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Antioxidant activities and mutagenic compounds in coconut and palm sugar from special region of Yogyakarta Indonesia

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Abstract

Coconut and palm saps containing antioxidant components, protein and various components of sugar undergo high heat treatment to produce hard and brown-colored sugar. Despite such treatment, the coconut and palm sugar still possess antioxidant activity through the formation of melanoidin. However, the treatment leads to the formation of mutagenic compounds such as 5–hydroxyethylfurfural (HMF), and furfuryl alcohol (FA). The antioxidant activity is measured using [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) assay and mutagenic compounds are measured using gradient HPLC methods with a UV detector. Coconut and palm sugar have higher antioxidant activity compared to total HMF. The mutagenic compounds found in sugar and coconut are HMF and furfural, but furfuryl alcohol is not present in the sugar. Trolox equivalent antioxidant capacity (TEAC) of coconut and palm sugar are 55.37 ppm and 110.74 ppm, respectively. Total HMF content in coconut and palm sugar is 3.25 ppm and 2.97 ppm, respectively. Total furfural content in coconut and palm sugar are 462.03 ppm and 371.87 ppm, respectively.

1. Introduction

Food with carbohydrate and protein contents undergo heat treatment with evaporation and it causes browning. The browning reaction is called the Maillard reaction (Tamanna and Mahmood, 2015). The brown color formed during food processing creates a specific flavor (Eskin and Shahidi, 2013). Scientists recently have identified that browning reactions in food produce antioxidant activity (Liu *et al*., 2014) and also some mutagenic compounds such as furfuryl alcohol (FA) (Wherry *et al*., 2019), 5-Hydroxymethyl-2-furfuraldehyde (HMF) (Lin *et al*., 2021), and furfural (Srivastava *et al*., 2018a).

Non-enzymatic reactions that can form HMF are Maillard reaction (Richards, 2015), caramelization (Lewkoski 2014; Chang *et al*., 2021), α-dicarbonyl cleavage, and recombination glyceraldehyde and methylglyoxal (Cämmerer *et al*., 1999). Furfural is formed through the Maillard reaction and caramelization (Srivastava *et al*., 2018a). Furfuryl alcohol mostly is generated by the degradation of 1,2-enediols during the high-heat process (Brands and Van Boekel, 2001).

Brown sugar could be made of coconut or palm sap. Coconut sap contains 0.26% protein and 6.91% sugar (Azlan *et al*., 2020). Coconut sap contains 339±47.2 mM sucrose, 9.6±7.8 mM fructose, 5.5±1.2 mM glucose (Nakamura *et al*., 2004). Alanine is the main amino acid in coconut sap (Yong *et al*., 2009). Compared to coconut sap, palm sap contains total sugar 10.36-18.94%, total protein 0.034%, total sucrose 9.40-17.44%, glucose 0.5-1.85%, and 0.48-1.81% fructose (Naknean *et al*., 2010). The main amino acid in palm sap is glutamine (Ho *et al*., 2008). The boiling of the coconut and palm sap evaporates the water content, and it leads to the creation of the brown color, antioxidant compound, and mutagenic compounds of brown hard sugar.

2. Materials and methods

2.1 Coconut and palm sap

The coconut sap and sugar were obtained from Karang Village, Bantul District, Special Region of Yogyakarta, Indonesia. The palm sap and sugar were obtained from Kulon Progo District, Special Region of Yogyakarta, Indonesia. Coconut and palm sugar were produced by heating coconut and palm sap.

2.2 Coconut sap, palm sap and sugar preparation

Coconut sap, palm sap and sugar preparation were

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performed using the method of Muriel *et al*. (2019). Approximately 1 L of the coconut and palm sap are taken after 10 hrs tapping from the tree flower. The sap is cooked for 1 hr until it reaches a certain viscosity and then poured into the mold made of coconut shell. Let the sugar palm cool and thicken.

2.3 Extraction methods for coconut and palm sugar

Coconut and palm sugar extraction was performed using the method of Azlan *et al*. (2020). Approximately 5 g sugar is mixed with 50 mL distilled water before the extraction with sonicator Elmasonic for 10 mins with a frequency of 32 kHz. Then, the sugar is filtered using filter paper Whatman 40. The supernatant is separated from the solid by centrifugation at 14000 rpm for 15 mins.

2.4 Determination of chemical compound of coconut and palm sap

Reducing sugars and total sucrose was determined using colorimetric method with spectrophotometer Thermo Scientific Genesys 10S UV-Vis (Başkan *et al*., 2016). The soluble solid was determined using the fusion method (Savjani *et al*., 2012) with Atago N-3E. The pH was determined using accurate electrochemical methods (Karastogianni *et al*., 2015) with a Hanna pH meter (serial number 1205609).

2.5 Determination of mutagenic compound

Determination of mutagenic compound was performed using high performance liquid chromatography (HPLC) method (Swasti and Murkovic, 2012). In brief, a 20 µL sample from coconut and palm sugar extract is injected into HPLC Shimadzu LC-10AT VP with a gradient time of 0-5 mins in 25% methanol (HPLC grade was obtained from Merck Darmstadt Germany), column temperature 30°C, column Purospher® RP-18 5 µm LiChroART® 125-4 (Merck, Darmstadt, Germany). Furfuryl alcohol (Sigma Aldrich, USA) and HMF standard (Fluka Chemikals, Buchs, Switzerland) curve is set from 0.5 ppm until 5 ppm and detected at 228 nm. The Furfural standard (Sigma Aldrich, Buchs, Switzerland) curve is set from 100 ppm until 500 ppm and detected at 277 nm.

2.6 Determination of antioxidant activity

Determination of antioxidant activity using radical [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) scavenging assay was performed using the method of Hu *et al*. (2005). ABTS (Sigma Aldrich, USA) is oxidized (7 mM) with 2.45 mM potassium persulfate (Merck, Darmstadt, Germany) for 16 hrs. Approximately 10 mL ABTS radical solution diluted with ethanol until 100 mL. The control solution was 1 mL ABTS radical cation solution and 10 µL ethanol. Both the sample (10 µL) and standard (10 µL) were added with 1 mL ABTS radical solution, respectively. Then, it was incubated for 1 min and detected at 734 nm with a spectrophotometer. The Trolox standard curve was made from 40 ppm to 200 ppm. Trolox stock solution was made by diluting 200 mg trolox (Sigma Aldrich, China) with ethanol until 100 mL.

3. Results and discussion

3.1 Chemical compounds of the coconut and palm sap

3.1.1 Sucrose concentration of coconut and palm sap

The sucrose concentrations of coconut and palm sap are 12.60±0.48% and 12.36±2.05%, respectively (Table 1). It is much higher than the findings of the previous research of which concentration is 6.91% (Asghar *et al*., 2020). The sucrose level is the highest concentration compared to fructose and glucose concentrations in coconut sap. Nevertheless, the sucrose concentration is much lower (15-17%) than those reported elsewhere (Mustaufik *et al*., 2022). This condition could be due to the higher yeast activity in this research.

Table 1. Characteristic of coconut and palm sap.

Type of Sap	Sucrose (%)	Reducing sugars (%)	°Brix	pH
Coconut	12.60±0.48	3.94±0.51	15	7.50±0.01
Palm	12.36±2.05	4.33±0.67	16	5.74±0.01

Values are presented as mean±SD of triplicate determinations.

3.1.2 Reducing sugars concentration of coconut and palm sap

The reducing sugars of coconut sap is 3.94±0.51% (Table 1). The reducing sugars of palm sap is 4.33±0.67%. These are higher than the previous study by Nurhadi *et al*. (2022) which found that the reducing sugars of coconut sap is 1.23%, but are similar to another research by Haryanti *et al*. (2020) which found that the reducing sugars of coconut sap is 3.78%. The difference in concentration is likely due to the difference in the type of preservative used during tapping time. The increase in tapping time may increase the concentration of reducing sugars due to the microbial activity (Mustaufik *et al*., 2021).

3.1.3 Soluble solid concentration of coconut and palm sap

The soluble solid coconut sap is 15°Bx. The soluble solid in palm sap is 16°Bx (Table 1). These results are in agreement with other researchers who reported that the concentration is around 12-18°Bx for coconut sap and 12.85-13.70 for palm sap (Haryanti *et al*., 2017;

Gunawan *et al*., 2020; Mustaufik *et al*., 2022). The high soluble solid is related to the high sucrose and reducing sugars concentration.

3.1.4 Degree acidity (pH) of coconut and palm sap

The pH of coconut sap is 7.50±0.01 and pH of palm sap 5.74±0.01 (Table 1). Previous research found that the pH of palm sap is 6.1 (Fariadatul Ain *et al*., 2014). The pH values in this research are nearly the same. The acidity of palm sap is contributed by lactic acid which was about 0.24-0.86% (Phaichamnan *et al*., 2010). The pH in coconut sap is contributed by citric acid, tartaric acid, malic acid, lactic acid, ferulic acid and pyroglutamic acid (Tomomatsu *et al*., 1996) and also ascorbic acid 20.6 mg/L (Xia *et al*., 2011).

3.2 Characteristic physic of coconut and palm sugar

The lightness of palm sugar (60.30±0.53%) is lower than the of coconut sugar (60.6±1.99%) (Table 2). It is likely attributed to the higher intensity of the browning reaction of the palm sugar (Tamanna and Mahmood, 2015). The higher intensity of the brown color of palm sugar is likely attributed to the higher melanoidin content (Apriyantono *et al*., 2002). The higher melanoidin content in palm sugar is brought about by the higher reducing sugars and total soluble solids in palm (Table 1).

3.3 Mutagenic compounds in coconut and palm sugar

In this research, there is no furfuryl alcohol (FA) (Figure 1) found in coconut and palm sugar (Table 3) although the other research found 0.1 ppm of furfuryl alcohol in palm sugar (Ho *et al*., 2007). However, this research found furfural (Figure 2) and HMF (Figure 3) in coconut and palm sugar. The non-enzymatic browning reaction induces the generation of mutagenic compounds such as HMF with the concentration of 5-500 ppm induce toxicity (Godfrey *et al*., 1999), furfural with LD_{50} 650-950 ppm in dogs (Hoydonckx *et al*., 2012), and furfuryl alcohol (Skog and Alexander, 2006). At the concentration of 100 ppm and inhaled for 16 weeks, furfuryl alcohol is able to induce cell degeneration (Savolainen and Pfäffli, 1983). Non-enzymatic reactions which are able to form HMF are Maillard reaction (Richards, 2015), caramelization (Lewkoski, 2014), α-dicarbonyl cleavage and recombination glyceraldehyde and methylglyoxal (Cämmerer *et al*., 1999). HMF is carcinogenic (Monien *et al*., 2009), but at the level of 80-100 mg/kg bw/d it is safe for some animals (Abraham *et al*., 2011). The HMF concentrations in this research are within the safe levels - 3.25 ppm of coconut sugar and 2.97 ppm of palm sugar.

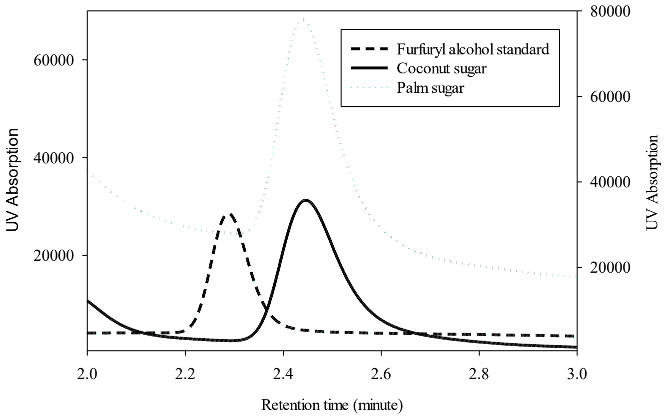

Figure 1. Chromatogram coconut and palm sugar compared to standard furfuryl alcohol.

Furfural is formed due to glucose in the sap (Rasmussen *et al*., 2014). The formation of furfural is less competitive than HMF (Hu *et al*., 2018). Furfural could be formed by heating ascorbic acid at 98°C (Nursten, 2005). Furfural is formed in the earlier

Table 2. Lightness of coconut and palm sugar.

Product	Sugar	
	Coconut	Palm
Sugar appearance		
Lightness (L*)	60.6±1.99	60.3±0.53

Values are presented as mean±SD of triplicate determinations.

Table 3. Antioxidant activity and mutagenic compounds of coconut and palm sap and sugar.

Tree	Trolox Equivalent Antioxidant Capacity (TEAC)		Mutagenic Compounds in Sugar (ppm)		
	Sap (ppm)	Sugar (ppm)	Furfuryl alcohol	HMF	Furfural
Coconut	332.51±0.04	55.37±0.01	0	3.25	462.03
Palm	117.15±0.02	110.74±0.01	0	2.97	371.87

Values are presented as mean±SD of triplicate determinations.

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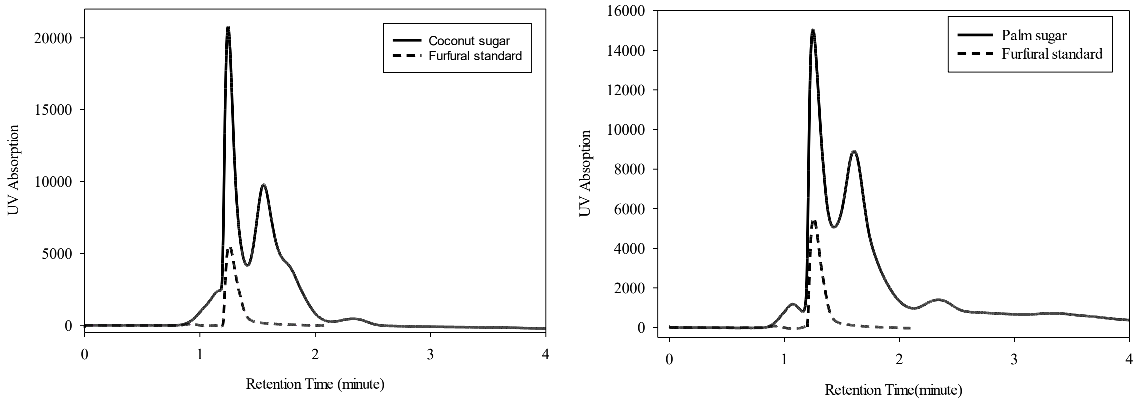

Figure 2. Chromatogram coconut and palm sugar compared to standard furfural standard.

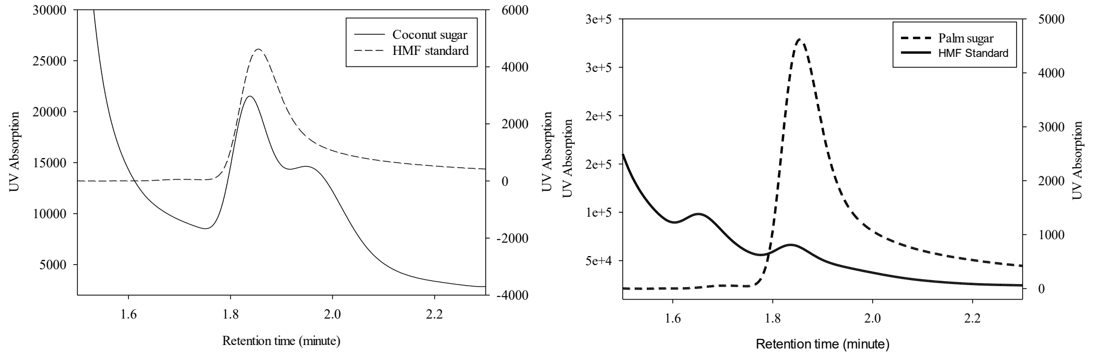

Figure 3. Chromatogram coconut and palm sugar compared to standard HMF standard.

Maillard (Srivastava *et al*., 2018b) reaction starting at 100°C with glucose as the model system. The addition of leucine is able to increase furfural content, but the furan content remains low. Furfural is mainly formed by the 1,2-enolization pathway via the 3-deoxyosone (Kroh, 1994) because 1,2-enolisation reaction is more favorable than 2,3-enolization. The formation of furfural is faster than furan (Srivastava *et al*., 2018b). The furfural content of coconut sugar (462.03 ppm) and palm sugar (371.87 ppm) are below the threshold for lethality.

HMF and furfural concentration are higher in coconut sugar than in palm sugar. It is because of the higher content of sucrose in coconut sap than in palm sap and accordingly, it increases the production of the mutagenic compounds. The sucrose produces a higher content furfural than glucose and fructose (Locas and Yaylayan, 2008). The high sucrose concentration in the high-heat process is also sufficient to produce furfural because the furfural production is attributed to the Maillard reaction and caramelization (Srivastava *et al*., 2018a).

3.4 Antioxidant activity of sap and sugar from coconut and palm tree

Sap and sugar from coconut and palm tree have antioxidant activity (Table 3). The antioxidant activity of coconut (332.51 ppm) and palm sap (117.15 ppm) have higher antioxidant activity than the coconut sugar (55.37 ppm) and palm sugar (110.74 ppm). The high antioxidant of sap is attributed to the content of ascorbic acid in the sap (Xia *et al*., 2011). The concentration of vitamin C in coconut sap is 116.19±0.87 mg/L and the coconut sap also contains high phenolic compounds of 0.34 g GAE/L (Devi *et al*., 2015), especially gallic acid and protocatechuic acid (Xia *et al*., 2011). The antioxidant activity of coconut sap is 21.85% (Asghar *et al*., 2020). During the heating, the concentration of ascorbic acid decreases.

After the heating process from 30 to 60 mins, coconut and palm sap still have antioxidant activity. It is due to the interaction of amino acid and sugar content which generates melanoidin as the end product of the Maillard reaction. The rise of the melanoidin content in coconut and palm sugar is concurrent with the decrease of sucrose, glucose, fructose and also amino acid during the sugar processing (Apriyantono *et al*., 2002; Ho *et al*., 2008). Melanoidin has antioxidant activity (Delgado-Andrade *et al*., 2010). Maillard reaction products also maintain the health of the liver (Oh *et al*., 2016). Although coconut sap has higher antioxidant activity than palm sap, palm sugar's antioxidant activity is higher than coconut sugar. The higher antioxidant activity in palm sugar is likely attributed to the higher melanoidin. Palm sugar has higher melanoidin than coconut sugar due the the higher concentration of reducing sugars and soluble solid in the palm sap. The higher intensity of the browning reaction during the heating enhances the antioxidant capacity (Açar *et al*., 2009; Zieliński *et al*., 2010). The high antioxidant activity of the palm sugar is contributed also by the high content of total phenol (Choong *et al*., 2016).

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4. Conclusion

The antioxidant activity of coconut sugar (55.37 ppm) and palm sugar (110.74 ppm) was higher than the HMF concentration. HMF content in coconut sugar was 3.25 ppm and palm sugar was 2.97 ppm. The furfural content in coconut sugar was 462.03 ppm and in palm sugar, 371.87 ppm. Therefore, coconut and palm sugar are considered safe to consume.

Conflict of interest

The authors declare that there are no conflicts of interest.

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