

## Effect of pH and temperature on browning intensity of coconut sugar and its antioxidant activity

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### Abstract

Coconut sugar is produced by heating coconut neera. The brown color of sugar is derived from non-enzymatic browning Maillard reaction. It is strongly influenced by pH and temperature. In this study, the effect of pH and temperature on browning intensity and antioxidant activity of coconut sugar were examined. The pH of coconut neera was adjusted at 6 and 8 and the temperature of its heating was 100°C, 105 °C, 110°C, and 115°C, respectively. The browning intensity of sugar was determined by spectrophotometrically at 420 nm. Total phenolic content of sugar was estimated by Folin-Ciocalteu method and antioxidant activity was expressed as DPPH scavenging activity. The results showed that browning intensity and antioxidant activity of sugars was increased with increasing pH of coconut neera and temperature. It was found that the effect of pH at 8 and temperature at 115°C show highest total phenolics (0.48%) and browning intensity (0.35) of sugar. The treatment also exhibited good antioxidant activity (DPPH scavenging activity) as high as 40%. This result also indicates that there is a significant correlation between browning intensity and antioxidant activity of coconut sugar.

## 1. Introduction

Coconut sugar is natural sweeteners that commonly used in various types of traditional cuisine and beverages as a sweetener, flavor and color enhancer. The raw material for making coconut sugar is coconut sap, which is a sweet liquid obtained by taping of coconut flower. Coconut sap was known as nira (Indonesia), toddy (Sri Lanka), maprau (Thailand), and lagbi (North Africa) (Purnomo, 1992).

Coconut sugar is produced by heating coconut sap and further solidified or granulated. Coconut sugar is also known as one of the major ingredients of Indonesian sweet soya sauce, as well as in the Indonesian intermediate moisture meat. The coconut sugar gives a specific taste and flavour to the end products (Purnomo, 2007). The unique flavour of coconut sugar has made its popular as a flavoring reagent in confectionery and baking products.

The brown color of sugar is derived from non-enzymatic browning reaction called Maillard reaction and caramelization (Purnomo, 2007), that caused by condensation of an amino group and a carbonyl

(Manzocco *et al.*, 2011). Coconut sap contains high reducing sugars and amino acids, which are substrates of Maillard reaction. Purnomo (2007) found that glutamate acid, threonine, aspartic acid and serine as the major amino acids in the fresh coconut neera, while proline, methionine, tryptophan, and histidine in the amount of <4.00mg / 100 g. Furthermore, Kozaki (1974) explained that the physical and chemical composition of coconut sap indicate it is rich in amino acids, specifically glutamic acid. Amino acid content of freshly-gathered coconut sap (g/100g) on coconut sap were histidine 1.19 g, arginine 0.35 g, aspartic acid 11.22 g, threonine 15.36 g, serine 8.24 g, glutamic Acid 34.20 g, proline 3.52 g, glycine 0.47 g, alanine 2.5 g, valine 2.11 g, isoleucine 0.48g, tyrosine 0.31g and phenylalanine 0.78g, respectively. It was indicated that coconut sap is rich in amino acids, specifically glutamic acid.

The Maillard reaction requires reducing sugars and amino compounds as reactants (Jaeger, 2010). A series of chemical reactions between reducing sugars and amino compounds occurring during production and storage of foods can be summarized as Maillard reaction. The main variables affecting the extent of the Maillard

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reaction are temperature and time which depend on processing conditions as well as pH, water activity and type and availability of the reactants. It was also based on product properties, chemical composition but may be changed as a result of the processing of food and raw materials (Rufia'n-Henares, 2009).

In general, maximum browning occurs at  $a_w$  between 0.60 and 0.85 and the browning rate increases with increasing pH, up to a pH of around 10 (Morales *et al.*, 1997; Gerrard, 2002). Maillard reaction is one of the most important reactions which result from food processing. Maillard reaction products (MRPs) greatly influence essential food quality attributes such as flavor, aroma, color, and texture. Actually, this reaction can be used to design foods that present sensory attributes demanded by the consumer (Ames, 1990; Yu and Zhang, 2010).

In addition, the brown color from Maillard reaction also plays a role in the formation of antioxidant compounds. Some research showed that Maillard Reaction Products (MRPs) have potential as an antioxidant. Reducing strength and scavenging free radicals activity that considered as an antioxidant mechanism of MRPs increased with increasing heating time (Zhuang and Sun, 2011). In addition, the level of browning intensity is correlated with the ability to scavenging free radicals, lipid peroxidation inhibition activity, and reducing strength activity of the MRPs (Echavarria *et al.*, 2013; Phisut and Jiraporn, 2013).

So far, there is no clear information about the correlation between antioxidant activity and browning intensity in coconut sugar. Therefore, investigation of browning intensity regard to antioxidant activity in coconut sugar is worthwhile. Maillard reaction was influenced by several factors, such as temperature, pH, concentration and type of reducing sugar, concentration and types of amino acids, chemical compounds or inhibitors, and water content (Antony *et al.*, 2000). The high of water content, temperature, pH, reducing sugar, and amino acid concentration cause the high of brown intensity formed (Echavarria *et al.*, 2013). The presence of chemical compounds such as acids and surfactants may inhibit Maillard reactions (Zeb and Wedzicha, 2003). In this study, the initial pH of coconut neera and end point temperature of heating are factors that examined to observe their effect on the formation of brown color, especially via the Maillard reaction and its correlation with antioxidant activity.

Based on these ideas, the objective of this study was to determine the effect of initial pH of coconut neera and

end point temperature of heating to browning intensity, total phenolic contents and antioxidant activity of coconut sugar, and to evaluate the correlation of browning intensity with antioxidant activity on coconut sugar.

## 2. Materials and methods

Fresh coconut neera was obtained from coconut plantation in Karang Tengah village, Baturraden, Banyumas, Central Java. The pH, total solids, reducing sugar content, total protein, and soluble protein content of fresh coconut neera was measured. All chemical reagents were purchased from Sigma and Merck, except when stated in the text.

### 2.1 Producing coconut sugar at various pH and temperature

Fresh coconut neera was filtered and heated up to  $\pm 95^\circ\text{C}$  then the pH was adjusted to pH 4 and pH 6 by adding 33.3% citric acid or pH 8 by adding 10% lime solution. The process then continued to produce coconut sugar by setting temperature heating at  $100^\circ\text{C}$ ,  $105^\circ\text{C}$ ,  $110^\circ\text{C}$ , and  $115^\circ\text{C}$ , respectively. The process with temperature  $100^\circ\text{C}$  and  $105^\circ\text{C}$  produce liquid formed of coconut sugar, while the temperature at  $110^\circ\text{C}$  and  $115^\circ\text{C}$  produce solidified formed of coconut sugar.

### 2.2 Browning intensity determination

The browning intensity of coconut sugars was measured according to Phisut and Jiraporn (2013). The sugars were diluted (1:25 v/v) with distilled water and centrifuged at 3000 rpm for 15 min. The browning intensity was determined by monitoring the absorbance at 420 nm using a UV-Vis spectrophotometer (Shimadzu, model 1800).

### 2.3 Total phenolic contents determination

Total phenolic contents were estimated by Folin-Ciocalteu method (Shahidi and Naczka 2004). 100 mg samples were diluted with 70% ethanol and shook at 200 rpm for 2 h and then centrifuged at 1000 rpm for 15 min. 1 ml sample was mixed with 1.5 ml of 10% Folin-Ciocalteu. After 5 min incubation, 1.5 ml of 20% aqueous sodium bicarbonate were added, and the mixture was mixed and allowed to stand at room temperature in the dark for 90 min. The absorbance of all samples was measured at 765 nm using UV-Vis spectrophotometer (Shimadzu, model 1800). Results were expressed as percent of total phenolic content or as milligrams of tannic acid equivalent per gram of dry weight (mg TAE/g dw).

## 2.4 DPPH scavenging activity

DPPH scavenging activity was determined according to Tamrin (2012). 0.1 ml sample (100mg/ml) was added by 3.9 ml of 0.15 mM DPPH in methanol. Then the solution was mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of samples was measured at 517 nm using a spectrophotometer. 0.1 ml of absolute methanol with 3.9 ml DPPH was used as a control solution. The DPPH scavenging activity was calculated according to the equation:

$$\text{DPPH scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \%$$

## 2.5 Statistical analysis

Randomized Block Design method was used in this research. All parameters were conducted in triplicate and each experiment was performed at least 2 times. Data were analyzed with F test. Differences were considered significant at  $p < 0.05$ , and if the data show significantly different, then continued with Duncan's Multiple Range Test, 5% level.

## 3. Results and discussion

### 3.1 Browning intensity

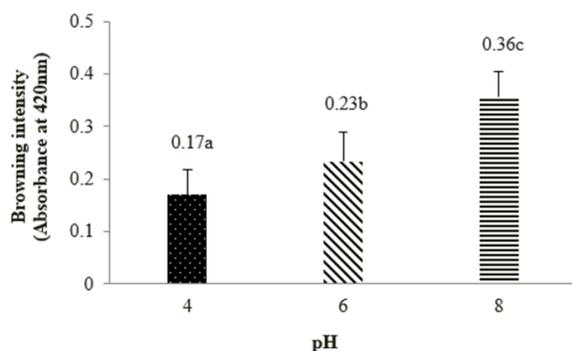


Figure 1. Effect of initial pH of coconut neera to the browning intensity of coconut sugar. Data obtained from the average of 3 replication. The value followed by the same superscript is not significantly different ( $p > 0.05$ )

Initial pH of coconut neera and end point temperature give a significant effect on the browning intensity of coconut sugar. Visually, increasing the pH of coconut neera and end point temperature further increase the brown color of coconut sugar. Increasing the initial pH of coconut neera further increase browning intensity of coconut sugar (Figure 1). The higher browning intensity showed the color of coconut sugar browner. It also indicated the browning reaction that occurs during heating coconut neera also increased. One of the

browning reaction that occurs during heating coconut neera is the Maillard reaction, which is nonenzymatic browning reaction that occurs via the reaction between the carbonyl group of a reducing sugar, especially with the primary amino group of amino acids, peptides, and proteins (Manzocco *et al.*, 2011).

Maillard reaction is strongly influenced by the pH which increases with increasing pH. At low pH, the amino group was protonation, so only a few amino groups were available for Maillard reaction (El-Ghorab *et al.*, 2010). Therefore, the formation of brown color was less at low initial pH than at high initial pH of coconut neera. Coconut neera contains sucrose that more easily hydrolyzed into glucose and fructose (reducing sugars) when heated at low pH conditions. Even though the presence of hydrolyzed sucrose can lead to reducing sugar more available for Maillard reaction, but it does not increase to Maillard reaction rate due to at low pH of the amine group is not available for the Maillard reaction itself.

The results of this study were in agreement with Wong *et al.* (2008) who examines the Maillard reaction between glucose and some amino acids under acidic conditions, where the majority of these reactions do not lead to the formation of brown color. Maillard reaction in model systems between glucose and glutamic acid (an amino acid predominant in coconut neera) forms brown color when the glucose medium pH 5, while at pH 3.2, no changes in color or in other words do not form a brown color (Wong *et al.*, 2008). El-Ghorab *et al.* (2010) also reported an increase in the intensity of browning of glucose-cysteine model system under alkaline conditions. In addition, the result of model Maillard reaction of fructose-lysine showed that the browning intensity also increased at higher pH (Ajandous *et al.*, 2001).

Maillard reaction is also strongly influenced by the temperature at which the higher temperature increases the Maillard reaction. Thus, the increase in temperature during the heating of coconut neera can increase browning reaction, therefore browning intensity value also was high.

Figure 2 shows that increasing the end point temperature of heating further increase browning intensity and reach a maximum when the temperature reached up to 115°C. This phenomena indicates that Maillard reaction rate increases at high temperatures than at low temperatures. Accordance with the theory that the reaction rate increases with increasing temperature. Heating coconut neera at high temperatures require more

time, so coconut neera was heated in a long time. This condition was implied to increase the browning reactions. This phenomenon was evidently proven by Phisut and Jiraporn (2013) through a model Maillard reaction between chitosan with 4 types of sugars (glucose, fructose, maltose, and lactose) that heated at 100°C for 1 to 7 hours and every hour measured for browning intensity. This study concluded that browning intensity of MRPs derived from chitosan-sugar model system was increased with the length of the heating time.

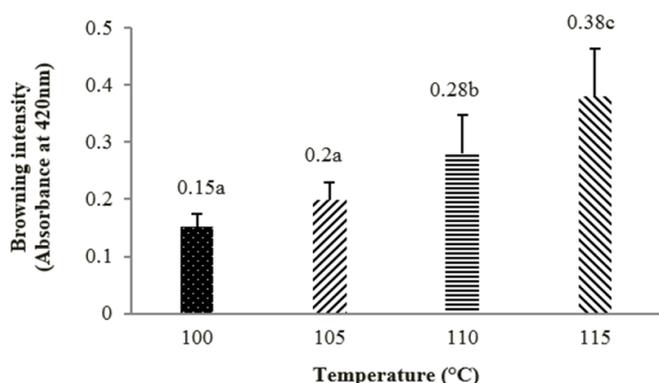


Figure 2. Effect of end point temperature on browning intensity of coconut sugar. Data obtained from the average of 3 replication. The value followed by the same superscript is not significantly different ( $p > 0.05$ )

### 3.2 Total phenolics content

In this study total phenolic content was expressed as percent of phenolics content on coconut sugar. Figure 3 shows that the total phenolic content of coconut sugar increases due to increased pH and temperature. This phenomenon indicated a correlation between the Maillard reaction with total phenolic contents. At high pH and temperature, Maillard reaction rate increases and the formation of MRPs also increases. Thus, there is the possibility that the phenolic group was produced from MRPs during heating coconut neera. El-Ghorab *et al.* (2010) also have demonstrated Maillard model system consisting of cysteine and glucose at low and high pH and resulted that a high total phenolic content was found in alkali conditions than in the neutral and acidic conditions. Some researchers showed a correlation between total phenolic with Maillard reaction products, especially melanoidin. There was a significant correlation between total phenolic with melanoidin on honey. The total phenolic on melanoidin fraction especially in the high molecular weight fraction increased after heating the honey (Brudzynski and Miotto, 2011). Our study also showed the same phenomenon where the total phenolics increased with increasing initial pH of coconut neera and the end point

temperature of heating. The presence of phenolic groups in the melanoidin fraction with high molecular weight has also been found in coffee brew due to the role of chlorogenic acid in the formation of melanoidin (Nunes and Coimbra, 2007; Bekedam *et al.*, 2008).

### 3.3 Antioxidant activity

The antioxidant activity of coconut sugar was measured using the method of DPPH free radical scavenging (2,2-diphenyl-1-picrylhydrazyl). The principle of the method is trapping hydrogen from antioxidant by free radicals. DPPH is the free radical source and will be reacted with samples containing antioxidants (Prakash, 2001). The presence of unpaired electrons in DPPH have strong absorption capability at a wavelength of 517 nm with a purple color. The presence of antioxidants causing change purple to yellow color that occurs due to changes of DPPH to DPPH-H (Xu and Hu 2004). The DPPH scavenging activity shown by the percentage reduction in the intensity of the purple color of DPPH (Prakash, 2001).

There is a significant effect between the treatment of initial pH and the end point temperatures with the ability to scavenge the DPPH radical on coconut sugar (Figure 4). Figure 4 shows an increase in activity of DPPH radical scavenging of coconut sugar along with the increase in the initial pH of coconut neera and the end point temperatures of heating. The antioxidant activity is thought to be played by Maillard reaction products. In this case, the high pH and temperature cause in high browning non-enzymatic reaction that is Maillard reaction. This reaction occurs during heating coconut neera so Maillard reaction products generated even higher. Thus, it can be said that in the heating process of coconut neera, high pH and high temperatures increased the Maillard reaction and increased the by-products with high antioxidant activity.

Most of the research on the antioxidant activity of Maillard reaction products were measured using the DPPH radical scavenging method. El-Ghorab *et al.* (2010) made a cysteine-glucose model system that reacted at a pH ranging from pH 4,5,7 to 10 and resulted in the highest antioxidant activity of the reaction products with pH 10. In addition, the system model of the Maillard reaction between D-glucose with L-asparagine and D-fructose with L-asparagine also produces a product that has scavenging radical DPPH activity (Echavaria *et al.*, 2013).

Based on the results of measurements of antioxidant activity was performed (expressed as percent DPPH

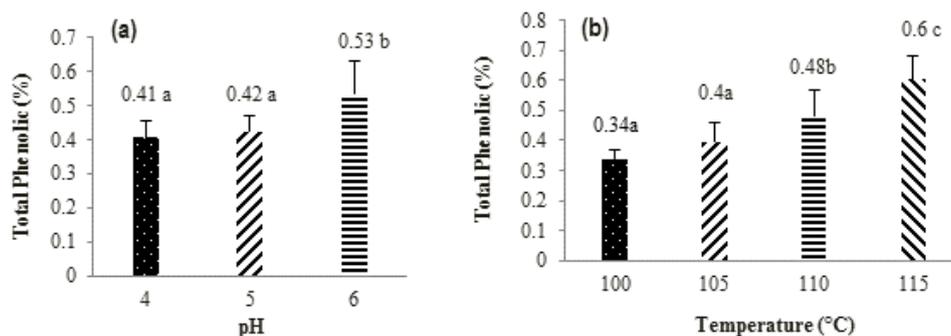


Figure 3. Effect of pH (a) and temperature (b) on total phenolics content of coconut sugar. Data obtained from the average of 3 replication. The value followed by the same superscript is not significantly different ( $p > 0.05$ )

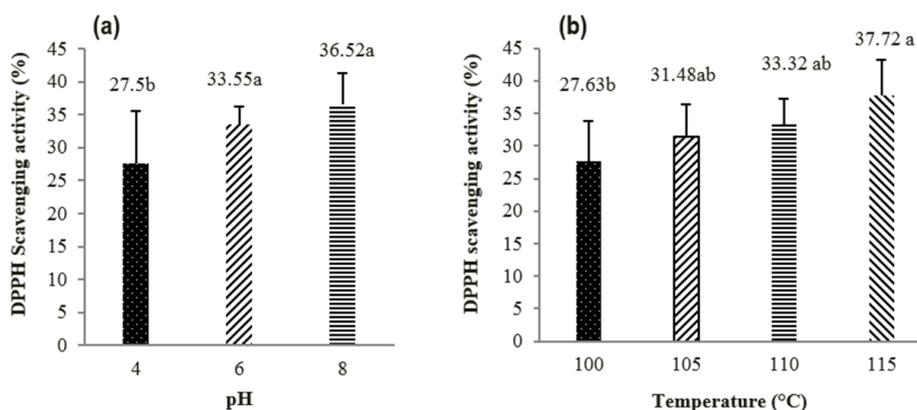


Figure 4. Effect of initial pH of coconut neera (a) and the end point temperature of heating (b) on DPPH radical scavenging activity of coconut sugar. Data obtained from the average of 3 replication. The value followed by the same superscript is not significantly different ( $p > 0.05$ )

radical scavenging), there is a strong correlation between the Maillard reaction products (which has brown color) to antioxidant activity. As the browning intensity increases, the antioxidant activity also increases. Correlation test between the browning intensity and the DPPH scavenging activity showed a positive correlation with a correlation coefficient ( $r$ ) of 0.93. The functional relationship between the browning intensity with radical scavenging activity was shown by the linear regression equation was  $Y = 8.44 + 71.68X$  (Figure 5).

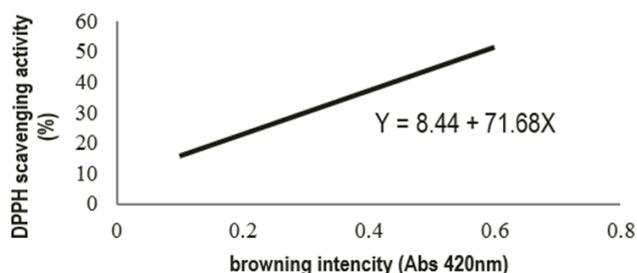


Figure 5. Linear correlation of browning intensity and DPPH radical scavenging activity

Correlation the browning intensity with antioxidant activity of Maillard reaction products have also been

proven by Phisut and Jiraporn (2013) when demonstrate a model Maillard system between chitosan with some kind of sugar, which the DPPH scavenging activity, hydroxyl radical scavenging, reducing power and inhibition of lipid peroxide by reaction products increases with increasing browning intensity. In addition, an increase in the browning intensity on the palm sugar-like flavouring also led to an increase in antioxidant activity (Amin *et al.*, 2010). El-Ghorab *et al.* (2010) also support this result by proving that DPPH radical scavenging activity increased with the increasing intensity of the Maillard browning reaction products.

#### 4. Conclusion

Browning intensity, total phenolic contents and antioxidant activity of coconut sugar increased with increasing an initial pH of coconut neera and temperature of its heating. It was found that the effect of pH at 8 and temperature at 115°C show highest total phenolics (0.48%) and browning intensity (0.35) of sugar. The treatment also exhibited good antioxidant activity (DPPH scavenging activity) as high as 40%. This result also indicated that there is a significant correlation between

browning intensity and antioxidant activity of coconut sugar. Further study should be conducted to explore and identify the components that responsible for antioxidant activity on coconut sugar.

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