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The effect of liquefaction time and temperature on the quality and anthocyanin content of purple sweet potato maltohemidextrin

The effect of liquefaction time and temperature on the quality and anthocyanin content of purple sweet potato maltohemidextrin

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Abstract. Purple sweet potato is one type of tuber from the Convolvulaceae family and is one of the food commodities with high productivity and not yet optimally utilized in processing. Purple sweet potato contains starch and crude fiber, which are carbohydrate components that made it potential to be used as the substrate in maltohemidextrin production. Maltohemidextrin production can be done by hydrolysis of carbohydrate and fiber substrates using acid catalysts or enzyme catalysts. In this study, the enzymatic method was used in which the three different temperatures and several different liquefaction time were applied. This study resulted that there was no relationship between both the temperature and liquefaction time to the antioxidant content and activity. However, it showed a relationship with the value of reducing sugar and dextrose equivalent. It concluded that the best liquefaction temperature was 70°C.

1. Introduction

Potential carbohydrat[29]ources that have high availability are tubers, one of which is sweet potato. Some of the most abundant varieties of sweet potato are purple sweet potato (*Ipomoea batatas* L.). Purple sweet potato is one type of tuber with high productivity. According to FAO statistics in 2010, more than 100 countries plant sweet potatoes around the world. Production in Asia was the highest at 91.4%, followed by Africa 5.1%, Latin America, and then Europe. In Asia, countries with large production regions are China, Japan, South Korea, Vietnam, and Indonesia [1]. In Indonesia, the total purple sweet potato production in 2015 was 2,261,124 tons, with productivity of 160.53 quintal/hectare [2]. Increased consumption of purple sweet potatoes can be done through the promotion of sweet potatoes as a functional food and healthy food. Purple sweet potato has a pur[36] color, which is quite concentrated in the tuber flesh. Sarwono (2005) stated that the purple color of sweet pota[20]s is caused by the presence of anthocyanin pigments that are spread from the skin to the tubers [3]. Purple sweet potato contains a high amount of anthocyanin at around 110-210 mg/100g [4]. Anthocyanin compounds function as antioxidants, and free radicals antidote so that it plays a role in preventing aging, cancer, and degenerative diseases. Besides, anthocyanin also has the ability as an antimutagenic and anticarcinogenic, prevents impaired liver function, antihypertension, and reduces blood sugar levels [5], thus maintain the anthocyanin content during processing is necessary.

To date, the processing of purple sweet potato is still not optimal. The processing of purple sweet potato is still limited to sweet potato flour. Whereas, purple sweet potato contains starch and crude fiber, which are carbohydrate components that are still potential to be utilized. Proper handling of purple sweet potato processing needs to be done to improve the properties of the resulting product. Purple sweet potato starch can be converted or modified into a variety of starch-derived products. Hydrolysis can be used as an alternative process of processing purple sweet potato to optimize its utilization. Through this process, several stages of the process, such as extraction and drying of starch, as well as handling byproducts or waste, can be reduced. Hydrolysis products can be in the form of starch hydrolyzate and dietary fiber. Starch hydrolyzate can be used for industrial purposes of making glucose syrup, high fructose syrup, maltodextrin, and others, while food fiber can be applied to food processing industries. This potential makes sweet potato can be used as an ingredient in the production of maltohemidextrin.

Maltohemidextrin production can be done by hydrolysis of carbohydrate and fiber substrates using acid catalysts or enzyme catalysts. The use of enzyme catalysts as biocatalysts can reduce the effects of environmental impacts and food safety from the acidic process used. In making this maltohemidextrin, the α-amylase enzyme is used as enzyme catalysis. However, the hydrolysis process of carbohydrate and fiber substrate using enzymes is very susceptible to high heating, so it is necessary to know the optimal temperature and duration of the liquefaction reaction in the production process of maltohemidextrin in maintaining its bioactive compounds.

2. Methods

2.1. Purple sweet potato preparation

Purple sweet potatoes are sorted and separated, and for this research, only the good quality was used, which were undamaged and no injuries sweet potato. The sweet potatoes were then washed using flow water and then drained. After that, purple sweet potatoes are peeled and sliced to a thickness of 3 cm and then directly put in a container filled with water to avoid oxidation. Then the blanching process is carried out (T: 70^{o}C, t: 15 minutes), then the sweet potatoes were grated. The grated sweet potato then used as a primary ingredient in the production of maltohemidextrin.

2.2. Maltohemidextrin production

Purple sweet potato suspension with a concentration of 15% w/v was made in a volume of 100 mL, and the pH was adjusted ± 5.5-6.5 with the addition of 0.1N HCl or NaOH solution. After that, it was gelatinized, and then 40 ppm of CaCl2.H2O cofactor and amylase enzyme was added to the solution. Liquefaction was performed through heating and stirring using a water bath shaker according to the temperature treatment (60 ° C; 70 ° C; 80 ° C), with speed (ω= ± 200 rpm). The hydrolysis results are then sampled according to the research design. Maltohemidextrin products obtained were analyzed for their contents.

2.3. Buffer making

In this study was used two types of buffers which were buffer pH 1.0 and a buffer with pH 4.5. To make a buffer with a pH of 1 was used 1.86 g of KCl mixed with 980 mL of distilled water and adjusted to reach pH = 1 using concentrated HCl. Then the solution was transferred into a 1 L measuring flask and added distilled water to the mark. While to make a buffer with a pH of 4.50 was used 54.43 grams of $CH_3CO2Na.3H_2O$ mixed with 950 mL demineralized water. Then the pH measure and adjusted with the addition concentrated HCl until a solution with a pH of 4.50 was obtained. Then the solution was transferred into a 1 L measuring flask and added distilled water until the total volume of the solution is 1 L.

2.4. Anthocyanin extraction

Anthocyanin extraction is done by weighing 1 gram of sample and then extracted by wet maceration technique using 96% ethanol solvent and 3% HCl with a solvent and sample volume ratio of 4: 1. Maceration was performed for 24 hours, then filtered using filter paper.

2.5. Research design

In this study was used two factors which were liquefaction temperatures (60°C, 70°C, and 80°C) and liquefaction times (0, 20, 40, 60, 80, 100, and 120 minutes).

2.6. Observation parameters

2.6.1. Anthocyanin content (pH differential method)

The anthocyanin content measurement was performed following a method used by Ticoalu *et al.* (2016) in which two extracted sample solutions were prepared as much as 0.5 ml [6]. The sample in the first test tube was added with an HCl-KCl buffer solution with a pH of 1 ml of 2.5 ml. Samples in the second test tube were added Na-acetate buffer solution with a pH of 4.5 as much as 2.5 ml. Then the Absorbance of both samples was measured at wavelengths of 510 and 700 nm. The wavelength of 510 nm is the maximum wavelength for cyanidin-3- glucoside, while the wavelength of 700 nm is to correct deposits that are still present in the sample. If the sample is clear, then the Absorbance at 700 nm is 0. The Absorbance is then determined by the formula:

$A = [(A_{510}-A_{700})\ pH\ 1 - (A_{510}-A_{700})\ pH4.5]$

The anthocyanin pigment content in the sample is calculated by the formula:

$$MAP\ (mg/L) = \frac{A \times Mw \times DF \times 1000}{£ \times 1}$$

where

A : Absorbance
Mw : Molecular weight = 449.20 (expressed as cyanidin-3-glycosides)
DF : Dilution factor
£ : Molar absorption coefficient = 26900 (expressed as cyanidin-3-glycosides)
MAP : Monomeric Anthocyanin Pigment

2.6.2. Antioxidant activity

The antioxidant activity of maltohemidextrin made from purple sweet potato is determined by the spectrophotometer method using DPPH [7]. Maltohemidextrin sample extracts were tested in several concentrations, namely 200, 400, 600, and 800 ppm. The DPPH solution used was made by dissolving DPPH crystals in a methanol solvent with a concentration of 1 mM, which was carried out at low temperatures and protected from light. The extract solution that has been made, then each of 4.5 ml was reacted with 0.5 ml of 1 mM DPPH solution in a test tube and labeled. The mixture was incubated at 37oC for 30 minutes. Then Absorbance was measured using UV-Vis spectrophotometry at a wavelength of 517 nm. The Absorbance of the blank solution is measured to calculate percent inhibition. A blank solution was made by mixing 4.5 ml of methanol with 0.5 ml of 1 mM DPPH solution in a test tube. The antioxidant activity of each sample is expressed by the percentage of free radical inhibition (percent inhibition) which can be calculated with the following formulation:

$$\% \text{ inhibition} = \frac{A0-A1}{A0} \times 100\%$$

where:
A0 = Absorbance of the blank
A1 = Absorbance of the sample

The values of sample concentration and percent inhibition were plotted respectively on the x and y-axis in the linear regression equation. The linear regression equation obtained in the form of an equation: y = b (x) + a, is used to find the value of IC_{50} (inhibitor concentration 50%) of each sample, by expressing the value of y by 50 and the value of x as IC_{50}. IC_{50} value states the concentration of the sample solution needed to reduce the DPPH by 50% so that the value of 50 is substituted in the line equation y = ax + c for the value of y. After substituting the value of 50 on the value of y, we will get the value x as the value of the IC_{50}. An antioxidant compound is said to be a powerful antioxidant if the IC_{50} value is less than 50 ppm, strong if the IC_{50} value is 50-100 ppm, while if the IC_{50} value is 100-150 ppm, it is weak if the IC_{50} value is between 150-200 ppm, and very weak when the value IC_{50} is more than 200 ppm [8].

2.6.3. *Reducing sugar*
Reducing sugar analysis performed using the same method applied by Djalal (2019) by adding dinitrosalycilyc acid (DNS) to the sample and applied heating to let the DNS reacted with sugar in the sample and create a color that further analyzed with a spectrophotometer in the wavelength of 550 [9].

2.6.4. Dextrose equivalent
Dextrose equivalent is the ratio between reducing sugars and the initial substrate used that can be obtained with the following formula:

$$DE = \frac{\sum \text{reducing sugar formed } \left(\frac{b}{v}\right)}{\sum \text{initial substrate used } \left(\frac{b}{v}\right)} \times 100\%$$

2.7. Data analysis
All parameters tested were analyzed using Analysis of Variance (ANOVA) using *Microsoft Excel* 2016 dan IBM SPSS *Statistics* Version 23 software, with two replications. Then followed by Duncan's test to see the differences.

3. Result and discussion

3.1. Total Anthocyanin levels
Anthocyanin is a secondary metabolite compound from the flavonoid group and is a derivative of 2-phenylbenzopyrilium, which is responsible for the purple base color in purple sweet potatoes. Anthocyanin compounds have the characteristics of two absorption regions at wavelengths of 260-280 nm (UV) and 490-550 nm (visible) and are measured by UV-Vis spectrophotometer [10]. Anthocyanin is water-soluble and unstable during the heating process. Anthocyanin purple sweet potato is easily oxidized and denatured so that biological activity can be easily reduced during processing [11]. The observation result showed that the highest anthocyanin content was at 80°C and 0 minutes of liquefaction time, which was 14.19 mg/L, and the lowest anthocyanin content was at 70°C and 20 minutes of liquefaction time which was 6.89 mg/L.

Table 1. Total anthocyanin in maltohemidextrin made from purple sweet potatoes (mg/L)

Liquefaction Temperature (°C)	Liquefaction time (Minutes)							Average
	0	20	40	60	80	90	120	
60	12.73	12.11	8.77	12.11	10.85	11.69	10.02	11.18
70	8.56	6.89	10.02	9.39	7.10	8.56	11.48	8.86
80	14.19	11.69	7.31	9.60	7.93	7.31	6.89	9.27
Average	11.83	10.23	8.70	10.37	8.63	9.19	9.46	9.77

The results of the analysis of variance showed that the temperature and the liquefaction time did not significantly affect the degradation of anthocyanin content of maltohemidextrin purple sweet potato at a 5% level. The content of anthocyanin at 60°C ranges between 8.77-12.73 mg/L; at 70°C ranges between 6.89-11.48 mg/L; and 80°C ranges between 6.89-14.19 mg/L (Table 1). Anthocyanin levels of maltohemidextrin based on temperature range between 9.27-11.18 mg/L while anthocyanin level based on the liquefaction duration ranged between 9.46-11.83 mg/L (Table 1). The result obtained in this research is not in line with Santoni *et al*., in which the longer the heating duration and the higher the temperature cause the increase in the degradation rate of anthocyanin [10].

3.2. IC_{50} Antioxidant activity

Testing the antioxidant activity of maltohemidextrin samples made from purple sweet potato in this study was carried out using the DPPH test method. This method is one of the most widely used methods for estimating the effectiveness of the performance of substances that act as antioxidants [30] This testing method is based on the ability of these antioxidant substances to neutralize free radicals (1,1-diphenyl-2-picrylhydrazyl (DPPH)) [12].

The observations showed that the highest antioxidant activity of IC_{50} at treatment temperature of 70 °C and 0 minutes liquefaction, namely 868.28 mg / L and the lowest antioxidant activity of IC_{50} at treatment temperature of 60 °C at 20 minutes liquefaction was 7165.90 mg / L. The antioxidant activity of IC_{50} at 60 °C ranges between 1950.61-7165.90 mg / L; the temperature of 70 °C ranges from 868.26-5563.34 mg / L; and a temperature of 80 °C ranging from 2544.09-5889.24 mg / L. The results of the antioxidant activity of IC_{50} maltohemidextrin purple sweet potato substrate obtained based on temperature variations ranging from 2124.67-4193.71 mg / L. Whereas IC_{50} antioxidant activity based on liquefaction duration ranged from 2342.94-4592.11 mg / L. The smaller IC_{50} value indicates higher antioxidant activity in the material being tested [8].

The results of the analysis of variance showed that the liquefaction temperature had a significant effect, and the length of the liquefaction did not show any significant effect on the antioxidant content of purple sweet potato ($p<0.05$). While the interaction between the two also has no real effect. Duncan further test of the liquefaction temperature was carried out to see significantly different results. Liquefaction temperature affects the results of the antioxidant activity of IC_{50} maltohemidextrin caused by the presence of a large number of anthocyanin pigments and the synergistic effect of other substances present in the extracted sample [13].

The antioxidant activity was different from the difference liquefaction temperature applied. Theoretically, the degradation of antioxidant activity is greatly influenced by the liquefaction temperature. However, in this study, we can draw any relation to the temperature and antioxidant activity since it is not

showing a positive or an inverse relationship. The result obtained in this study is not in line with Carlos *et al.* (2012) statement that said that the heating process decreases the carotene, total phenol, and antioxidant activity [14]. The antioxidant activity value of Maltohemidextrin in different Liquefaction temperatures is presented in figure 1.

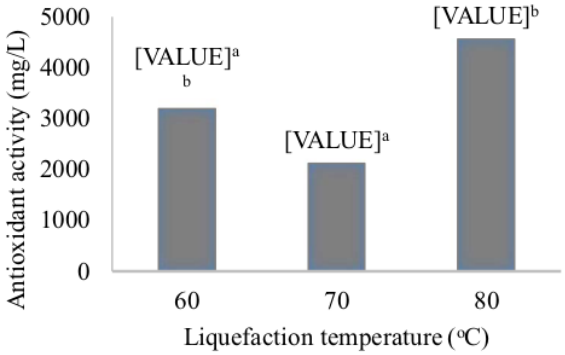

Figure 1. Antioxidant activity value of maltohemidextrin in different liquefaction temperature
The numbers that followed by different letter showed a significant difference ($p<0.05$)

Figure 1 showed that the lowest antioxidant activity was in the sample that received the highest liquefaction temperature. However, as the highest antioxidant activity is showed by a sample with 70°C liquefaction temperature and not the lowest liquefaction temperature, which was 60°C, we cannot draw any relation of the antioxidant activity and the liquefaction temperature. In general, in this study, the antioxidant activity of the tested maltohemidextrin still had weak antioxidant activity in counteracting free radicals. This can be seen in the antioxidant activity of IC_{50} at 70°C, which is 2124.67 ppm or 2.12 mg/ml. This is based on the statement of Blois (1958) in Molyneux (2004) which states that a compound is said to be a powerful antioxidant if it has an IC_{50} value of less than 0.05 mg/ml, strong if the IC_{50} value is between 0.05-0.10 mg/ml, while the IC_{50} value is between 0.10-0.15 mg/ml, and weak when the IC_{50} value is between 0.15-0.20 mg/ml [8].

3.3. Reducing sugar

Reducing sugar is a sugar that can reduce electron-accepting compounds. This is due to the presence of aldehyde and ketone groups. All types of monosaccharides (glucose, fructose, and galactose) and disaccharides (lactose and maltose) except sucrose and starch are types of reducing sugars. The results of observations of the lowest reducing sugar levels at a treatment temperature of 60 °C with 0 minutes liquefaction time are 2.71 g / L, and the highest reducing sugar is at 80 °C and 120 minutes liquefaction, which is 12.00 g / L.

The results of the analysis of variance showed that the liquefaction temperature and the duration of the liquefaction reaction significantly affected the maltohemidextrin reducing sugar made from purple sweet potato ($p < 0.05$) while the interaction between the two had no significant effect. Duncan further test of the liquefaction temperature and the length of the liquefaction was carried out to see significantly different results. These results indicate that the liquefaction temperature of 60 °C, 70 °C, and 80 °C and the liquefaction time have a significant effect on the reducing sugar produced. Liquefaction temperature affects the activity and stability of the process. The higher the temperature used, the enzyme activity will increase and decrease enzyme stability. Conversely, low temperatures can increase stability but decrease

productivity in hydrolyzing the carbohydrate component. In this research, a temperature of 60°C was found as the best treatment to obtain the amount of desired reduced sugars. This is in line with research conducted by Devita (2013) where the value of the best reduction levels is also in the treatment with a temperature of 60 °C because the higher the temperature used will cause more glycosidic bonds that can be broken due to increased enzyme activity so that the resulting product not only destrins and oligosaccharides but also glucose and maltose are formed [15]. The relationship of liquefaction temperature to reducing maltohemidextrin sugar is presented in Figure 2.

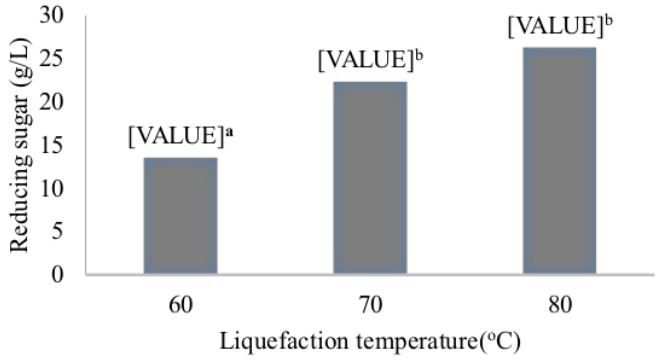

Figure 2. The reducing sugar content in different liquefaction temperature
The numbers that followed by different letter showed a significant difference ($p<0.05$)

The activity of the α-amylase enzyme experiences instability at high temperatures so that it affects the value of the reducing sugars produced. Enzyme activity is also influenced by the duration of the starch hydrolysis reaction, the longer the liquefaction process, the higher the reducing sugar produced.

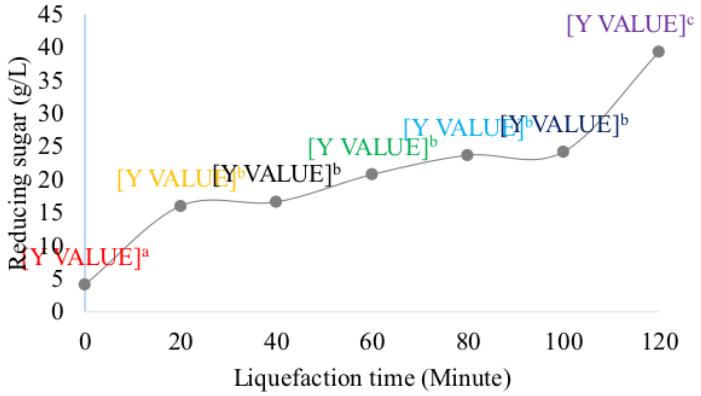

Figure 3. The reducing sugar content in different liquefaction time process
The numbers that followed by different letter showed a significant difference ($p<0.05$)

Figure 3 shows that reducing sugar levels have increased during the hydrolysis process. Liquefaction reaction time causes an increase in reducing sugar levels during the hydrolysis process. This is because the

more starch granules are broken down due to the hydrolysis proce to form a greater reducing sugar. Each hydrolyzed sugar chain has one reducing sugar group so that an increase in the number of simple sugars as the enzyme hydrolysis activity increases will cause the value of reducing sugars to increase. This is in accordance with Ambriyanto (2010), which states that the longer the interaction between the enzyme and the substrate causes more and more simple sugars to form [16].

3.4. Dextrose equivalent

DE value affects the characteristics of maltodextrin. If the DE value is high, then the value of hygroscopicity, plasticity, sweetness, solubility, and osmolality is also high. Besides, starch will be easier to experience the browning process. However, if the DE value is low, it will increase the molecular weight, viscosity, cohesiveness, and film-forming properties. Also, a low DE value results in the formation of preventable large sugar crystals [17].

The lowest DE was observed at a treatment temperature of 60 °C with 0 minutes liquefaction was 3.87%, and the highest DE at a treatment temperature of 80 °C and 120 minutes liquefaction was 60.00%. DE maltodextrin values based on temperature variations ranged from 19.21 to 37.43%, while DE value based o quefaction duration is around 5.83-56.09%.

The results of the analysis of variance showed that the liquefaction temperature and the reaction time of liquefaction had a significant effect on the DE value of maltohemidextrin so that Duncan further tests were conducted to see the effect of temperature and duration of the liquefaction reaction on DE maltohemidextrin values.

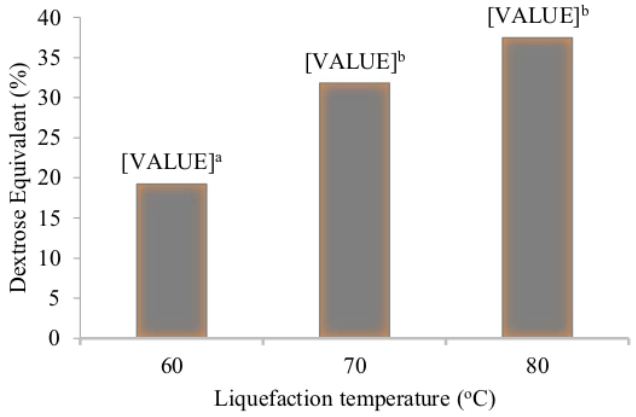

Figure 4. The dextr equivalent value in different temperature of the liquefaction process
The numbers that followed by different letter showed a significant difference ($p<0.05$)

Maltohemidextrin hydrolysis process made from purple sweet potato at high temperatures causes more starch to be converted so that the DE value produced will be higher along with the liquefaction process. This process causes amylopectin compounds to be reduced so that the resulting maltohemidextrin becomes more soluble in water, and more starch is hydrolyzed into a simple compound, namely dextrose (glucose).

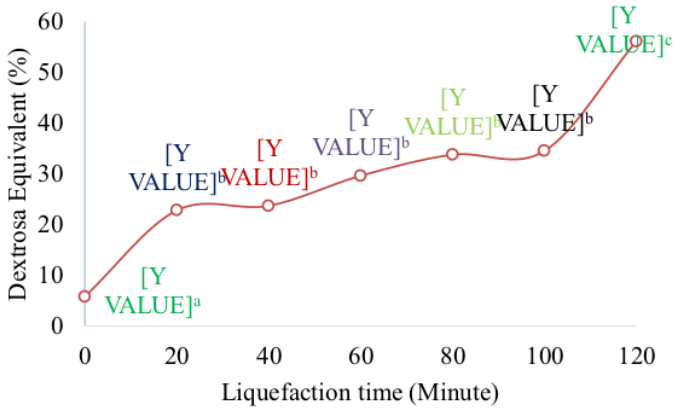

Figure 5. The dextrose-equivalent value in different liquefaction time process
The numbers that followed by different letter showed a significant difference ($p<0.05$)

Figure 5 shows that the liquefaction time causes a significant increase in the value of dextrose equivalent. This is because the longer the hydrolysis reaction, the higher the DE value obtained. Purple sweet potato maltodextrin obtained following USPNF XVII value standards for maltodextrin products that have a DE value range of 5-20 [18]. The high DE value obtained is due to the large number of starches hydrolyzed into reducing sugars. Also strengthened by Triyono (2008) in a paper by Berghmans (1981) that the hydrolysis of starch from sweet potatoes at the liquefaction stage with the α-amylase enzyme in a few minutes could reach DE more than 20% [19].

4. Conclusion

It can be concluded that the best liquefaction temperature is 70°C, considering the dextrose equivalent value, reducing sugar and the antioxidant activity, as well as considering the energy needed for the liquefaction. While about the liquefaction time, we can see that the reducing sugar and dextrose equivalent value is increasing with the increasing of liquefaction time, but for the effect of liquefaction time to the antioxidant content, we cannot draw any conclusion from the result of this study.

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