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Citric Acid Production from Toba Banana Peel (*Musa acuminata* Colla) through Submerged Fermentation using *Aspergillus niger*

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Toba banana peel waste is derived from Toba banana fruit (*Musa acuminata* Colla) processing. Local people utilized banana peel waste usually as livestock feed. The waste also can cause an environmental problem if it is not handled well. Banana peel waste has a high content of carbohydrate that can be fermented to produce more valuable product, one of which is citric acid. Citric acid is an organic acid that is consumed globally and produced in large quantities. In food and beverages industries, citric acid is used for various purposes due to its high solubility, non-toxic, and good taste characteristics. The objective of this research is to determine the optimum conditions of submerged fermentation of banana peel to produce citric acid using *Aspergillus niger*. The treatments were various banana peel concentrations (5%, 10% and 15% w/v) added with 5% sucrose or 5% glucose (w/v). During the fermentation, pH was measured to determine pH changes indicated the production of citric acid. The results showed that the variation concentration of banana peel substrate and type of sugars affect citric acid production. The optimum condition of submerged fermentation by *Aspergillus niger* was obtained at 15% substrate concentration by adding 5% sucrose to produce 0.651% (w/v) of citric acid.

Key words: *Aspergillus niger*, citric acid, *Musa acuminata* Colla, submerged fermentation

Kulit pisang Toba merupakan limbah buangan dari hasil pengolahan buah pisang Toba (*Musa acuminata* Colla). Umumnya pemanfaatan kulit pisang oleh masyarakat lokal hanya digunakan sebagai bahan pakan ternak. Limbah tersebut juga dapat menimbulkan masalah lingkungan apabila tidak ditangani dengan baik. Kandungan karbohidrat yang tinggi pada limbah kulit pisang Toba dapat diolah melalui fermentasi untuk menghasilkan produk komersial yang lebih bernilai, salah satunya adalah asam sitrat. Asam sitrat adalah asam organik penting yang dikonsumsi secara global dan diproduksi dalam jumlah besar. Asam sitrat di industri pangan banyak digunakan untuk berbagai keperluan karena kelarutan asam sitrat yang tinggi, tidak beracun dan rasanya yang disukai. Penelitian ini dilakukan dengan tujuan untuk mengetahui kondisi optimum *submerged fermentation* kulit pisang Toba dalam menghasilkan asam sitrat dengan menggunakan *Aspergillus niger*. Perlakuan yang diberikan adalah variasi konsentrasi substrat kulit pisang Toba (5, 10, dan 15% b/v) serta variasi jenis gula 5% sukrosa dan 5% glukosa (b/v). Selama fermentasi dilakukan pengukuran pH, bertujuan untuk mengetahui perubahan pH yang mengindikasikan produksi asam sitrat. Hasil penelitian menunjukkan adanya pengaruh konsentrasi substrat kulit pisang Toba dan jenis gula pada produksi asam sitrat. Kondisi optimum *submerged fermentation* kulit pisang Toba oleh *Aspergillus niger* diperoleh pada konsentrasi substrat 15% dengan penambahan 5% sukrosa yaitu menghasilkan konsentrasi asam sitrat sebesar 0,651% b/v.

Kata kunci: asam sitrat, *Aspergillus niger*, *Musa acuminata* Colla, *submerged fermentation*

Banana is a tropical fruit plant that is liked by most of the world's population. Banana production continues to increase annually by 4.16% and in 2015 reached 7.3 million tons (Kementerian Pertanian Indonesia 2016). Abundant of banana production affects the increase in banana peel waste. Banana peel is a waste from processing bananas. In general, the use of banana peel by local people are used as animal feedstock ingredients such as cows, goats, buffaloes and several others species. The banana peels waste also can make environmental problems if it is not handled well.

The banana peels waste contains high carbohydrates. Carbohydrates found in banana peel waste can be used to produce more valuable commercial products. Along with the increase in industrial technology, the review regarded to utilizing banana peel waste is increasing. Several studies have been conducted on banana peel waste as a substrate to produce useful products such as xylitol, bio methane, bioethanol, and absorbers in removing minerals in waste. This is very interesting for scientists because banana peels can be used as raw materials that are very beneficial for the community.

High carbohydrate content in banana peel has the

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potential to produce more valuable commercial products, one of them is citric acid. Citric acid is an important organic acid that is consumed globally and produced in large quantities. Citric acid in the food and beverage industry is widely used for various purposes because of the high solubility of citric acid and nontoxic. Some of studies on banana peel have been done in producing citric acid through a submerged fermentation process (Abbas *et al*. 2016; Khairan, Makstum and Yulvizar 2019) and others through solid state fermentation (Iralapati and Kummari 2015; Monrroy *et al*. 2019). In these studies, banana peel was fermented using *Aspergillus niger* at 30°C to produce citric acid. Fermentation of various species of banana peel (*Musa canabalisa*, *Musa paradisiaca*, *Musa acuminata*, and *Musa balbisiana*) obtained citric acid yield average of 21,76 g kg^{-1} (Iralapati and Kummari 2015), 29 g kg^{-1} (Monrroy *et al*. 2019), 52 g L^{-1} (Abbas *et al*. 2016), 69.84% (Khairan*et al*. 2019), and 87% (Dhandayuthapani *et al*. 2008).

Production of citric acid from banana peel is conducted as on one of the solution to increase the quality value of banana peel and reduce environmental problems caused by banana peels. In the fermentation research conducted by Varshey (2016) using mixed fruit peels, it showed a high concentration of citric acid at 30 °C fermentation conditions for 5 days, and the addition 0.25% w/v NH_4NO_3 and 4% methanol. Based on the previous studies, the objective of this research is to determine the optimum conditions of submerged fermentation of Toba banana peel to produce citric acid using *Aspergillus niger*. The treatments were various banana peel concentrations (5%, 10% and 15% w/v), and the addition of 5% sucrose and 5% glucose (w/v). During the fermentation, pH was measured to determine pH changes that indicates the production of citric acid.

MATERIALS AND METHODS

Pretreatment of Substrates. Toba banana peels were washed using water, chopped and dried in the oven at 60 °C for 2 days. The dried banana peel was mashed using a blender until it becomes powder.

Cultivation of Microorganism. Prepared 100 mL of Potato Dextrose Agar (PDA) media and poured into 2 sterile petri dish. Then, the inoculum was grown from stock culture into a petri dish containing PDA and incubated at 37 °C for 3 days.

Preparation of Inoculum. Prepared 100 mL of Yeast Pepton Glucose (YPG) media with a composition of 1% yeast extract, 2% peptone, and 2% glucose. Then, 3 inoculating loop of fungal colonies on culture media, inoculated into the media and incubated for 36 hours at 37 °C.

Preparation of Substrate. Prepared 100 mL of fermentation media with composition of 5% sugar, NH_4NO_3 0.25%, KH_2PO_4 0.1%, $MgSO_4$ 0.025%, methanol 4%, and Toba banana peels substrate 5%, 10%, and 15%.

Fermentation. The fermentation media of banana peel was inoculated by aseptically transferring 5% of starter culture to the fermentation media. The media was stirred and then incubated at 30 °C for 5 d. During the fermentation, pH measurements were conducted to determine the pH changes every 24 h using a pH meter.

Separation of Fermentation Results. Fermentation media that has been incubated for 5 d was cooled at room temperature. Then filtered using filter paper to obtain the filtrate and saved at -4 °C.

Analysis of Citric Acid with HPLC. The citric acid was analyzed using the High Performance Liquid Chromatography (HPLC). The HPLC column used was Zorbax Eclipse Plus C-18 with a mobile phase mixture of methanol and acetonitrile, pH 2.5 KH_2PO_4 buffer as a solvent, and citric acid as a standard. Mobile phase mixing is carried out in a ratio of 60:40. Mobile phase and solvent solution was filtered using a membrane with the help of a vacuum pump.

The standard citric acid curve is carried out by diluting 10% citric acid using a buffer solution. The concentrations of citric acid made are 0.05%, 0.1%, 0.5%, 1%, and 1.5%. HPLC analysis results produced peaks at certain retention times of each sample and indicated citric acid compounds. The linear equations from standard solutions, used to obtain the concentration of citric acid**.**

RESULTS

The pH value of banana peel fermentation media in both additional of glucose 5% or sucrose 5% shown in Figure 1. During the fermentation process there was a decrease in pH reached 3.79 for addition of glucose 5% and for addition of 5% sucrose reached 3.59, for 120 hours or five-day fermentation times.

The concentration of citric acid produced in the fermentation of Toba banana peel is obtained from the results of HPLC analysis (Table 1). From the analysis of the value of citric acid concentration, there is a significant difference in each of the concentration of the banana peel substrate 5%, 10% and 15% in the addition

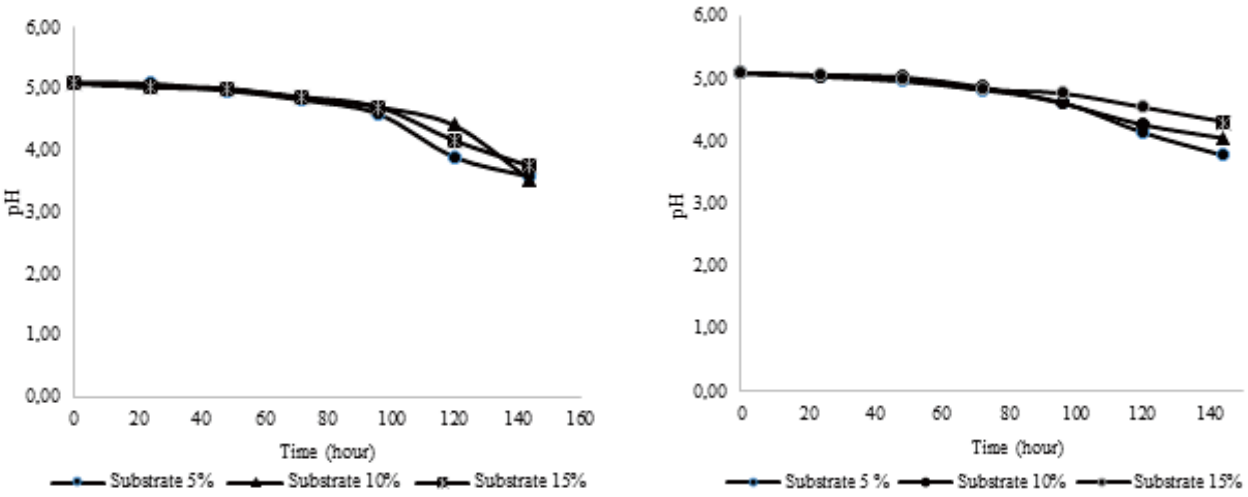

Fig 1 Fermentation medium pH value in addition of 5% glucose (left) or 5% sucrose (right).

Table 1. Citric Acid Concentration

Banana peel substrate % (w/v)	Citric acid concentration % (w/v)	
	5% sucrose added (w/v)	5% glucose added (w/v)
5	0.379 a (a)	0.257 a (a)
10	0.523 b (a)	0.383 b (a)
15	0.651 c (a)	0.474 c (a)

Note: Different characters in the same column indicates a significant difference among data. The same character in parentheses in the same row does not indicate a significant difference among data.

of 5% sucrose or in the addition of 5% glucose. Citric acid concentration was increase significantly with the increasing concentration of Toba banana peel substrate, whereas the addition of 5% sucrose tended to increase citric acid concentration compared to the addition of 5% glucose.

DISCUSSION

Decreased pH is one of the factors indicating the formation of organic acids. In this research, pH was decrease in the range of 3 – 4. The pH of the media changes continuously during the fermentation process as a result of the metabolic activity of microorganisms, due to the secretion of organic acids such as citric acid, gluconic acid, and oxalic acid (Show *et al*. 2015). To accumulate citric acid, the pH of fermentation media is the most important factor for two reasons, the sporulation phase and production. In the sporulation phase, the spores need pH 5. The germinated spores absorb nitrogen derived from ammonia and release the proton (H^+), resulting in increased acidity in the media and supporting the production of citric acid. At low pH ($pH \leq 2$), the formation of unexpected products such as oxalic acid and gluconic acid is inhibited, and the possibility of contamination by other microorganisms is also reduced which makes the production of citric acid higher (Max *et al*. 2010). Due to the pH range of 3-4, it is indicated that in the end of fermentation there are still other products in the fermentation media beside citric acid, such as gluconate acid or oxalic acid.

In this study the concentration of nitrogen sources from ammonium nitrate (NH_4NO_3) was added to the solution of 0.25% w/v equivalent to 0.124 N (Rohr *et al*. 1981). The concentration of nitrogen sources necessary for the fermentation of citric acid is 0.1-0.4 N L^{-1}. This suggests that the concentration of nitrogen sources from NH_4NO_3 0.25% w/v supports in terms of pH reduction but it should still be improved. In addition to the source of nitrogen, it indicates that the pH decrease was slowly during the 120 h fermentation period, it is suspected that the pH will decrease along with the length of fermentation time. Therefore, the addition of fermentation time, should be conducted to achieve

lower pH in the fermentation media.

The results of citric acid concentration analysis in Toba banana peels showed a significant difference in each concentration of banana peels 5%, 10% and 15%. This research showed that the increasing concentration of Toba banana peel substrate will increase the production of citric acid. The highest concentration of citric acid (0.621 % w/v) was obtained in 15% of Toba banana peel substrate which was added with 5% sucrose. Nutrition is one of the important growth factors for fungal growth. Adequate nutrients will stimulate the growth and biosynthesis of metabolite products (Sukandar 2002). In the *A. niger* metabolic pathway in producing citric acid, phosphoenol pyruvate will be converted to oxaloacetate with the activity of phosphoenol pyruvate carboxylase enzyme. This reaction requires ATP as an energy source that can be obtained from manganese, potassium, or ammonia (Papagianni 2007). Banana peel substrate contains manganese mineral which is one of the nutritional sources for *A. niger* in the incorporation of citric acid. Based on research conducted by Abbas *et al*. (2016), an increasing concentration of banana peel substrate did not always cause an increasing production of citric acid by *A. niger*. The highest citric acid production was in 25% concentration of banana peel substrate, whereas in 30% and 35% concentration of banana peel substrate the citric acid production was steeply decreased. This phenomenon showed that too much substrate would be a barrier for enzyme activity in citric acid cycle.

In this study, fermentation with the addition of 5% sucrose resulted a higher concentration of citric acid compared to the addition of 5% glucose, due to the efficiency of *A. niger* to metabolize certain types of sugar to produce citric acid. In the metabolism of *A. niger* to produce citric acid, the metabolic pathway used is the glycolysis pathway and the Entner-Doudoroff pathway which provides pyruvic acid compounds as intermediate compounds. Sucrose is a disaccharide composed of glucose and fructose units. On the path of glycolysis, sucrose will be inserted into the cells through the mechanism of sucrose hydrolysis invertase to break down sucrose into glucose and fructose (Boddy *et al*. 1993), and the two sugars are then converted to fructose 6-phosphate before being converted to acid pyruvate. Fermentation of banana peel with the addition of 5% sucrose results in a higher concentration of citric acid because in the steps of glycolysis, the amount of fructose 6-phosphate is obtained from glucose and fructose, while at the addition of 5% glucose, fructose 6-phosphate will only be obtained from glucose. This results in a higher number of fructose 6-phosphate molecules that it will affect the amount of pyruvate obtained in the glycolysis process.

In conclusion, although 15% of Toba banana peel substrate and the addition of 5% sucrose produced a high concentration of citric acid in this research, the fermentation condition still has to be optimize to reach a higher citric acid concentration. Those conditions are Toba banana peel substrate concentration, sucrose concentration, ammonium nitrate concentration, and fermentation time.

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