Optimization of Vanillin Extraction from Biodegradation of Oil Palm Empty Fruit Bunches by *Serpula lacrymans*

Optimasi Ekstraksi Vanillin Dari Biodegradasi Tandan Kosong Kelapa Sawit oleh *Serpula lacrymans*

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Abstract

This research aims to determine the combination of the ethyl acetate solvent volume and the extraction time that resulted in the optimum response of vanillin content and vanillin yield from the degradation of lignocellulosic components from oil palm empty fruit bunches (OPEFB). First, OPEFB degraded using *Serpula lacrymans* to break down lignocellulosic components. The research design used a centralized composite design with two factors, the volume of ethyl acetate solvent (ml) and the extraction time (minutes). The responses of the experiment are vanillin content and vanillin yields. The optimization analysis results showed that the volume of ethyl acetate solvent and extraction time have a quadratic effect on the vanillin content and vanillin yields. The optimal solution was obtained by treatment with ethyl acetate volume 101.1 ml and extraction time 123.5 minutes. The optimal solution prediction results obtained vanillin content 0.014% and vanillin yield 7.302 μg/g with desirability of 92.8%. Validation based on the optimal solution’s prediction brought response vanillin content 0.013% and vanillin yield 6.950 μg/g. The validation content and yield validation results differed respectively by 4.081% and 4.826% lower when compared to predictions on the optimal solution.

Keywords: ethyl acetate, vanillin content, vanillin yield

Abstrak

Penelitian ini bertujuan untuk mengetahui kombinasi dari volume pelarut etil asetat dan lama waktu ekstraksi yang menghasilkan respon kadar vanillin dan yield vanillin optimum dari hasil degradasi komponen lignoselulosa tandan kosong kelapa sawit. Tandan kosong kelapa sawit terlebih dahulu diidegradasi menggunakan jamur pelapak *Serpula lacrymans* untuk memecah komponen lignoselulosa. Rancangan penelitian menggunakan rancangan komposit terpusat dengan dua faktor, yaitu volume pelarut etil asetat (ml) dan lama waktu ekstraksi (menit). Respon dari percobaan tersebut adalah kadar vanillin dan yield vanillin. Hasil penelitian untuk analisis optimasi menunjukkan bahwa faktor volume pelarut etil asetat dan lama waktu ekstraksi berpengaruh secara kuadratik terhadap respon kadar vanillin dan yield vanillin. Hasil solusi optimal diperoleh pada perlakuan dengan volume pelarut etil asetat sebesar 101,1 ml dan lama ekstraksi selama 123,5 menit. Hasil prediksi solusi optimal diperoleh kadar vanillin 0,014% dan yield vanillin 7,302 μg/g dengan ketepatan 92,8%. Validasi yang dilakukan berdasarkan pada prediksi solusi optimal diperoleh respon kadar vanillin 0,013% dan yield vanillin 6,950 μg/g. Hasil validasi kadar dan yield vanillin tersebut memiliki perbedaan masing-masing 4,081% dan 4,826% lebih rendah jika dibandingkan dengan prediksi pada solusi optimal.

Kata kunci: etil asetat, kadar vanillin, yield vanillin

INTRODUCTION

Oil palm empty fruit bunches (OPEFB) are the main waste in the palm oil-processing industry, with about 23%, followed by 14% mesocarp fiber; and 7% shells (Omar et al., 2011). Indonesia is one of the largest palm oil producing countries in Southeast Asia and the world (Isroi et al., 2012; Respati, 2016). Based on data from the Indonesian Ministry of Agriculture, total palm oil production in 2017 reached 37.97 tonnes and is estimated to increase to 42.87 tonnes in 2019 (Kementerian Pertanian, 2019). This estimate can be the basis for evaluating OPEFB produced from 8.73 tonnes in 2017 to 9.86 tonnes in 2019. OPEFB currently has a low economic value...
because its utilization is still limited, including natural fertilizers and garden mulch (Suhartati et al., 2016). OPEFB is also a problem in disposal because of its large number. Generally, OPEFB is burned, dumped in landfills, or composted (Isroi et al., 2012). OPEFB, as lignocellulosic biomass is composed of 24-65% cellulose, 21-34% hemicellulose, and 14-31% lignin (Suksong et al., 2019). Lignin is the most abundant amorphous aromatic polymer in lignocellulosic biomass (Tang et al., 2015) and needs to be broken down because the lignin polymer is irregular and very complex. The breakdown of lignin structures or pretreatment can be done biologically using the weathering fungus *Serpula lacrymans* (Korrippally et al., 2013).

The biotransformation process of lignin through degradation will produce lignin derivative products, including aromatic aldehyde monomers (such as vanillin, p-hydroxybenzaldehyde, and syringaldehyde) and hydroxycinnamic or phenolic acids (such as vanillic acid, ferulic acid, and p-coumaric acid) (Gomes & Rodrigues, 2019; Tang et al., 2015). Vanillic acid is a phenolic compound formed from oxidized vanillin (Mahendra et al., 2019). Ferulic acid is a phenolic acid that is a component of plant cell walls (Sibhatu et al., 2021) converted to vanillin (Gallage et al., 2014). P-coumaric acid, a phenylpropanoid type is a natural antioxidant that acts as an antidote to free radicals. P-coumaric acid can be converted into ferulic acid (Katsuragi et al., 2010).

This study focuses only on vanillin. Vanillin is an essential phenolic aldehyde and gives it the distinctive aroma of vanilla. Vanillin is a popular and valuable flavoring compound. Vanillin is the main constituent of natural vanilla flavor obtained from the vanilla plant (Gallage et al., 2014). Vanillin (4-hydroxy-3-methoxybenzaldehyde) is widely used worldwide in food, beverages, and medicines (Kundu, 2017; Wang et al., 2018). An alternative of vanillin production from renewable sources, one of them is lignin (Gomes & Rodrigues, 2019; Wang et al., 2018). Lignin has advantages as a raw material for vanillin production such as a unique aromatic structure, high reserves, low cost, and is more environmentally friendly when compared to guaiacol (Wang et al., 2018).

The extraction process using an organic solvent can be alternatively performed to obtain vanillin (Di Gioia et al., 2011). The solubility of phenolic compounds such as vanillin is determined or influenced by factors such as the type of solvent (Medini et al., 2014), the extraction time (Aires, 2017), and the volume of solvent used (Czemplik et al., 2017). The resin extraction containing vanillin was carried out using ethyl acetate (1:2, w/v) and shaken at 200 rpm 30 °C for 2 hours (Lun et al., 2014). Ethyl acetate is used as a solvent because of its low dielectric constant (ε = 6.0); and is widely used as an extractant from food samples in many interesting organic analytes from food samples (Agüi et al., 1999). Isolation of vanillin from vanilla fruit by extracting Soxhlet using ethyl acetate resulted in 2.01% vanillin with a melting point of 81 °C (Handayani et al., 2011). This method gives good results because generally, the vanillin content in vanilla fruit ranges from 1.5 - 3% (Medina et al., 2009). Process optimization is a crucial thing to consider in the design of the extraction process. Process optimization is also important for the transition from laboratory scale to commercial scale (Sanz et al., 2017).

Optimization is defined as a way to find the best solution by considering the existing constraints (Karamba et al., 2016; Sanz et al., 2017). Response Surface Method (RSM) is an experimental approach to find optimal and efficient conditions for various factors that produce the best operation or process. RSM is used to evaluate the relationship between experimental factors that can be controlled and the observed results (Wang, 2011). RSM is used in this study because RSM can optimize several variables or factors simultaneously. RSM can also analyze the interaction between factors using statistics to obtain a regression equation for the optimization model. RSM provides a large amount of information and a more economical approach (Wani et al., 2012). The advantages of RSM are that it can determine the interaction between independent variables, mathematical modeling of the system, and save time and costs by reducing the number of trials (Aydar, 2018). Optimization was carried out in this study on adding of a volume of ethyl acetate solvent and extraction process time to the response of vanillin content and vanillin yield from OPEFB degraded by rotting fungus *Serpula lacrymans*. The aim was to determine the combination of ethyl acetate solvent volume and the length of extraction time which resulted in an optimum response of vanillin levels and vanillin yield.

**METHODS**

The primary material in this research is OPEFB, taken from PT Sawit Arum Madani (Blitar, East Java, Indonesia). OPEFB dried first
by drying for 5-7 days at a temperature range of 27-30 °C. Reducing the size is then done using scissors and cut to a size of ~ 1 cm. The rotting fungus used for pretreatment was *Serpula lacrymans* obtained from the Bio-Industry Laboratory, Department of Agro-Industrial Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, East Java, Indonesia.

The solvent used for extraction is ethyl acetate. The chemicals for analysis are ethyl alcohol, sodium hydroxide (NaOH), and Merck’s vanillin standard. The materials used as a culture-growing medium for *Serpula lacrymans* are Malt Extract Agar (MEA) from Merck, barley, water, calcium carbonate (CaCO₃), gypsum (CaSO₄), and glucose. MEA media materials consist of 2% w/v malt extract, 1.2% w/v agar, and distilled water that is added up to 1 liter. The grain spawn medium’s ingredients consist of barley cooked with a ratio of barley and distilled water 1: 1 then added with 0.001% w/w CaSO₄ and 0.0003% w/w CaCO₃.

The tool used for this research is the Kern brand analytic scales type ABJ 220, Erlenmeyer tube, glass spatula, Hirayama brand autoclave type HVE-50, disposable petri dish, glass bottle, inloop, tweezers, Bunsen, Laminar Air Flow (LAF) with 50 Watt power and 220 Volt voltage, centrifuge tube, measuring cup, test tube, filter cloth size 15 x 15 cm, filter paper with a pore size of 10 microns, Julabo brand waterbath shaker type SW 22 with a power/voltage of 2000 Watt/230 Volt and a speed of 150 rpm, Thermo Scientific brand UV-Vis spectrophotometer type Genesys 10 UV with a wavelength scan speed of 4200 nm/minute, Accumax brand micropipette 20-200 µl, Accumax brand micropipette 100-1000 µl, centrifugation (800 electric, Japan) and Rotary Vacuum Evaporator type RV 10, temperature 20-40 °C with 20-200 rpm (IKA, Malaysia).

The RSM experimental design uses a central composite design (CCD). The factors studied were ethyl acetate solvent volume (X₁) and extraction time (X₂). The conditions of each factor are as follows:

1. Factor I: the volume of ethyl acetate solvent with a lower limit value of 80 ml and an upper limit of 120 ml produces a mean value of 100 ml.
2. Factor II: extraction time with a lower limit value of 60 minutes and an upper limit of 180 minutes delivers a mean value of 120 minutes.

The water bath temperature for the extraction process as a control variable was 40 °C. Optimization level conditions based on the requirements of each of these factors can be seen in Table 1. The experimental design used Design Expert 7.0.0 software based on the optimization level conditions are shown in Table 2.

### Table 1. Optimization level conditions of a centralized composite experimental design

| Optimization Factor | Optimization Level |
|---------------------|--------------------|
| Ethyl acetate solvent volume (ml) | -α | -1 | 0 | 1 | α |
| Extraction time (minutes) | 71.72 | 80 | 100 | 120 | 128.28 |
| | 35.16 | 60 | 120 | 180 | 204.84 |

### Table 2. Independent variables and codes in a centralized composite experimental design

| No | Code Variables | Original Variable | Response |
|----|----------------|-------------------|----------|
|    | X₁  | X₂  | Ethyl Acetate Volume (ml) | Extraction Time (minutes) | Vanillin Content (%) | Vanillin Yield (µg/g) |
| 1  | -1  | -1  | 80  | 60  | Y₁  | Y₂  |
| 2  | 1   | -1  | 120 | 60  | Y₁  | Y₂  |
| 3  | -1  | 1   | 80  | 180 | Y₁  | Y₂  |
| 4  | 1   | 1   | 120 | 180 | Y₁  | Y₂  |
| 5  | -1.414 | 0  | 71.72 | 120 | Y₁  | Y₂  |
| 6  | 1.414 | 0   | 128.28 | 120 | Y₁  | Y₂  |
| 7  | 0   | -1.414 | 100 | 35.15 | Y₁  | Y₂  |
| 8  | 0   | 1.414 | 100 | 204.85 | Y₁  | Y₂  |
| 9  | 0   | 0   | 100 | 120  | Y₁  | Y₂  |
| 10 | 0   | 0   | 100 | 120  | Y₁  | Y₂  |
| 11 | 0   | 0   | 100 | 120  | Y₁  | Y₂  |
| 12 | 0   | 0   | 100 | 120  | Y₁  | Y₂  |
| 13 | 0   | 0   | 100 | 120  | Y₁  | Y₂  |

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RESULTS AND ANALYSIS

Optimization of the degradation results of OPEFB with Serpula lacrymans was carried out at an incubation time of 35 days and a temperature of 22 ± 2 °C. Table 3 shows the factor and response data based on a centralized composite design for OPEFB degradation using Serpula lacrymans.

Response Vanillin Levels

Table 3 shows that the highest vanillin content in the extraction of degradable EFB using Serpula lacrymans was 0.015% in the combination treatment of adding 100 ml volume of ethyl acetate with the extraction time of 120 minutes. The lowest vanillin level was 0.007% in the combination treatment of adding 80 ml volume of ethyl acetate with an extraction time of 60 minutes. The Fit Summary analysis results show that the suggested model for optimization of the response to vanillin levels is quadratic with a p-value <0.0001 and a significance value of p <0.05. ANOVA analysis results also show that the model is significant with an F value of 89.62. The volume factor of ethyl acetate solvent had a significant effect on the vanillin content with a

The volume of ethyl acetate in levels is quadratic with a

The longer the extraction time. This increase is because the amount of phenolic content in the extracted material is getting more prominent due to contact with ethyl acetate solvent (Ghasemzadeh & Jaafar, 2014). The volume of ethyl acetate added was thought to affect the levels of vanillin produced. Jadhav et al. (2009) showed that vanillin extraction from vanilla beans gave the lowest yield at a solvent volume of 66.67 ml and increased by about 50% at 100 ml solvent volume, but only slightly increased at 200 ml solvent volume. The volume of solvent used in the extraction process is critical in the production of vanillin compounds (Dağdelen & Dağdelen, 2016). The vanillin yield will increase if the extraction time is longer. However, the vanillin yield tends to decrease at a certain limit point because the solvent has reached its saturation point and is considered the optimal point. According to Jadhav et al. (2009), the extraction rate almost follows a linear path with extraction time and will decrease with longer extraction time.

Vanillin Yield Response

Table 3 shows that the highest vanillin yield was in the extraction of degraded OPEFB using Serpula lacrymans of 7.718 µg/g in the combination treatment of adding 100 ml volume of ethyl acetate with an extraction time of 120 minutes. The lowest vanillin yield was 3.002 µg/g in the combination treatment of 120 ml of ethyl acetate and an extraction time of 60 minutes. The results Fit Summary analysis showed that the recommended model for optimizing the vanillin yield response is quadratic with a p-value <0.0001 and a significant model with a p-value <0.05.

| No | X1 | X2 | Ethyl acetate Volume (ml) | Extraction Time (minutes) | Vanillin Content (%) | Vanillin yield (µg/g) |
|----|----|----|--------------------------|---------------------------|---------------------|----------------------|
| 1  | -1 | -1 | 80                       | 60                        | 0.007               | 3.885                |
| 2  | 1  | -1 | 120                      | 60                        | 0.011               | 3.002                |
| 3  | -1 | 1  | 80                       | 180                       | 0.009               | 3.827                |
| 4  | 1  | 1  | 120                      | 180                       | 0.010               | 4.138                |
| 5  | 1.414 | 0 | 71.72                    | 120                       | 0.007               | 4.235                |
| 6  | 1.414 | 0 | 128.28                   | 120                       | 0.011               | 2.223                |
| 7  | 0  | -1.414 | 100                      | 35.15                     | 0.010               | 3.335                |
| 8  | 0  | 1.414 | 100                      | 204.85                    | 0.009               | 4.743                |
| 9  | 0  | 0  | 100                      | 120                       | 0.015               | 7.778                |
| 10 | 0  | 0  | 100                      | 120                       | 0.014               | 7.190                |
| 11 | 0  | 0  | 100                      | 120                       | 0.015               | 7.598                |
| 12 | 0  | 0  | 100                      | 120                       | 0.014               | 7.311                |
| 13 | 0  | 0  | 100                      | 120                       | 0.013               | 6.690                |
Optimization of Vanillin Extraction

ANOVA analysis results also show that the model is significant with an F value of 42.48. The volume factor of ethyl acetate soluble had a significant effect on vanillin levels' response with a p-value <0.0001. The extraction time factor also had a significant effect on vanillin yield with a p-value of 0.0493. The $R^2$ value is 0.9681 with an adjusted $R^2$ of 0.9453.

Figure 1 (B) shows a shape curved upwards in reddish yellow color at the apex. This results shows the effect of adding ethyl acetate volume and extraction time on the vanillin OPEFB yield by quadratic. The vanillin yield is higher until the ethyl acetate volume is higher and the extraction time is longer at a certain point or the optimum point. The increase in vanillin yield occurs along with the extraction time carried out. Therefore, the amount of phenolic content in the extracted material is more significant due to contact with ethyl acetate solvent (Bhanja Dey & Kuhad, 2014). The vanillin yield increases as the volume of solvent increases to a certain extent. It is assumed that due to the polarity of the solvent, the extraction will increase with the increasing polarity of the solvent. In the research of Jadhav et al. (2009), the optimal solvent volume for vanillin extraction is 100 ml. The solvent takes a particular time to enter between the matrices by breaking the matrix so that the compounds in the material will come out (Dong et al., 2014).
Table 4. The optimal solution for the predictions uses Design Expert 7.0.0

| Criteria          | Parameter                      | Prediction Standards |
|-------------------|--------------------------------|----------------------|
| Factor            | Ethyl acetate volume (ml)      | 101.10               |
| Factor            | Extraction time (minutes)      | 123.50               |
| Response          | Vanillin content (%)           | 0.014                |
| Response          | Vanillin yield (μg/g)          | 7.302                |
| Desirability      |                                | 0.928                |

Optimal Solutions and Validation

The response of vanillin content and vanillin yield with the factor of adding a volume of ethyl acetate and extraction time of OPEFB showed the optimal treatment combination on the volume of ethyl acetate solvent of 101.1 ml and extraction time of 123.5 minutes (Table 4). The prediction of the optimal solution obtained vanillin content of 0.014% and vanillin yield of 7.302 μg/g. The desirability of this prediction is 0.928 or 92.8%.

Validation aims to determine the accuracy of predictions against the quadratic equation model obtained (Yeni et al., 2014). Validation was carried out based on the optimal solution’s prediction using a solvent volume of ethyl acetate of 101.1 ml and extraction time of 123.5 minutes. The validation results showed that the response to vanillin levels was 0.013%, and the vanillin yield was 6.950 μg/g. The validation for vanillin levels had a difference of 4.081% lower. The vanillin yield’s validation results had a difference of 4.826% lower when compared to the predictions for the optimal solution. These results are still acceptable; because the difference is still below 5% (Jayakrishna et al., 2018). The difference of less than 5% between the validation value and the predicted value indicates that the optimal point independent variable’s value is sufficiently appropriate to produce an optimal response (Sugiono, 2015).

CONCLUSIONS

Optimization analysis of the degradation yield of OPEFB using Serpula lacrymans showed that the volume factor of ethyl acetate solvent and the extraction time had a quadratic effect on the response of vanillin content and the response to vanillin yield. The optimal solution results with the best response prediction were obtained at the solvent volume of ethyl acetate of 101.1 ml and extraction time of 123.5 minutes. The results of the prediction of the optimal solution obtained vanillin content and vanillin yield respectively 0.014% and 7.302 μg/g, with an accuracy of 92.8%. The validation results showed that the vanillin content and vanillin yield were 0.013% and 6.950 μg/g, respectively. This result is still acceptable, because the difference with the optimal solution is still below 5%.

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