Lactic Acid Fermentation from Durian Seeds (*Durio zibethinus Murr.*) Using *Lactobacillus plantarum*

Abdullah Abdullah¹*, Ima Winaningsih¹,² and Agus Hadiyarto¹

¹ Department of Chemical Engineering, Faculty of Engineering, Diponegoro University, Semarang, Central Java, 50275, Indonesia
² Department of Chemical Engineering, Bandung State Polytechnic, Pekalongan, Central Java, 51161, Indonesia

*Corresponding author: abd_busairi@yahoo.com

**Abstract.** Durian is one of the abundant seasonal fruits at harvest time. Generally, the part consumed in durian is its salutre fruit or its meat. Durian seeds contain 70-80% carbohydrates, but not yet utilized. Carbohydrates can be fermented into lactic acid using *Lactobacillus plantarum* bacteria. Lactic acid is a raw material for biodegradable plastic/poly lactic acid (PLA). The purposes of this study were to determine the effect of substrate concentration (7.5%, 10%, 12.5%, 15% and 17.5%), inoculum concentration (2%, 5%, 7%, 10% and 12%), and yeast extract concentration (0 g/L, 2.5 g/L, 5 g/L, 7.5 g/L, and 10 g/L) on the yield and the concentration of lactic acid produced. The research was initialized by making durian seed flour, testing the proximate value of the materials, bacterial inoculation, fermentation of durian seed flour at 37°C, agitation speed of 100 rpm for 72 hours, then the fermentation results were analyzed for lactic acid concentration using UPLC (Ultra Performance Liquid Chromatography). The highest yield and lactic acid concentration at substrate concentration of 15% were 12.51% and 13.75 g/L, at inoculum concentration of 2% were 19.44% and 21.38 g/L, while the highest yield and lactic acid concentration at 10 g/L yeast extract concentration were 21.76% % and 23.92 g/L.

**Keywords:** durian seed flour; fermentation; lactic acid; *Lactobacillus plantarum*

1. Introduction

Durian is the most popular seasonal fruit in Southeast Asia, particularly in Malaysia, Indonesia, Thailand, and the Philippines [1,2]. Durian is quite abundant in Indonesia. Based on the Central Bureau of Statistics Republic of Indonesia in 2017 regarding fruits and vegetables, durian production is increasing every year. A total of 735,423 tons in 2016 and 795,211 tons in 2017 [3]. Generally, the part of durian fruit that is more commonly consumed is its salutre fruit or its meat. Only about 30% of durian is edible while the rest is considered as waste, of which 20–25% of the whole fruit is seeds that have not been fully utilized [1,2].

[1.1]
Durian seeds (Durio zibethinus Murr.) which mainly consist of starch and sap are waste that can be used for food or non-food industrial applications [4]. Commonly, research on durian seed flour is still limited to food as an emulsifier for mayonnaise [2], yogurt [5], ice cream stabilizer [6], etc., there has been no research on lactic acid fermentation using durian seed flour.

Ripe durian seeds contain 51.1% water, 46.2% carbohydrates, 2.5% protein, and 0.2% fat [7]. Its high carbohydrate content can be used for lactic acid fermentation [8,9,10,11]. Lactic acid is the most important product due to its wide application, especially in the food, chemical, cosmetic, and pharmaceutical industries. It has great potential for the production of polylactic biodegradable and biocompatible polymer lactic acid (PLA). PLA products can be utilized in a wide variety of applications from packaging to fibers and foams. The production of lactic acid using microbial fermentation has several advantages, including high purity (90-95%) of lactic acid results [12].

*Lactobacillus plantarum* is a homofermentative lactic acid bacteria, lactic acid is the primary metabolite [13,14] thus it is expected to produce high lactic acid. Fermentation is highly influenced by operating conditions such as pH, temperature, substrate concentration, inoculum concentration, and the addition of other nutrients.

In lactic acid fermentation on durian seed flour substrate with *Lactobacillus plantarum* bacteria, the optimal operating conditions have yet to be known. This study aimed to determine the effect of variable substrate concentration, inoculum concentration, and yeast extract concentration on yield and concentration of lactic acid produced.

### 2. Materials and methods

#### 2.1. Equipment and Materials

Autoclave (Hiclave HVE-50), laf (Laminar air flow 1300 series A2), incubator shaker (Labnet), UV-VIS spectrophotometer (Genesys 10S UV-VIS), centrifuge, analytical balance, water bath, inoculation loop, petri dish, filter paper, and pH meter.

Durian fruit seeds were obtained from the Pekalongan Regency, *Lactobacillus plantarum* FWCL-0250 was obtained from the Laboratory of Center for Food and Nutrition Studies UGM. The other materials were MRS (De man, rogosa, sharpe), Broth (Merck), MRS Agar (Merck), yeast extract granulated (Merck), concentrated HCL, alcohol 70 %, distilled water, DNS (Merck), potassium sulfite (Merck), and Glucose Anhydrite (Merck).

#### 2.2. Production of Durian Seed Flour

Durian seed waste was cleaned from the rest of the meat and dirt, was boiled for 20 minutes and peeled, thinly sliced, then the slices were dried under the sun, after it had dried, it was mashed with a dry mill and sieved with a 100 mesh sieve, durian seed flour was then ready to be used [4,6].

#### 2.3. Inoculation and Rejuvenation of *Lactobacillus plantarum*

Inoculation was performed on MRS Agar. 6.82 g of MRS Agar powder was dissolved in 100 mL of distilled water, heated and stirred until it dissolved, then sterilized using autoclave at 121°C for 15 minutes. Then poured in a petri dish and rested to solidify. The *Lactobacillus plantarum* ampule was put in the MRS. The incubation process was done for 24 - 48 hours at 37°C for optimal growth. Culture rejuvenation was carried out by growing 1 loop of *Lactobacillus plantarum* culture on MRS Broth media in an incubator at 37°C for 24 hours with an agitation speed of 100 rpm [15].

#### 2.4. Growth Patterns of *Lactobacillus plantarum*

*Lactobacillus plantarum* 10% (v/v) culture that had been rejuvenated was grown on MRS Broth medium then observed for the optical density (OD) using a spectrophotometer at a wavelength (λ) of 625 nm every 1 hour for 24 hours. The OD value represented the number of cells grown in MRS B medium [16].
2.5. Making a Starter Culture

*Lactobacillus plantarum* starter culture was prepared by growing 10% (v/v) rejuvenated culture for 24 hours on MRS Broth medium in 250 ml Erlenmeyer and incubated in an incubator for 3 hours at 37°C with an agitation speed of 100 rpm [9,17].

2.6. Preparation of Fermentation Media

The suspension contained a substrate of durian seed flour (7.5, 10, 12.5, 15 and 17.5%), yeast extract (0, 2.5, 5, 7.5, and 10 g/L) and was set the pH to ± 5.5 with 1 N HCL then sterilized at 80°C for 10 minutes [9].

2.7. Lactic Acid Fermentation

The prepared fermentation medium was added to the *Lactobacillus plantarum* inoculum at temperatures reaching 37-40°C according to the concentration variables, namely 2, 5, 7, 10, and 12% (v/v). The acid fermentation process was executed in a fermenter using a batch system at 37°C, pH ± 5.5 for 72 hours, with a stirring speed of 100 rpm [9,10].

2.8. Samples and Proximate Analysis of Durian Seed Flour

The proximate analysis consisted of protein analysis (SNI 01 2354.42006), fat content (SNI 2891 01 1992), water content (SNI 2354.2:2015), ash content (SNI 01-2354.1-2006), and carbohydrates by difference. The fermentation solution was centrifuged at a speed of 4000 rpm for 15 minutes, then filtered using Whatman filter paper no.1 and stored at -4°C. The supernatant result from the centrifugation was measured for the lactic acid concentration by Ultra Performance Liquid Chromatography (UPLC) [19].

3. Results and Discussion

3.1. Results of Proximate Analysis of Durian Seed Flour

Durian seed waste was obtained from local durian fruit traders in the Kajen area of Pekalongan Regency, durian seeds contain starch and sap [4], hence the seeds needed to be boiled to remove them. Data from proximate analysis of durian seed flour can be seen in Table 1.

| Proximate composition of Durian Seed Flour (% w) |  |
|-----------------------------------------------|---|
| Moisture                                      | 9.861 |
| Ash                                           | 6.826 |
| Fat                                           | 1.187 |
| Protein                                       | 8.828 |
| Carbohydrates                                 | 73.298 |

Table 1 shows the nutritional value of durian seed flour, namely moisture content, ash content, fat, protein and carbohydrates, respectively 9.861%, 6.826%, 1.187%, 8.828%, and 73.298%. The high carbohydrate content in durian seed flour can be made into glucose syrup [7]. High carbohydrate is a macronutrient source of carbon for microbial growth, it can be used by lactic acid bacteria to produce lactic acid [8,9,11].

3.2. Growth Curve of Lactobacillus plantarum Bacteria

The growth phase of *Lactobacillus plantarum* was used to determine the incubation time during lactic acid production. Bacterial growth was observed for optical density/OD value every 1 hour using the turbidimetric method with a wavelength of 625 nm [9]. LAB growth had increased with increasing OD
value, the more turbid the bacterial suspension, the more the number of bacteria would grow. The growth of Lactobacillus plantarum bacteria can be seen in Figure 1.

![Figure 1. Growth Curve of Lactobacillus plantarum Bacteria](image)

The growth curve of Lactobacillus plantarum consisted of a lag phase, a log phase (exponential), a stationary phase. The lag phase occurred at 0 to 2 hours. In the lag phase, bacteria carried out an adaptation process to their environmental conditions, the increase in the number of bacterial cells is slow. The second phase was the exponential phase, the growth of bacteria was very fast, starting at the 2nd hour until the 21st hour, in this phase a type of microbe multiplied by dividing itself. The next phase was the stationary phase, which occurred from the 21st hour to the 24th hour. In this phase there was no increase in the number of bacteria, the number of cells that grew was the same as the number of cells that died because food reserves were running low.

Lactic acid was produced by Lactobacillus plantarum as a primary metabolite [13,14], it was a growth-associated product, so its production had a linear relationship with the growth rate. Lactobacillus plantarum at the 3rd hour was used as the incubation time before fermentation because at that time the bacteria began to grow optimally [20].

### 3.3. Effect of substrate concentration

The durian seed flour produced was used as a substrate for growth media in lactic acid fermentation using Lactobacillus plantarum bacteria. Table 2 show that lactic acid increased with increasing substrate concentration. At a substrate concentration of 7.5%, 10%, 12.5%, 15% and 17.5%, the lactic acid obtained were 2.31 g/L, 5.49 g/L, 8.67 g/L, 13.75 g/L, and 15.02 g/L. The yield was calculated by dividing the lactic acid obtained to the total carbohydrates in the substrate, the yield increased at the substrate concentration of 7.5%, 10%, 12.5% and 15% by 4.2%, 7.49%, 9.46%, and 12.51% but decreased at the substrate concentration of 17.5% for 11.71%, therefore, the best (efficient) lactic acid and yield was at a substrate concentration of 15%.
Table 2. Effect of substrat concentration

| Substrat concentration (%) | Lactic acid production (g/L) | Yield (%) |
|----------------------------|-----------------------------|-----------|
| 7.5                        | 2.31                        | 4.20      |
| 10                         | 5.49                        | 7.49      |
| 12.5                       | 8.67                        | 9.46      |
| 15                         | 13.75                       | 12.51     |
| 17.5                       | 15.02                       | 11.71     |

3.4. Effect of inoculum concentration

The inoculum had a strong influence on the produced lactic acid and yield. Table 3 show that the higher the inoculum concentration, the lower the lactic acid and the production yield would decrease [21]. At the concentration of 2%, 5%, 7%, 10%, 12%, the obtained lactic acid and the yield were 21.38 g/L, 19.44%; 13.75 g/L, 12.51%; 5.49 g/L, 4.99%; 2.95 g/L, 2.68%; 1.67 g/L, 1.52% respectively. The highest lactic acid concentration and the yield were obtained at an inoculum concentration of 2%. The addition of the inoculum at a low concentration caused the fermentation rate to be slower, but it could produce a higher concentration of lactic acid because after the cells reproduce themselves, the cells would gradually convert sugar into lactic acid. High inoculum concentration resulted in weakened lactic acid production and reduced cell viability [22].

Table 3. Effect of inoculum concentration

| Inoculum concentration (%) | Lactic acid production (g/L) | Yield (%) |
|-----------------------------|-----------------------------|-----------|
| 2                           | 21.38                       | 19.44     |
| 5                           | 13.75                       | 12.51     |
| 7                           | 5.49                        | 4.99      |
| 10                          | 2.95                        | 2.68      |
| 12                          | 1.67                        | 1.52      |

3.5. Effect of yeast extract concentration

Yeast extract is a source of macronutrients (nitrogen) for the development of bacteria that can produce higher lactic acid compared to urea, corn steep liquor, malt sprout, and ammonium sulfate [21,23].

According to Table 4, the higher concentration of yeast extract, the higher the lactic acid and the yield produced. Yeast extract concentrations of 0 g/L, 2.5 g/L, 5 g/L, 7.5 g/L and 10 g/L produced 2.31 g/L, 2.1%; 7.39 g/L, 6.73%; 8.67 g/L, 7.88%; 15.02 g/L, 13.66% and 23.92 g/L, 21.76%. The highest concentration of lactic acid and yield was obtained at a concentration of yeast extract 10 g/L. Yeast extract was not only a macronutrient for bacteria to produce lactic acid but also reduce the time required for the completion of fermentation, this was because yeast extract contained substances such as amino acids, peptone, vitamins, and several organic acids, for instance, pyruvic acid and glycerin [21].
Table 4. Effect of yeast extract concentration

| Yeast extract concentration (g/L) | Lactic acid production (g/L) | Yield (%) |
|-----------------------------------|-----------------------------|-----------|
| 0                                 | 2.31                        | 2.1       |
| 2.5                               | 7.39                        | 6.73      |
| 5                                 | 8.67                        | 7.88      |
| 7.5                               | 15.02                       | 13.66     |
| 10                                | 23.92                       | 21.76     |

4. Conclusion
Flour from waste durian seeds had a high carbohydrate content of 73.298%, so it can be used as a source of macronutrients (carbon sources) for the growth of lactic acid bacteria. The number of substrate concentrations, inoculum concentrations, and yeast extract concentrations affected lactic acid production. *Lactobacillus plantarum* bacteria could produce lactic acid in durian seed flour media as much as 23.92 g/L with a yield of 21.76% at the best fermentation conditions with substrate concentration, inoculum concentration, and yeast extract concentration respectively 15%, 2%, and 10 g/L.

Acknowledgments
This research was financially supported by The Faculty of Engineering, Diponegoro University, Indonesia through Strategic Research Grant 2020.

References
[1] A.M. Amin et al., *J. Food Hydrocolloids*, 21, 273–279 (2007).
[2] M. Cornelia, T. Siratantria, and R. Prawitaa, *J. Procedia Food Science*, 3, 1-18 (2015).
[3] Central of Bureau Statistics (BPS), Statistics of Annual Fruit and Vegetable Plants Indonesia (Jakarta: Central of Bureau Statistics Indonesia) (2017).
[4] S. Baraheng and T. Karrila, *J. Food Bioscience*, 30, 100412 (2019).
[5] I.R. Kartika, *J. Mesomeri*, 1, 86-97 (2011).
[6] E. Sistanto, Sulistowyati and Yuwana, *J. Sains Peternakan Indonesia*, 12, 9-23 (2017).
[7] M. Djieni and A. Prasetyaningrum, *J. Aspek Nutrisi dan Tekno Ekonomi*, 4, 11 (2010).
[8] M.A. Abdel-Rahman, Y. Tashiro and K. Sonomoto, *J. Biotechnol. Adv.*, 31, 877–902 (2013).
[9] N. Istianah and S. Gunawan, Jurnal Rekayasa Bahan Alam dan Energi Berkelanjutan, 1, 49-55 (2017).
[10] F. Nurdiansyah and U.H.A. Hasbullah, *Journal of Biology*, 11, 64-71 (2018).
[11] A. Djukić-Vuković et al., *J. Renewable and Sustainable Energy Reviews*, 108, 238–252 (2019).
[12] M.A. Abdel-Rahman, Y. Tashiro and K. Sonomoto, *J. Biotechnol*, 156, 286-301 (2011).
[13] K. Okano, S. Yoshida, T. Tanaka, H. Fukuda and A. Kondo, *J. Appl Environ Microbiol*, 75, 5175–8 (2009a).
[14] K. Okano, S. Yoshida, R. Yamda, T. Tanaka, C. Ogino, H. Fukuda and A. Kondo, *J. Appl Environ Microbiol*, 75, 7858–61 (2009b).
[15] Wardani, A. Krisna, F. Nurtyastuti dan E. Pertiwi, *J. Agritech*, 33 (2013).
[16] J.G. Cappuccino and N. Sherman, *Microbiology: a Laboratory Manual Seventh Edition* (San Francisco: Pearson Education, Inc.) (2005).
[17] R.A. Speers, W. Yong-Quan, J. Yu-Lai and J.S. Robert, *Journal of Instrumental Brewing*, 112, 246–254 (2006).
[18] A. Abdullah, L. Lutfi, B. Muliajaya, C.N. Minasti, *Int. Conf. on Information Technology And Engineering Application* p 19-20 (2016).
[19] M. Zerbiba et al., *J. Food Chemistry*, 266, 441–448 (2018).
[20] G. Vrancken, T. Rimaux, L.D. Vuyst and F. Leroy, *International Journal of Food Microbiology*, **128**, 58–66 (2008).

[21] A. Abdullah and I. Winaningsih, *AIP Conference Proceedings* 2197, 060002 (2020).

[22] K. Mukhtar, M. Asgher, S. Afghan, K. Hussain and S. Ziaul-Hussnain, *Journal of Biomedicine and Biotechnology*, 1-5 (2010).

[23] M.T. Gao, M. Hirata, E. Toorisaka and T. Hano, *J. Bioresource Technology*, **97**, 2414–2420 (2006).