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Original Research

Study on the Banana Peel Hydrolysate for Lactic Acid Bacteria Growth Media

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Abstract—Banana peel is food waste material that may contain certain beneficial nutrition and be useful for bacterial media production. This experiment aims to determine the effect of several variables which may have influence on the ability of banana peel to support probiotic growth. The thermal treatment used autoclave. In the hydrolysis step for probiotic growth, the variables studied were pH and temperature. The best result of hydrolysis was obtained by the initial steam treatment, followed by hydrolysis process at pH 4.5 and 95°C. The reducing sugar content obtained was 0.96 g/L. In the fermentation process, pure banana peel hydrolysate media was able produces to support *Lactobacillus plantarum* growth better than mixed media. The pure banana peel hydrolysate media could achieve up to 12.89 log CFU/ml. The amylase activity produced in the pure banana peel hydrolysate media was 8.14 DP.

Keywords: banana, probiotic, hydrolysis, growth, amylase

Abstrak—Kulit pisang merupakan bahan limbah yang memiliki kandungan nutrisi menguntungkan dan berguna untuk produksi media pertumbuhan bakteri. Penelitian ini bertujuan menentukan pengaruh beberapa variabel yang dapat mempengaruhi kulit pisang untuk mendukung pertumbuhan probiotik. Kulit pisang tersebut diolah melalui hidrolisis enzimatik menggunakan enzim amylase termofilik. Variabel sebelum proses hidrolisis adalah penggunaan pengolahan termal menggunakan uap air dan tanpa pengolahan. Pada langkah hidrolisis, variabel yang dipelajari adalah pH dan suhu. Hasil hidrolisa terbaik adalah dengan penggunaan pengolahan awal dengan uap air diikuti dengan hidrolisis pada pH 4,5 dan suhu 95°C. Kandungan gula pereduksi yang diperoleh sebesar 0,96 g/L. Pada proses fermentasi, media dari hidrolisat kulit pisang murni dapat menghasilkan pertumbuhan *Lactobacillus plantarum* sampai 12,89 log CFU/mL. Aktivitas amylase yang dihasilkan dari media hidrolisat kulit pisang tersebut adalah 8,14 DP.

Keywords: pisang, probiotic, hydrolysis, growth, amylase

INTRODUCTION

Food demand constantly increases with the world's population, and this will also cause the increase in waste generation. The food waste can be regarded as organic waste that originates from several sources such as residence, retail and restaurant food outlets, and cafeterias [1]. Several by-products from food production facilities which includes the supply, handling, and processing vegetables, fruits, cereals, edible and uncooked raw materials from traditional market, homes, and restaurants considered as food waste [2], [3]. In Indonesia, 44% of waste generation in 2018 was from food waste [4]. According to FAO, food waste production was estimated about 180 – 200 kg per capita per year [4], [5]. Meanwhile, in Indonesia the value reached 115 – 184 kg per capita per year during 2000 – 2019 [4]. The estimated food waste value had been reported also by FAO to achieve $680 billion including $310 billion misspent by underdeveloped countries that generated 1.6 gigatons [3]. The impact of food waste on the environmental quality had also been concerned. Food waste in Indonesia was reckoned to contribute 1,702.9 Mton CO_2-eq, which was about 7.29% average GHG emission in Indonesia over 20 years [4]. More stringent laws and regulation concerning sustainability has driven waste management to reduce environmental impact. Valorization of food waste for energy and value-added products is then worth to be explored. Several products yielded from food such as biofertilizer, animal feed, organic acids, bioplastics and enzymes had been reported [3].

Depending on the source from which the food wastes are collected, they may contain 30 – 60% starch, 5 – 10% proteins, and 10 – 40% lipid which can be potential for biorefinery [6],

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[7]. Hydrolysis of such food waste results in fermentable sugar containing hydrolysate, and amino acid [8]. The hydrolysate can be utilized to produce succinic acid and lactic acid, in addition to energy-rich compounds such as hydrogen and biogas. Lactic acid is a prominent compound because of its utilization in cosmetics, food and chemical industries [9]. Besides lactic acid-producing fungi, amylolytic lactobacilli can directly convert complex compound into lactic acid [10]. Unlike fungi, lactic acid-producing bacteria has ability to grow in anaerobic facultative environment, a condition in which simultaneous saccharification and fermentation (SSF) normally occurs.

Despite its prominent health effect as probiotic, lactic acid bacteria (LAB) starter culture is commonly used to produce fermented foods, cereals, milk, fish, and meat because of their characteristic flavor and taste generation and preservative effect [11]. Food preservation can be carried out using LAB as these bacteria exhibits wide spectrum of activity against unfavorable microflora growth including bacteriocin production [12]. Yin (2013) investigated on the ability of *Lactobacillus* sp. to grow on kitchen waste which contained 60.4 and 24.2 % dry weight of total sugar and protein, respectively. Lee (2024) [13] utilized LAB isolated from kimchi in the malt wort beverage and elaborated several flavor compounds that contributed to flavor and aroma. *Lactobacillus plantarum* is lactic acid-producing bacteria that can grow on various substrate with comparable characteristic to food waste such as coffee husk, wheat bran, rice brans, and oat [14].

Banana (Musa spp.) is massively cultivated food plant in tropical countries. Indonesia is among several countries such as China, Brazil, and Ecuador which produce large amount of banana [15], [16]. In 2023, banana production in Indonesia achieved [17]. As leading export comodity, banana production in Indonesia is always encouraged to contribute to international trading [18]. Raw banana peel still contains nutrition compounds, consisting of 3.5 – 6.3 % starch, 5.5 – 7.87 % crude protein, 9 – 11 % ash, and 47 – 53 % dietary fiber. Besides nutrition compound, banana also shows antioxidant activity [19]. Despite its beneficial content, raw banana peel which constitutes 35 – 50 % of the total ripe fruit mass is still discarded (Abdullah, 2022, Gomes, 2019) [20]. Nevertheless, several utilizations in food or feed sector have been explored. Fatmawati (2018) [21] studied the protein content increase of fermented banana peel for feed ingredient. Abdullah (2022) [15] investigated LAB fermentation of banana peel to produce lactic acid. The prebiotic activity of banana peel against acne causing bacterium has also been studied (Rusdi, 2023) [22]. However, the amylase activity produced by LAB such as *L. plantarum* on the banana peel has not been reported. In this research the potential of banana peel for LAB growth media is investigated by exploring the growth and amylase production of *L. plantarum* on banana peel.

MATERIALS AND METHODS

Materials

Banana peel used was collected from local banana processing industry (East Java, Indonesia). α-Amylase enzyme from *Bacillus licheniformis* (CAS:9000-85-5, Sigma Aldrich) was used in enzymatic hydrolysis experiment. The lactic acid bacteria investigated was *Lactobacillus plantarum* FNCC0026 (FNCC, UGM, Indonesia). MRS broth and agar media were used to grow inoculum and cultivate as well as plate count. Dinitrosalycilic acid (DNS) reagent (Sigma Aldrich) was used for reducing sugar within banana peel powder hydrolysate.

Banana Peel Preparation

Prio to utilization, the collected banana peel was washed to remove the adhered dirt and sap. It is then steamed at 100 ^{o}C within a kitchen boiler for 15 minutes, dried and cut into $\pm$ 5 cm pieces. The peel cuts were then milled using a disc mill machine (FFC 23A, Chine) at 5800 rpm and screened to obtain banana peel powder with size of 0.149 – 0.21 mm.

Banana Peel Hydrolysis

Banana peel hydrolysis was performed using α-amylase from *Bacillus licheniformis* at varied temperature (85, 95, and 105 ^{o}C) and pH (3.5, 4.5, and 5.5). The pH variation was achieved

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by using 0.1 M citrate buffer formulated from 0.1 M sodium citrate and citric acid solution. The hydrolysis was carried out using 250 mL Erlenmeyer flask containing 100 mL citrate buffer solution, 25%w/v banana peel powder, and 0.3 mL enzyme. The flasks were placed within orbital shaker incubator in which the temperature was kept constant for 1 hour. The hydrolysate produced was then filtered out from the spent solid for fermentation use.

Fermentation

Lactobacillus plantarum was cultivated using MRS agar. The inoculum was prepared aseptically transferring 1 loopful of *L. plantarum* colony from agar plate into a 50 mL McCartney glass bottle containing 40 mL MRS broth. The broth was placed within an incubator set at 37^{o}C for 16 hours. Samples were taken every hour to measure the number of cells produce by using plate count. The fermentation was carried out within a 250 mL Erlenmeyer containing 90 mL banana powder hydrolysate and 10 mL *L. plantarum* inoculum. The flask was shaken at 70 rpm and 37^{o}C for 24 hours. At the end of the fermentation time, sample was withdrawn to measure the cell number produced.

Chemical Analysis

The reducing sugar content of banana peel powder hydrolysate were measured using DNS reagent [23], [24]. Amylase activity was expressed using dextrinizing power (DP) as developed by [25]. Initially, this was performed by preparing reagent A which was a mixture of 0.2% iodine solution and 2% potassium iodide solution. Acetate buffer solution with concentration of 0.5 M was used to maintain pH at 6.0. One milliliter of acetate buffer solution and enzyme solution mixture made up to 2.5 mL using distilled water was placed into Tube A. Another tube called Tube B was filled with 2.5 mL distilled water. Both tubes were brought to 37^{o}C for 10 minutes using water bath. Two and half milliliters of 0.2% amylose solution was added into each of the tubes. The tubes were let stand for 30 minutes. Five milliliters of 1 N acetic acid solution was then added to each of the tubes and followed by shaking the tube. The content of the tubes was transferred into 250 mL volumetric flask and added with 200 mL of distilled water, 5 mL of reagent A, and diluted to the mark with water. The both mixtures absorbance were analyzed using UV-VIS spectrophotometer at 300 nm. The dextrinizing power (DP) can then be determined using the following equation:

$$DP = \frac{\frac{D_0 - D}{D_0} \times 100\%}{10} \quad (1)$$

According to Eq. (1) 1 DP is defined as the amount of amylase which will produce 10 percent fall in the intensity of the amylose-iodine blue color [25].

Alkaline protease inhibitor of banana powder hydrolysate was measured by mixing 1 mL trypsin and 1 mL sample solution. The mixture was incubated at 37^{o}C for 15 minutes. Two milliliters of 1% Hammerstein casein solution was added, and the mixture was incubated at 37 ^{o}C for 30 minutes. A 2.5 mL of 0.44 M Trichloroacetic acid (TCA) solution was then added. The mixture was then centrifugated at 10,000 rpm for 5 minutes. The absorbance of supernatant was measured using UV-VIS spectrophotometer at 280 nm.

RESULTS AND DISCUSSION

In this work banana peel was employed for producing *L. plantarum* growth media by converting the peel into fermentable reducing sugar. Producing low-cost growth media for such probiotic culture from kitchen or food industry waste like banana peel may be economically beneficial as besides being well-known as human health-promoting agent used in functional food, probiotic is also considered key elements in farm animal feed formulation [26], [27]. Much research has been focused on the fish diets in freshwater fish farming, and the results suggested the positive impact of probiotics on the feed conversion ratio [27], [28].

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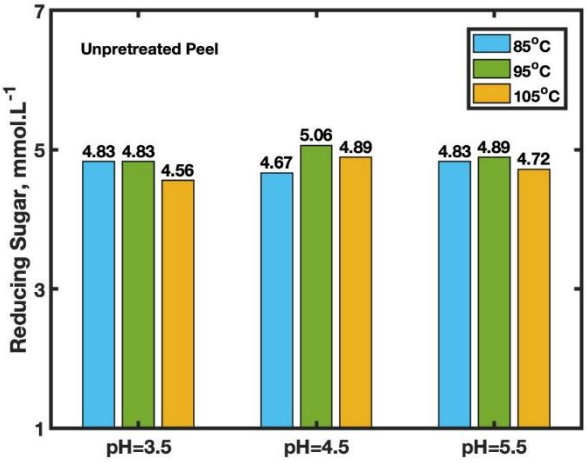

Figure 1. Reducing sugar produced from unpretreated-banana peel hydrolysis.

The starch content of banana peel can be hydrolyzed using starch-hydrolyzing enzyme to produce reducing sugar (glucose) that can support the probiotic growth. The hydrolysis reaction is as follows:

$$(C_6H_{10}O_5)_n + nH_2O \xrightarrow{\alpha-amylase} nC_6H_{12}O_6$$

Figure 1 shows the reducing sugar concentration produced from unpretreated banana peel after being hydrolyzed using α-amylase at varied temperature and pH for 1 hour. The trends of reducing sugar production are similar for all the varied pH where the reducing sugar concentration produced at 95°C is the highest. The hydrolysis at pH of 4.5 produced the highest reducing sugar at 95 and 105°C while it produced the lowest one at 85°C. However, the reducing sugar produced does not significantly differ with the variation of temperature and pH (p>0,05).The highest reducing sugar concentration ($5.06\ mmol.L^{-1}$ or $0.9\ g.L^{-1}$) produced from enzymatic hydrolysis at 95°C and pH of 4.5. This means that the highest glucose productivity at that condition is $0.9\ g.L^{-1}.h^{-1}$. The reducing sugar produced from steamed pretreated banana peel are shown in Figure 2. For steam pretreated banana peel (Fig. 2), the highest reducing sugar are produced at pH of 4.5 for temperature at 85 and 95°C while it is the lowest for temperature at 105°C. The highest reducing sugar concentration produced from the hydrolysis of steam-pretreated banana peel was $5.33\ mmol.L^{-1}$ or $0.96\ g.L^{-1}$ which was obtained at 95°C and pH of 4.5. The reducing data shown for steam pretreated banana peel are the same as those of unpretreated banana peel that variation of pH and temperature do not significantly give difference reducing sugar concentration (p>0,05). Left over rice saccharification using *Aspergillus niger* glucoamylase had been studied for *Lactiplantibacillus plantarum* fermentation and resulted 10.16 $g.L^{-1}$ total reducing sugar (Lee, 2024) [29].

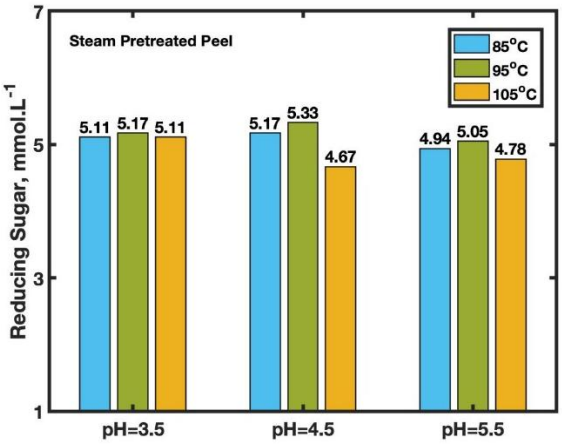

Figure 2. Reducing sugar produced from steamed-banana peel hydrolysis.

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The fermentation of banana peel hydrolysate started by feeding the hydrolysate with *L. plantarum* inoculum. The inoculum contained *L. plantarum* cells grown within MRS broth medium. Figure 3 shows the batch growth curve of *L. plantarum* in MRS broth. The exponential phase started after 5 h incubation and ended after 14 h. The cells for inoculum were harvested after 16 h which contained 7.26×10^9 CFU/mL (9.9824 log CFU/mL). Figure 4 shows the final *L. plantarum* cell concentration after grown on steamed-pretreated banana peel hydrolysate. As depicted on the figure, pure steam-pretreated banana peel hydrolysate gave up to 12.89 log CFU/mL while the hydrolysate and MRS broth mixture only produced 9.02 log CFU/mL cells. The steam-pretreated banana peel may contain richer growth supporting compounds for *L. plantarum* cells that it managed to provide higher cell number after 24 h fermentation.

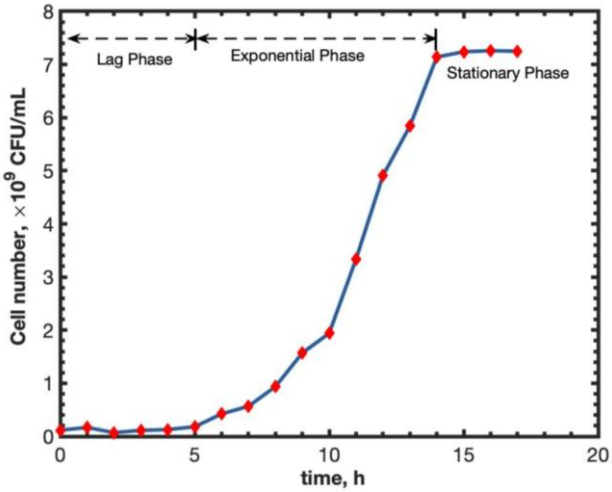

Figure 3. L. plantarum inoculum growth curve on MRS broth media.

The growth of *L. plantarum* on saccharified sorghum malt extract media resulted in 9 log CFU/mL cells from 4 log CFU/mL inoculum [11]. The ability of *L. plantarum* to produce amylase enzyme during fermentation within banana peel hydrolysate was measured and expressed as dextrinizing power (DP). The DP value resulted from the growth on pure hydrolysate is higher than that from hydrolysate–MRS broth mixture. *L. plantarum* is an amylolytic LAB that can breakdown starch molecule into fermentable sugar [30], [31]. The hydrolysate may still contain unhydrolyzed glucose oligomer chains that caused *L. plantarum* cells to produce amylase enzyme. The presence of MRS broth may impede the amylolytic activity of *L. plantarum* and therefore the DP value resulted from hydrolysate–MRS broth mixture is lower than that of pure hydrolysate.

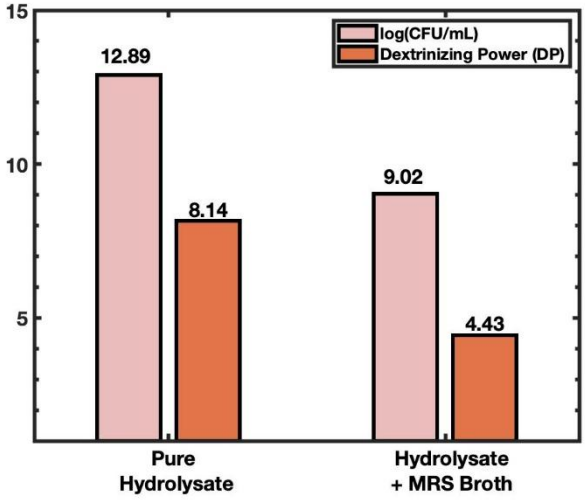

Figure 4. Cell concentration and dextrinizing power produced from hydrolysate fermentation.

CONCLUSION

Banana peel waste from banana processing industry is potential to be used as cheap probiotic growth substrate or media. In this work, 12.89 log CFU/mL probiotic cell can be achieved by using pure banana hydrolysate as growth media, which is enough for health improvement. *L. plantarum* has been shown to exhibit amylolytic activity after grown on banana peel hydrolysate and therefore the direct use of the cells to breakdown and grow on the peel can offer a low-cost process for probiotic of lactic acid production.

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