Optimization of Lactic Acid Fermentation from Durian Seeds (*Durio zibethinus Murr.*) Using Response Surface Method

Abdullah Abdullah*, 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. Lactic acid production in Indonesia has not been able to meet the needs of the industry, especially as the raw material for biodegradable plastic/poly lactic acid (PLA), thus an inexpensive and abundant alternative material is needed, based on previous research, durian seed waste has the potential as a raw material to produce lactic acid by fermentation using *Lactobacillus Plantarum* bacteria, but there is no optimization yet. This study aimed to determine the optimization mathematical modeling of the variables that affected yield and concentration of lactic acid, namely substrate concentration (15%), inoculum concentration (2%), and yeast extract concentration (10 g/L), using the Response Surface Method (RSM) and Central Composite Design (CCD) to save time and costs. This research was conducted in several stages, preparation of raw materials, testing the proximate value of the materials, inoculation and rejuvenation of bacteria, fermentation at 37°C, agitation speed of 100 rpm for 72 hours, then the fermentation results were analyzed using UPLC (Ultra Performance Liquid Chromatography) for its lactic acid concentration. Response Surface Method (RSM) was executed by using software Minitab 17. The obtained mathematical equation model for the fermentation reaction of lactic acid production was

\[
Y = 22.49 + 1.883X_1 + 1.305X_2 + 1.380X_3 - 3.273X_1X_1 - 2.558X_2X_2 - 1.475X_3X_3 - 0.968X_1X_2 + 0.390X_1X_3 + 0.954X_2X_3
\]

with the optimum conditions for substrate concentration, inoculum concentration, and yeast extract concentration respectively of 15.14%, 2.16% and 10.3 g/L.

Keywords: Durian seeds; RSM; Fermentation; lactic acid; *Lactobacillus plantarum*

1. Introduction

Lactic acid is 2-hydroxypropanoic acid present in two optical isomers, the L (+) and the D (−) enantiomers. The enantiomer of L-Lactic acid is produced and metabolized in humans by L-lactate dehydrogenase [1]. Lactic acid has many applications in the food, chemical, cosmetic, and pharmaceutical industries, making it the most important product. Lactic acid is also a raw material to
produce other chemical compounds, for instance, acrylic acid, lactate ester, 1-2-propanediol, and pyruvic acid [2]. It has great potential for the production of polyactic biodegradable and biocompatible polymer lactic acid (PLA). PLA products can be used in a wide variety of applications from packaging to fibers and foams.

Lactic acid can be produced by fermentation with lactic acid bacteria or synthesis by hydrolysis of lactonitrile, degradation of sugar with an alkaline catalyst, oxidation of propylene glycol, etc. More than 90% of the lactic acid in the global market is produced by lactic acid bacteria (LAB) which can stereoselectively produce L or D-LA [1,3] depending on the bacteria used, whereas synthesis produces a racemic mixture of the enantiomeric LA [4,5]. Lactobacillus plantarum is a lactic acid bacterium that plays the role of homofermentative metabolism of pentose to lactic acid [6,7], which can produce lactic acid with a high yield of 85% [8].

Lactic acid fermentation requires a substrate as a medium for bacterial growth, the main obstacle that many industries face is the high price of raw materials, hence renewable, cheap, and environmentally friendly raw materials are required [9]. Agricultural waste containing carbohydrates can be an alternative material to produce lactic acid [10].

Durian is the most popular seasonal fruit in Southeast Asia, especially in Malaysia, Indonesia, Thailand, and the Philippines [11,12]. Durian production in Indonesia is quite abundant. The Central Bureau of Statistics Republic of Indonesia stated that for fruits and vegetables (2017), durian production is increasing every year. A total of 735,423 tons in 2016 and 795,211 tons in 2017 [13]. As yet, the salutary fruit or the meat is the most consumed pars of durian. It takes about 30% of the durian fruit while the rest is waste, of which the other 20–25% of the whole fruit are seeds that have not been fully utilized [11,12]. Ripe durian seeds contain 51.1% water, 46.2% carbohydrates, 2.5% protein, and 0.2% fat [14]. The high carbohydrate content of the seeds can be used for lactic acid fermentation [8,10,15,16].

The efficiency of the lactic acid fermentation process is highly dependent on the lactic acid producer (LAB), a fermentation substrate, and operational models [15], fermentation with durian seed substrate using Lactobacillus plantarum has been studied before, but the optimization of variables has not been studied. Therefore, this research discussed the optimization of independent variables that affected the yield of lactic acid production using durian seed flour as the substrate. The RSM method is one of the effective methods used as a modeling in the optimization of biochemical and biotechnological processes related to food processes [17,18].

The optimization design in this study used the Response Surface Method (RSM) of the Central Composite Design (CCD) system in Minitab 17 software.

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 [19,20].
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. 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 [8,22].

2.5. 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 [8].

2.6. 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 [8,16].

2.7. 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) [23].

2.8. Design of Experiment

The variable optimization stages were carried out based on the Central Composite Design (CCD) of the Response Surface Methodology (RSM) experimental design which aimed to determine the optimum point for the variables of the lactic acid fermentation process. The design used in the second-order experiment was the $2^k$ factorial design, a central point design, and an axial runs design, carried out in 20 experimental units. The design of axial runs is a design with the combination of ± $\alpha$* value and the center point value. The value of ± $\alpha$* is obtained from $\alpha*=(nf)^{1/4}$, in this case, $nf=2^3=8$, thus obtaining $\alpha*=1.682=\pm 1.682$ [24]. C is the code in the central composite design method to determine the model from the independent variable (X). The relationship between the two is presented in Eq. (1) [24].

$$C = \frac{X-x_{cp}}{\Delta X}$$  (1)

Where $X_{cp}$ is the center point variable and $\Delta X$ is the difference between the boundary variable and the center point.
Table 1. Determination of independent variables and treatment code in the study.

| Independent Variable        | Code | Range and Level |
|----------------------------|------|-----------------|
| Substrate concentration    | X1   | 14.5 15 15.5    |
| Inoculum concentration     | X2   | 1.5 2 2.5       |
| Yeast extract concentration| X3   | 9.5 10 10.5     |

3. Results and Discussion

3.1. Optimization Results of Lactic Acid Fermentation
In this study, the effect of variable X1 (substrate concentration) and variable X2 (inoculum concentration) on Y1 (lactic acid concentration) were observed using the RSM CCD. The results of the experiment are presented in Table 2.

Table 2. Results of Lactic Acid Fermentation

| Run | X1 | X2 | X3   | Y (%) |
|-----|----|----|------|-------|
| 1   | -1 | -1 | -1   | 12.566|
| 2   | 1  | -1 | -1   | 12.623|
| 3   | -1 | 1  | -1   | 12.681|
| 4   | 1  | 1  | -1   | 12.739|
| 5   | -1 | -1 | 1    | 12.797|
| 6   | 1  | -1 | 1    | 18.289|
| 7   | -1 | 1  | 1    | 20.601|
| 8   | 1  | 1  | 1    | 18.347|
| 9   | -1.682 | 0 | 0 | 6.727 |
| 10  | 1.682 | 0  | 0   | 20.023|
| 11  | 0   | -1.682 | 0 | 12.508|
| 12  | 0   | 1.682 | 0   | 18.289|
| 13  | 0   | 0   | -1.682 | 18.636|
| 14  | 0   | 0   | 1.682 | 18.289|
| 15  | 0   | 0   | 0    | 23.203|
| 16  | 0   | 0   | 0    | 23.492|
| 17  | 0   | 0   | 0    | 21.179|
| 18  | 0   | 0   | 0    | 21.758|
| 19  | 0   | 0   | 0    | 22.336|
| 20  | 0   | 0   | 0    | 22.914|

3.2. Model Equations Using Response Surface Methods (RSM)
The process of variable optimization was based on the central composite design which stated the relationship between the 3 independent variables and yield. The model equation was determined through Response Surface Methods (RSM) by using Minitab 17 program. The model is presented in mathematical Eq. (2).
\[ Y = 22.49 + 1.883X_1 + 1.305X_2 + 1.380X_3 - 3.273X_1X_1 - 2.558X_2X_2 - 1.475X_3X_3 - 0.968X_1X_2 + 0.390X_1X_3 + 0.954X_2X_3 \]  

Based on the Eq. (2), three effects affect the Y value (% w/w lactic acid yield), namely the linear effect, the quadratic effect, and the interaction effect. Their effect is known from the coefficient value.

In the linear effect, the coefficient \( X_1 \) (% substrate concentration) had the largest coefficient (+1.883). Thus, \( X_1 \) had a big effect on the increase of % yield of lactic acid. \( X_3 \) (yeast extract concentration) had the second-largest coefficient (+1.380) while \( X_2 \) (% inoculum concentration) was the third most influential variable (+1.305). The positive sign on the linear effect indicated that the higher concentration of the substrate, inoculum, and yeast extract variables would increase the % yield of lactic acid.

The overall coefficient on the quadratic effect was negative, which made the decrease in the substrate squared (\(-3.273 X_1X_1\)), inoculum squared (\(-2.558 X_2X_2\)), and yeast extract squared (\(-1.475\)) affected the increase of % yield. A decrease in the substrate-inoculum interaction effect (\(-0.968 X_1X_2\)) increased % yield, while an increase in the interactive effect of the substrate-yeast extract (\(+0.390 X_1X_3\)) and inoculum-yeast extract (\(+0.954 X_2X_3\)) increased the % yield lactic acid.

### 3.3. Analysis of Variance

Analysis of variance is used to evaluate the accuracy and significance of the experimental results obtained. This study used ANOVA in determining the analysis of variance. From the ANOVA table, the P-value, F-value, and \( R^2 \) can be obtained as the indicators. Analysis of variance of experimental results is presented in Table 3.

**Table 3.** Analysis of variance minitab software output

| Source      | DF | Adj SS  | Adj MS  | F-Value | P-Value |
|-------------|----|---------|---------|---------|---------|
| Model       | 9  | 353.814 | 39.313  | 5.18    | 0.008   |
| Linear      | 3  | 97.656  | 32.552  | 4.29    | 0.035   |
| X1          | 1  | 48.420  | 48.420  | 6.38    | 0.030   |
| X2          | 1  | 23.242  | 23.242  | 3.06    | 0.111   |
| X3          | 1  | 25.994  | 25.994  | 3.42    | 0.094   |
| Square      | 3  | 240.159 | 80.053  | 10.54   | 0.002   |
| X1*X1       | 1  | 154.407 | 154.407 | 20.33   | 0.001   |
| X2*X2       | 1  | 94.291  | 94.291  | 12.41   | 0.006   |
| X3*X3       | 1  | 31.337  | 31.337  | 4.13    | 0.070   |
| 2-Way       | 3  | 15.999  | 5.333   | 0.70    | 0.572   |
| Interaction | X1*X2 | 1 | 7.501 | 7.501 | 0.99    | 0.344   |
|            | X1*X3 | 1 | 1.218 | 1.218 | 0.16    | 0.697   |
|            | X2*X3 | 1 | 7.279 | 7.279 | 0.96    | 0.351   |

In Table 3, it can be seen that the P-value in the model = 0.008 is smaller than the degree \( \alpha = 5\% \), this shows that the polynomial model was the right model for this study. Apart from the P-value, the F-value is suitable to determine the effect of the variables on the model with the hypothesis \( H_0 \): there is no influence of the variable on the model and \( H_1 \): there is a variable effect on the model. The calculated F-value (\( F_{model} \)) was 5.18 greater compared to the F table value (\( F_{0.05; 9/10} = 3.02 \)). Because \( F_{model} > F_{table} \), \( H_0 \) was rejected, which means that the independent variables X influenced on the model [25].

The accuracy of the model can also be observed from the efficient value of determination, \( R^2 \) which reached 82.33%. The model can be declared accurate if the value of \( R^2 \) exceeds 70%, therefore, the value estimated by the model was close to the value obtained from the experimental results. It can be concluded that 82.33% of the total variation in the results obtained was represented in the model [25].
3.4. The Effect of Substrate Concentration, Inoculum Concentration, and Yeast Extract Concentration on Lactic Acid Yield

The optimum operating conditions with the RSM method can be interpreted with three-dimensional images (surface plot and contour plot). The three-dimensional optimization image consists of the x, y, and z axes where the x and y axes are the variables being tested, while the z-axis shows the response/yield value obtained from the interaction of the two variables tested. The contour plot in this study had a rising ridge type, on the surface contour plot, the color areas are drawn. Each color indicates a susceptible yield concentration. The highest lactic acid yields are represented by the darkest green area.
Figure 1. Surface plot (a) and contour plot (b); Effect of % substrate concentration vs % inoculum on % yield of lactic acid

According to Fig. 1. Surface plot and contour plot; Effect of % substrate concentration vs % inoculum on % yield of lactic acid, the dark contour surface color in the middle has a yield > 20%, the yield would increase with an increase in the substrate and a decrease in the inoculum. The substrate had a positive effect on increasing yield, where an increase in substrate caused an increase in yield, while inoculum concentration had a negative effect on an increase in yield, where an increase in inoculum concentration caused a decrease in yield. The substrate concentration (+1.883) had a greater effect than the inoculum concentration (+1.305). This was indicated by the coefficients in the model equation. The greater the coefficient, the greater the influence of the variable on the response. The greatest yield was produced at substrate concentrations between 14.6% - 15.6%, while inoculum concentrations were between 1.6% - 2.6%.

(a)
Based on Fig. 2. Surface plot and contour plot; Effect of % substrate concentration vs % yeast extract concentration (g/L) on % yield of lactic acid, it can be seen that the dark contour surface color in the middle has a yield > 20%, the yield would increase with an increase in the substrate and yeast extract. The substrate concentration had a greater effect than the concentration of yeast extract. This was indicated by the coefficients in the model equation. The greater the coefficient, the greater the effect of the variable on the response. The greatest yield resulted in the substrate concentration between 14.6% - 15.6%, while the concentration of yeast extract was between 9.5 g/L - 10.9 g/L.
Figure 3. Surface plot (a) and contour plot (b); Effect of % inoculum concentration vs % yeast extract on % yield of lactic acid

Fig. 3. Surface plot and contour plot; Effect of % inoculum vs concentration of yeast extract (g/L) on % yield of lactic acid illustrates a yield > 20% shown by the dark contour surface color in the middle, the yield would increase with an increase in inoculum and yeast extract. Yeast extract concentration had a greater effect than inoculum concentration. This was indicated by the coefficients in the model equation. The greater the coefficient, the greater the influence of the variable on the response. The highest yield resulted in inoculum concentration between 1.8% - 2.5%, while the concentration of yeast extract was between 9.8 g/L - 10.7 g/L.

3.5. The Optimum Conditions for the Fermentation Process
The resulting response fitted surface was parabolic and the contour plot was oval, indicating the type of process optimization was maximal. The critical/optimal values for each variable are shown in Fig. 4.

Figure 4. Critical value of the variable
The mathematical equation for the fermentation reaction of lactic acid in durian seed flour media using *Lactobacillus plantarum* is \( Y = 22.49 + 1.883X_1 + 1.305X_2 + 1.380X_3 - 3.273X_1X_2 - 2.558X_2X_3 - 1.475X_3X_3 - 0.968X_1X_2 + 0.390X_1X_3 + 0.954X_2X_3 \). Based on the coefficient value, the % substrate concentration was the variable that had a bigger effect on the increase in % yield of lactic acid compared to the % concentration of inoculum and yeast extract. The optimum conditions for the fermentation process were achieved at % substrate concentration, % inoculum concentration, and yeast extract concentration of 15.14%, 2.16%, and 10.3 g / L, respectively.

4. Conclusion
The mathematical equation for the fermentation reaction of lactic acid in durian seed flour media using *Lactobacillus plantarum* is \( Y = 22.49 + 1.883X_1 + 1.305X_2 + 1.380X_3 - 3.273X_1X_2 - 2.558X_2X_3 - 1.475X_3X_3 - 0.968X_1X_2 + 0.390X_1X_3 + 0.954X_2X_3 \). Based on the coefficient value, the % substrate concentration was the variable that had a bigger effect on the increase in % yield of lactic acid compared to the % concentration of inoculum and yeast extract. The optimum conditions for the fermentation process were achieved at % substrate concentration, % inoculum concentration, and yeast extract concentration of 15.14%, 2.16%, and 10.3 g / L, respectively.

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

References

[1] A. Carlsten et al., *J. Clin. Lab. Investig*, 13, 418–28 (1961).
[2] C. Gao, C.G. Ma, and P. Xu, *J. Biotechnol. Adv.*, 29, 930–939 (2011).
[3] L. Axelsson and S. Ahme, Lactic acid bacteria. In: Priest FG, Goodfellow M, editors Applied microbial systematics (Dordrecht: Springer) p. 367–88 (2000).
[4] M.G. Adsul, A.J. Varma, D.V. Gokhale, *J. Green Chem.*, 9, 58–62 (2007).
[5] R.P. John, R.K. Sukumaran, K.M. Nampoothiri and A. Pandey, *J. Biochem Eng.*, 36, 262 (2007).
[6] K. Okano, S. Yoshida, T. Tanaka, H. Fukuda and A. Kondo, *J. Appl Environ Microbiol*, 75, 5175–8 (2009a).
[7] K. Okano, S. Yoshida, R. Yamda, T. Tanaka, C. Ogino, H. Fukuda and A. Kondo, *J. Appl Environ Microbiol*, 75, 7858–61(2009b).
[8] N. Istianah and S. Gunawan, Jurnal Rekayasa Bahan Alam dan Energi Berkelanjutan, 1, 49-55 (2017).
[9] M.A. Abdel-Rahman, Y. Tashiro and K. Sonomoto, *J. Biotechnol*, 156, 286–301 (2011).
[10] A. Djukić-Vuković et al., *J. Renewable and Sustainable Energy Reviews*, 108, 238–252 (2019).
[11] A.M. Amin et al., *J. Food Hydrocolloids*, 21, 273–279 (2007).
[12] M. Cornelia, T. Siratantria, and R. Prawitaa, *J. Procedia Food Science*, 3, 1-18 (2015).
[13] Central of Bureau Statistics (BPS), Statistics of Annual Fruit and Vegetable Plants Indonesia (Jakarta: Central of Bureau Statistics Indonesia) (2017).
[14] M. Djaeni and A. Prasetyaningrum, *J. Aspek Nutrisi dan Tekno Ekonomi*, 4, 11 (2010).
[15] M.A. Abdel-Rahman, Y. Tashiro and K. Sonomoto, *J. Biotechnol. Adv.*, 31, 877–902 (2013).
[16] F. Nurdyansyah and U.H.A. Hasbullah, *Journal of Biology*, 11, 64-71 (2018).
[17] M. Vázquez and A.M. Martin, *J. Biotechnology Bioengineering*, 57, (3) 314 (1998).
[18] J.A. Ramirez et al., *Journal of Food*, 3, (1) 21-28 (2000).
[19] S. Baraheng and T. Karrila, *J. Food Bioscience*, 30, 100412 (2019).
[20] E. Sistanto, Sulistiyowati and Yuwana, *J. Sains Peternakan Indonesia*, 12, 9-23 (2017).
[21] Wardani, A. Krisna, F. Nurtyastuti dan E. Pertiiwi, *J. Agrüech*, 33 (2013).
[22] R.A. Speers, W. Yong-Quan, J. Yu-Lai and J.S. Robert, Journal of Instrumental Brewing, 112, 246–254 (2006).
[23] M. Zerbiba et al., J. Food Chemistry, 266, 441–448 (2018).
[24] Kutner et al., Aplied Linear Statistical Models 5th edition (Boston: Mc Graw-Hill) (2005).
[25] L. Yu, T. Lei, X. Ren, X. Pei and Y. Feng, Biochemical Engineering Journal, 39, (3):496–502 (2008).
[26] A. Abdullah, L. Lutfi, B. Muliajaya, C.N. Minasti, Int. Conf. on Information Technology And Engineering Application p 19-20 (2016).
[27] R. Trihaditia, M. Syamsiah, A. Awaliyah, J. Agroscience, 8, 212-230 (2018).