut sap has a total phenolic concentration of 53.37±5.04 mg GAE/100 g, or 0.46±0.07% db. Since the MRPs and melanoidin were generated during the heating process, total phenolic content increased more than twofold from coconut sap to granulated sugar. After thermal heating, the natural phenolic compound in coconut sap diminished. This finding is confirmed by Wiriyawattana *et al*. (2018), who found that thermal heating at temperatures higher than 110°C can reduce the phenolic content of rice berry flour. In this step, however, the amount of melanoidin increased. Indeed, an increase in melanoidin was the most common cause of an increase in phenolic content in coconut sugar. Melanoidin and MRPs both include phenolic groups, which are formed during the heating process (Delgado-Andrade *et al*., 2005; Brudzynski and Miotto, 2011).

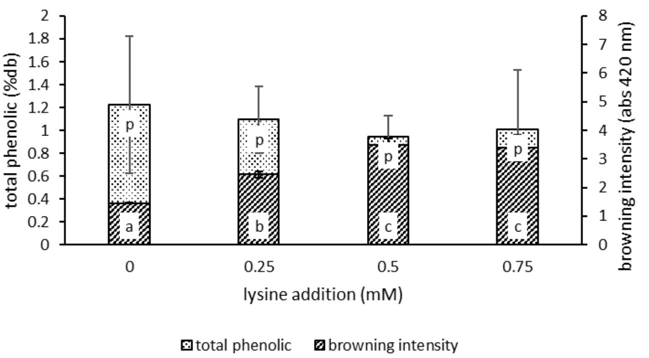

Figure 4. Total phenolic content and browning intensity of granulated coconut sugar. The results are represented in term of mean±SD from six experiments. Bars carrying same letters at the same parameter indicate mean values have no significant difference at P > 0.05.

Browning intensities of sugar generated from sap varied greatly depending on the lysine concentration. The dark hue of granulated coconut sugar increased as the lysine concentration increased. The addition of 0.5 mM of lysine increased the brown colour intensity of the granular coconut sugar. This treatment caused the most intense browning of granulated sugar, up to 42 times that of the initial phase. Furthermore, Sulistyo and Haryanti (2020) found that the changes in sap browning intensity during heating followed an exponential curve. Higher lysine concentrations, on the other hand, did not affect the browning intensity of sugar. The addition of more than 0.5 mM of lysine to sap may result in a lower pH, which may suppress the browning Maillard reaction. Maillard process is often encouraged under higher alkaline circumstances, according to Eskin and Shahidi (2013). Coconut sap's neutral and alkaline pH provided an ideal environment for accelerating C2 and C3 fragments from sugar breakdown, which have high reactivity. The sugar breakdown products were then polymerized and linked by amino compounds to form melanoidin, a brown-coloured substance (Ho *et al*., 2008).

3.2.5 Radical scavenging activity and chelating activity

Figure 5 shows the radical scavenging activity (RSA) and chelating activity of granulated coconut sugar applied to sap with varying lysine concentrations. Granulated coconut sugar made from coconut sap and supplemented with 0.5 mM lysine had a substantially higher RSA (73.96%) than granulated coconut sugar made without lysine (67.45%). The RSA of 10% granulated coconut sugar solution added with 0.5 mM lysine was similar to the RSA of 100 ppm BHT i.e. 74.95%. Lysine is the polar charged side chain of amino acid which might play role in the Maillard reaction by providing nitrogen atom sources (Ho *et al*., 2008). Because of its two reactive amino groups, lysine basic characteristics have a higher activity during the Maillard reaction. According to Delgado-Andrade and Rufian-Henares (2009) and Eskin and Shahidi (2013), lysine contains free amino groups (e-amino) that participate in the Maillard reaction. The Maillard reaction products (MRPs) produced by heating a lactose-lysine model system at 100°C for 8.5 mins have an RSA of 50% (Morales and Jimenez-Perez, 2001). Furthermore, Wiriyawattana *et al*. (2018) discovered that 110°C was the best temperature for producing pregelatinized riceberry flour with the maximum antioxidant activity. Another study by Boonmawat *et al*. (2019) found that heating riceberry bran in superheated steam at a very high temperature did not affect antioxidant activity. Furthermore, compared to other treatments, the addition of 0.5 mM lysine resulted in granulated sugar with a higher browning intensity. The RSA was likewise higher with the granular coconut sugar that had a higher browning intensity. According to Karseno *et al*. (2018), there was a strong link between DPPH radical scavenging activity and browning intensity.

As this treatment also boosted the chelating activity of coconut sap after heating, the chelating activity of sugar obtained from coconut sap combined with 0.25 mM of lysine (7.01%) was much higher than that of other treatments. The addition of a high concentration of lysine raised the concentration of amino groups, which altered the production of pyrazine, a volatile molecule. However, an increase in pyrazine production, which is controlled by nitrogen atoms in lysine, did not result in an increase in granulated coconut sugar chelating ability. As a result, adding more than 0.25 mM of lysine to granulated coconut sugar had no influence on its chelating ability.

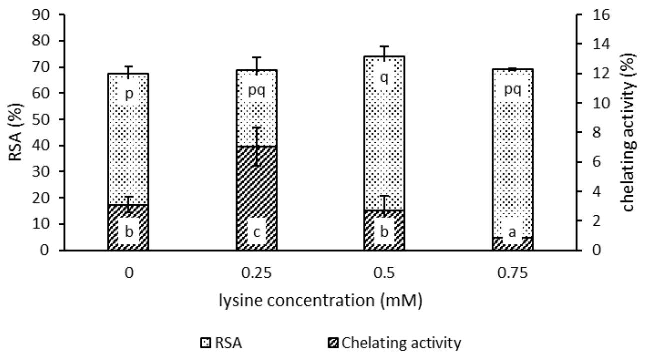

Figure 5. Radical scavenging activity and chelating activity of granulated coconut sugar. The results are represented in term of mean±SD from six experiments. Bars carrying same letters at the same parameter indicate mean values have no significant difference at P > 0.05.

As a comparison, the chelating ability of granulated coconut sugar was much lower than that of ethylenediaminetetraacetic acid (EDTA). The chelating activity of 10% granulated coconut sugar was 7.01%, while 20 ppm EDTA produced a chelating activity of 61.79%. The final stage of the Maillard reaction, melanoidin, may be responsible for the chelating activity of granulated coconut sugar. MRPs have chelating action, according to Delgado-Andrade *et al*. (2004). Furthermore, MRPs ion chelating affinity is crucial to the antioxidant action mechanism (Jing and Kitts, 2004). Melanoidin's chelating activity, according to Verzelloni *et al*. (2010), may help to avoid oxidation. Melanoidin was shown to possess an anionic molecule capable of chelating metal transitions (Daglia *et al*., 2008; Rufian-Henares and De La Cueva, 2009; Tagliazucchi *et al*., 2010).

3.2.6 Spectroscopic analysis

Figure 6 shows the absorption spectra of granulated coconut sugar applied with varied concentrations of lysine on sap. As seen in Figure 6, the graph has two peaks, one at 264.5-268.5 nm and the other at 401.5 nm. Proteins, sucrose, reducing sugar, and MRPs like reductone are all detected at wavelengths between 264.5 and 268.5 nm. Meanwhile, the presence of melanoidin is indicated by a peak at a wavelength of 401.5 nm. According to Bekedam *et al*. (2006), a low molecular weight coffee molecule can absorb light at 280 nm, while melanoidin has a 405 nm absorption spectrum. The absorption at wavelengths of 264.5-268.5 and 401.5 nm has been proven to estimate the relative amount of melanoidin and other components in granulated coconut sugar based on these absorption spectra. In comparison to melanoidin (abs value at 401.5 nm = 0.287-0.303), granulated coconut sugar generated from coconut sap combined with all lysine concentrations is dominated by the chemical that absorbs light at 264.5-268.5 nm (abs value = 1.193-1.426).

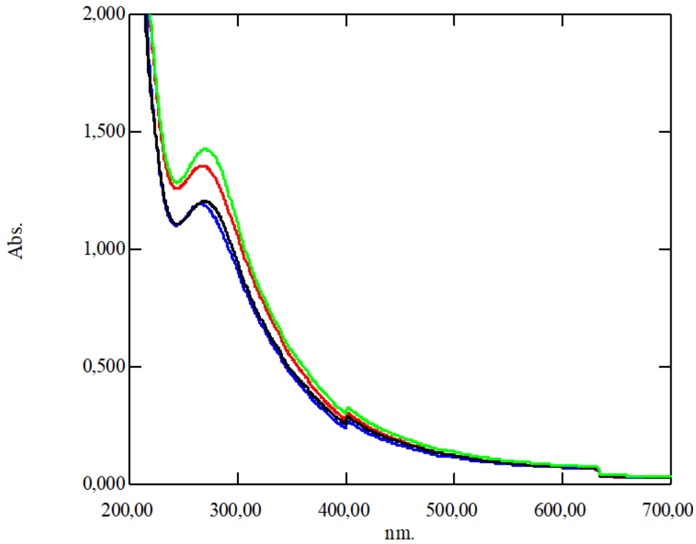

Figure 6. Absorption spectra of 25 mg/mL granulated coconut sugar produced added with 0 (black line); 0.25 (red line); 0.5 (blue line) and 0.75 mM (green line) lysine on sap.

The current study findings confirmed that increasing lysine concentration did not affect granulated coconut sugar's water and ash content, decreasing sugar, total sugar, sucrose content, free amino acid, or total phenolic content. Furthermore, the amount of lysine added to coconut sap has a substantial impact on granulated coconut sugar browning intensity, RSA, and chelating activity. The findings of this study can be used as a starting point for future research into the possibilities of adding amino acids to granulated coconut sugar to improve its health benefits.

Conflict of interest

The authors declare that there are no conflicts of interest.

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