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Utilization of banana peel waste for citric acid production by *Aspergillus niger*

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Abstract. The potential of Banana peel waste of Pisang Ayam (*Musa acuminata*), Pisang Raja (*Musa Paradisiaca* cv. Raja genom AAB), and Pisang Nipah (*Musa balbisiana*) as substrate for citric acid production by *Aspergillus niger* have been investigated. The main purpose of this research was to determine the percentages of citric acid production, pH, and total biomass at differences of media of banana peel waste. The results showed that the percentages of citric acid production using media of *Musa acuminata*, *Musa Paradisiaca* cv. Raja genom AAB and *Musa balbisiana* were 58.80, 69.84, and 46.80% respectively. The pH values of each media were 2.50, 1.50, and 2.0 respectively. The results also founded that the total biomass of each media were 2.442, 2.649, and 2.407 respectively. Overall, the results showed that banana peel waste of *Musa Paradisiaca* produce the highest amount of citric acid after 240 h fermentation.

1. Introduction

Citric acid plays an important role in modern industries. In modern industries, citric acid commonly used such as beverage, food, pharmaceutical, textile, metal, chemical and other industries. In the world, citric acid is commercially produced in millions of tons per year. Besides that, the number of requests and supply is increasing every year. Citric acid is contained in various types of fruits and vegetables, especially in the citrus fruit. Lemon juice has been known containing 7 - 9% of citric acid. Citric acid is responsible for the sour taste of fruits [1].

In technology fermentation, the citric acid is commonly produced by fermentation of sucrose or molasses using submerged method by *Aspergillus niger* [2,3]. Recently, solid-state fermentation (SSF) is considered as an alternative method in the production of citric acid due to higher yields, low water requirement and low operating costs.

Due to the large demand or request for citric acid in the industrial sector, especially in the pharmaceutical industries, a lot of efforts or methods have been made to multiply production of citric acid which is faster, cheaper, eco-friendly, environmentally safe, and does not have to use citrus fruit as the main source of citric acid. Many researchers have been used various microorganisms to produce citric acid. According to Gandjar and Sjamsuridzal [4], various types of microorganisms that are capable to produce citric acid are *Penicillium janthinellum*, *Penicillium restrictum*, *Trichoderma viride*, *Mucor piriformis*, *Ustilina vulgaris*, *Aspergillus niger* (*A. niger*), *A. wentii*, *A. saito*, *A. usami*,

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A. fumigatus, *A. funicus*, *A. lanosus*, and *A. flavus*. However, the fungal strains of *A. niger* is widely used for citric acid production due to ease to handling, high yields of citric acid [5] and can be used with a cheap raw agricultural wastes materials such as pineapple, pear, carrots, orange, kiwi peels, corn pods [6], cotton waste, okara soy-residue and cane molasses [3,7].

In Aceh province, banana is most widely used in daily life. However, most people only take part of the fruit, while the peels of banana are discarded a waste. A banana peels waste is currently posing disposal problem in Aceh. Banana peels are known contain higher levels of catecholamine and gallic acid than the fruit. Other ingredients contained in banana peels are water, protein, anthocyanin, cyanidin, carotenoids, sterols, fibre, lipid peroxidation inhibitors and vitamin B₆ [8]. The aim of this study was to determine the production of citric acid from *A. niger* by using several of banana peels waste as media through solid state fermentation (SSF).

2. Materials and method

Pisang Ayam (*Musa acuminata*), Pisang Raja (*Musa Paradisiaca* cv. Raja genom AAB), and Pisang Nipah (*Musa balbisiana*) were obtained from banana plantation in Lamreung, Aceh Besar Regency, Aceh Province, Indonesia. Isolate of *Aspergillus niger* were obtained from the Faculty of Veterinary Medicine, Universitas Syiah Kuala. Potato dextrose agar (PDA) was purchased from Sigma Aldrich. HCl, NaOH, MgSO₄·7H₂O, ZnSO₄·7H₂O, and FeSO₄·7H₂O were provided by Laboratorium of Organic Chemistry, Faculty of Science, Universitas Syiah Kuala.

2.1. Pre-treatment of Banana peels

Banana peels waste of Pisang Ayam (*Musa acuminata*), Pisang Raja (*Musa Paradisiaca* cv. Raja genom AAB), and Pisang Nipah (*Musa balbisiana*) (Figure 1) were used are naturally old and marked with golden yellow on the skin. Each of banana peels were cut into 1 - 2 cm and dried in oven-vacuum at 60°C for 2 h and powdered using a mixer and filtered into 2 mesh size [7].



Figure 1. (A) Pisang Ayam (*Musa acuminata*) (B) Pisang Raja (*Musa paradisiaca* cv. Raja genom AAB); and (C) Pisang Nipah (*Musa balbisiana*)

Table 1. The compositions of media for production of citric acid using several bananas peel waste by *A. niger*.

Supplements	Compositions		
	Media I	Media II	Media III
Substrate of <i>M. acuminata</i> (g)	30.0	-	-
Substrate of <i>M. paradisiaca</i> (g)	-	30.0	-
Substrate of <i>M. balbisiana</i> (g)	-	-	30.0
NH ₄ NO ₃ (g)	0.32	0.32	0.32
CaH ₂ PO ₄ (g)	0.06	0.06	0.06
MgSO ₄ ·7H ₂ O (g)	0.10	0.10	0.10
ZnSO ₄ ·5H ₂ O (g)	0.01	0.01	0.01
Fe (g)	0.20	0.20	0.20
Aquadest (mL)	100	100	100
pH	3.5	3.5	3.5

2.2. Production of citric acid

30 g of each banana peel powder were added into Erlenmeyer flask 200 mL to prepare a basal medium. The composition of each media is listed in Table 1. The flasks contained media were plugged with cotton and autoclaved at 121°C and 15 psi for 15 min and allowed to cool at room temperature. After cooling, each medium was inoculated with the suspension of *A. niger* (9.0×10^6) and incubated at 30°C in a rotary shaking incubator at 60 rpm for 10 days [9].

2.3. Citric Acid determination

The percentage of citric acid was determined by titrimetric method [7,10] by using 0.1 M of sodium hydroxide (NaOH), and phenolphthalein as indicator. Percentage of citric acid was calculated according to equation 1.

$$\% \text{ citric acid} = \frac{\text{Normality} \times \text{Volume of 0.1M NaOH} \times \text{Equivalent weight of citric acid} \times \text{Dilution factor}}{\text{Weight of sample (g)} \times 10} \quad (1)$$

2.4. Biomass and pH determination

Percentage of biomass and determination of the pH values were determined according to AOAC [10] with slight modification. The percentage of biomass was determined by centrifuged the whole of fungal culture at the end of fermentation at 1200 rpm for 10 min to precipitate cells. The precipitate cells then dried in the oven at 85°C to obtain of cell-dried or pellet. The pellet is then weight constantly using analytical balance. The supernatant that obtained from centrifugation were used to determine the pH of media by using pH meter. Each analysis was performed in triplicates.

2.5. FTIR spectroscopy analysis

After fermentation process, the suspension of each media then centrifuged at 1200 rpm for 10 min to obtain supernatant. The supernatant was used to determine the profile of citric acid using the FT-IR spectroscopy (Agilent Technologies Cary 630 model).

3. Results and discussion

Citric acid has the chemical formula $C_6H_8O_7$ with two functional group that is carboxylic acid (-COOH) and hydroxyl (-OH). Citric acid is a weak organic acid occurs naturally in citrus fruits. In biochemistry, citric acid is an intermediate in the citric acid cycle. In metabolism, citric acid occurs in all aerobic organisms including bacteria and fungi. In this study we used fungi of *A. niger* for citric acid production by utilizing of three kinds of bananas peels waste that are Pisang Ayam (*Musa acuminata*), Pisang Raja (*Musa Paradisiaca* cv. Raja genom AAB), and Pisang Nipah (*Musa balbisiana*).

3.1. The pH profiles

Table 2 presents the pH profile of several banana peel waste in media for production of citric acid by *A. niger*. The table showed that pH of peel media of Pisang Ayam (*Musa acuminata*) and Pisang Nipah (*Musa balbisiana*) were decreased at 8th day of fermentation however increased until 10th days of fermentation. While, the pH of peels media of Pisang Raja (*Musa Paradisiaca* cv. Raja genom AAB) was continues decreased during fermentation (figure 3). Table 2 also showed that the decrease in pH in each time of fermentation is proportional to the citric acid production by *A. niger*, this result agreed with Kareem et al., [7] that mentioned that decreasing in pH was caused by formation or accumulation of citric acid by *A. niger* at the time of incubation, where the optimum pH for citric acid formation is at around 2.0. The lower pH value, the more citric acid produced [11].

Table 2 and Figure 2 showed that media supplemented with peel of pisang raja (*Musa Paradisiaca*) was continues decreased during fermentation process of *A. niger*. According to Emeka [14], this is due to differences of nutritional and minerals contents and of each fermentation medium. Babayemi [15] mentioned that peel of Pisang Raja (*Musa Paradisiaca*) contains higher minerals such as iron (Fe), magnesium (Mg), and zinc (Zn) compared with other banana peels. Soccol stated that the minerals of

iron (Fe), magnesium (Mg), and zinc (Zn) supports for optimizing of citric acid production because these minerals are very important to increase the acidity of *A. niger* in fermentation process [16]. Besides that, Yigitoglu has been found that peel of Pisang Raja (*Musa Paradisiaca*) has higher fat contain compared with peel of Pisang Ayam (*Musa acuminata*) and Pisang Nipah (*Musa balbisiana*) [17].

Table 2. The pH profile using several banana peel waste in media for production of citric acid by *A. niger*

Days	pH Profile		
	Media I	Media II	Media III
2	3.50	3.50	3.50
4	3.00	3.00	3.25
6	2.75	2.25	3.00
8	2.00	1.50	2.00
10	2.50	2.00	1.50

Note: Media I supplemented with *M. acuminata* peel; Media II supplemented with *Musa balbisiana*; Media III supplemented with *Musa Paradisiaca*

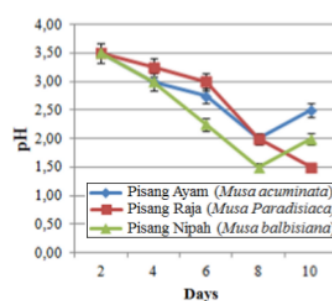


Figure 2. The pH profile using several banana peel waste in media for production of citric acid by *A. niger*.

3.4. The biomass profiles

Biomass is a basic parameter in microbial growth characterization [12]. Table 3 and Figure 3 presented the percentage of biomass of banana peel media for production of citric acid by *A. niger*. The results showed that the medium contain peel of *Musa Paradisiaca* has higher biomass compared with others.

Table 3. The percentage of biomass using several banana peel waste in media for production of citric acid by *A. niger*.

Days	Percentage of Biomass		
	Media I	Media II	Media III
2	2,231	2,183	2,164
4	2,256	2,289	2,302
6	2,321	2,343	2,343
8	2,479	2,490	2,503
10	2,442	2,407	2,649

Note: Media I supplemented with *M. acuminata* peel; Media II supplemented with *Musa balbisiana*; Media III supplemented with *Musa Paradisiaca*

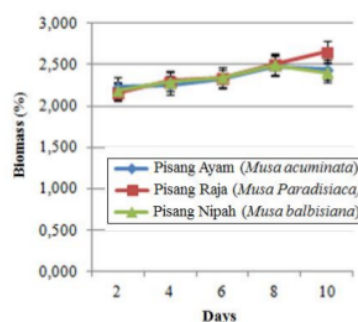


Figure 3. The percentage of biomass using several banana peel waste in media for production of citric acid by *A. niger*.

Table 3 and Figure 3 showed that the medium contain peel of *Musa Paradisiaca* has higher biomass compared with other medium. This is because peel of *Musa Paradisiaca* has a higher contain of sugar as source of carbon. Babayemi mentioned that peels of *Musa Paradisiaca* has a sugar contain of 31,6% compared with *Musa acuminata* and *Musa balbisiana* which is 2.88 and 1.9 % respectively [15]. Yigitoglu and Emeka stated that source of carbon is essential for citric acid production by *A. niger* [14, 17]. Kareem also stated that source of carbon influence of biomass and citric acid formation [7].

In addition of each of peel waste banana to media as source of carbon such as sugar enhanced citric acid production. As substrate, sugar plays important role intracellular enzymes [18, 19]. Increasing of biomass and citric acid values is correlative decreasing of source of carbon or consumption of sugar along the incubation time. The yield of citric acid during fermentation by *A. niger* is strongly dependent on the concentration and type of source of carbon. Concentration of carbon influences citric acid accumulation because carbon plays roles in process of transport and phosphorylation. The results showed that media supplemented with peel of *M. paradisiaca* under solid-state fermentation produce a higher citric acid production reach until 69.84%. However, Hang and Woodams reported that apple and grape pomace produce citric acid until 88 and 60% respectively [20].

3.3. The percentage of citric acid

The percentage of citric acid utilization of banana peel waste is presented in Table 4 and Figure 4. The results showed that the percentage of citric acid was increased during fermentation in each media. In this study, we found a parallel relationship between biomass and citric acid production. This result was unanimous with the results of El-Holi and Al-Delamy [13] that the production of citric acid approximately paralleled with the biomass production or sugar consumption.

Table 4. The percentage of citric acid using several banana peel waste in media for production of citric acid by *A. niger*.

Days	Percentage of Citric Acid		
	Media I	Media II	Media III
2	11,04	16,32	17,04
4	18	41,52	28,32
6	41,76	55,68	38,88
8	59,76	63,6	50,4
10	58,8	46,8	69,84

Note: Media I supplemented with *M. acuminata* peel; Media II supplemented with *Musa balbisiana*; Media III supplemented with *Musa Paradisiaca*

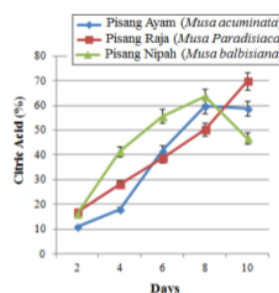


Figure 4. The percentage of citric acid using several banana peel waste by *A. niger*

Besides that, Figure 4 also showed that citric acid was increased after 8th days of fermentation or at 192 h of fermentation. Ashour stated that citric acid was formed in maximum level above 8th days of fermentation [21], while Dhandayuthapani mentioned that citric acid was advanced in optimum after 20th days of time fermentation. Meanwhile, minerals such as zinc (Zn), manganese (Mn), iron (Fe), magnesium (mg), phosphorus (P), ammonium (NH₄), and Fe were also specified in citric acid formation by *A. niger* were responsible for citric acid production [9]. Wherea manganese (Mn) and phosphate (PO₄) plays role in the growth of *A. niger* [16,17,22].

The agitation in fermentation is also influenced in production of citric acid. Agitation plays role in spreading of *A. niger*. Agitation also has a role in avoiding of hyphal sedimentation in fermentation of *A. niger*. Hyphal sedimentation plays a role in preventing excessive production of citric acid [11]. In addition to agitation, temperature has a major effect in citric acid production due to temperature greatly influences the work of enzymes for the formation of citric acid. The optimum temperature to citric acid production is 30°C [7,9,14,17].

4. The FTIR profiles

Citric acid is a weak organic acid that has the chemical formula C₆H₈O₇. Citric acid has three functional groups of carboxylic acid (COOH) that contain three functional group of carbonyl group (C=O) and one of hydroxyl group (OH). The FTIR spectra were performed at the end of fermentation (10th days of fermentation). The FTIR spectra were presented in Figure 5. The functional group of

carbonyl of citric acid in fermented media was obtained in the range of 1690-1760 cm^{-1} . The FTIR analysis also showed the hydroxyl group (OH) absorption bands at the range of 3610-3640 cm^{-1} .

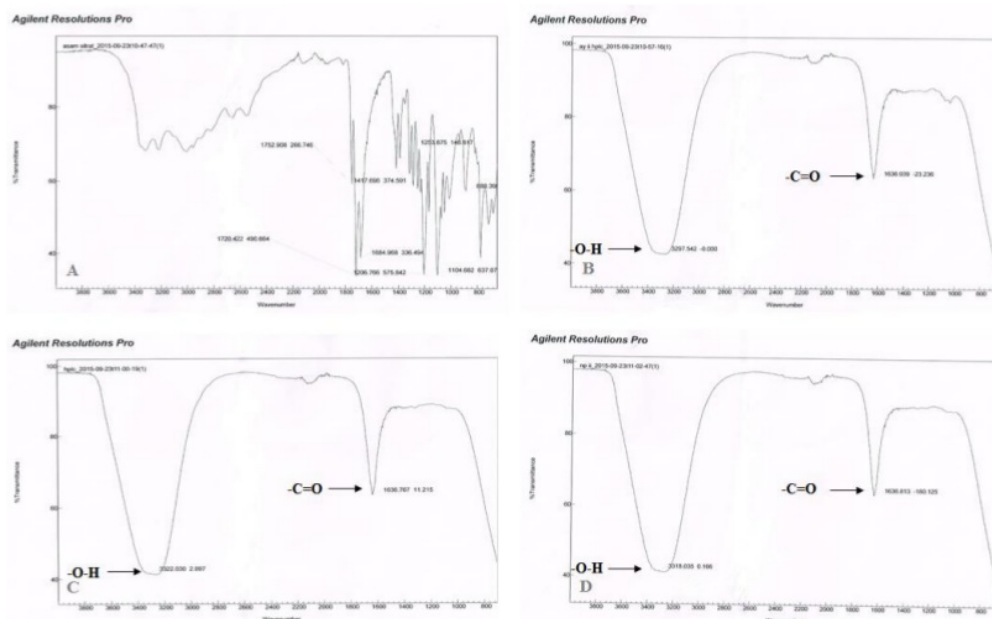


Figure 5. The FTIR spectra; A. citric acid standard; B. supernatant utilization of banana peel waste of pisang ayam (*Musa acuminata*) media ; C. supernatant utilization of banana peel waste of pisang nipah (*Musa balbisiana*) media; and D. supernatant utilization of banana peel waste of pisang raja (*Musa Paradisiaca* cv. Raja genom AAB) media..

4. Conclusion

Banana peel waste of pisang ayam (*Musa acuminata*), pisang raja (*Musa Paradisiaca* cv. Raja genom AAB), and pisang nipah (*Musa balbisiana*) can be used as substrate for citric acid production by *Aspergillus niger*. The media supplemented with pisang raja (*Musa Paradisiaca*) has a lower pH at 1.5 and a higher biomass and citric acid production at 2.64 and 69.84 % respectively compared to pisang ayam (*Musa acuminata*) and pisang nipah (*Musa balbisiana*).

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