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Optimization of fulvic acids extraction from oil palm empty fruit bunches using microwave extractor

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Abstract

Fulvic acid (FA) derives from a non-renewable source, Shilajit, known as highly commercial values for its benefit for human health. Fulvic acid can also be extracted from materials such as coal, lignite, and peat. Extraction methods of FA generally use solid acids and bases, ion exchange chromatography, and their combinations. However, these methods cause corrosion, low purity, and environmental pollution. The FA extraction using organic solvents is common, but low yielded, and many organic solvents are toxic. Therefore, an effective way to separate organic solvents from FA must be determined. This research aims to extract the FA from renewable biomass, namely oil palm empty fruit bunches (OPEFB), using a microwave extractor combined with hydrogen peroxide. The advantage of using a microwave is its quick and efficient extraction process. Hydrogen peroxide is an environmentally friendly solvent that can be converted into water and oxygen. Fulvic acid extraction was optimized using expert design with the Response Surface Methodology method with optimization of four 4 factors (H_2O_2 concentration and volume, reaction time, and microwave power). The extracted FA was then characterized using FTIR, H-NMR, and Fluorescennce spectroscopy. The highest FA concentration namely 24.716%, was obtained using H_2O_2 at a concentration of 30.46% with a volume of 137.4139 mL, reaction time of 9.384 minutes, and microwave power of 351.39 W. *Fourier-Transform Infrared Spectroscopy* peaks at 3213 cm^{-1}, 2935.47 cm^{-1}, and 2825.13 cm^{-1} in the OPEFB-FA sample indicate existence of FA. The fluorescent emission intensity ratio between 450/500 nm wavelengths of OPEFB-FA was 0.719.

[Keywords: fluorescence spectroscopy, *FTIR, humic substance, hydrogen peroxide, H-NMR*]

Introduction

Oil palm empty fruit bunches (OPEFB) waste has been widely used, including converting OPEFB into paper (Rafidah et al., 2017), helmets (Nikmatin et al., 2017), fire-resistant composites (Suriani et al., 2021), and bioplastics (Faramitha et al., 2024). However, there has yet to be much research using OPEFB in the health sector. One of the valuable compounds that can be extracted from OPEFB is fulvic acid (FA). The naturally available FA for health is named Shilajit found in the Himalayan mountains. Shilajit is a semi-hard black resin formed through the long-term humification of several types of plants, especially mosses. The current market price of Shilajit as a medicine for most degenerative diseases is IDR 950.000 per 45 grams.

Fulvic acid can also be extracted from several sources, including peat, compost, and low-quality coal (Goenadi, 2021; Gong, 2020). Apart from being used in the health sector, FA also has benefits that are no less than humic acid (HA) in the agronomic sector. Several studies show the potential of FA as a growth stimulant in plants (Suh, 2014; Kandil et al., 2020), vegetables (Yang et al., 2014), coffee (Justi., et al., 2019), tobacco (Moradi et al., 2019), and wheat (Kumar et al., 2020). Application of FA to soil significantly increase P availability in soil. Mao (2019) reported that applying FA to livestock improve broiler chickens' growth, meat composition, and immunity. This material is a component of humic substances (HS) with low levels of aromatic compounds, small molecules, and many functional group, and has good water solubility. Of all HS components FA has

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the highest chemical, physiological, and physicochemical activities (Lotfi et al., 2015; Klučáková et al., 2017). Fulvic acid has several characteristics, including functioning as a matrix compound, easy to combine or react with other substances, having a micro to nanometer size, and having good biocompatibility (Swidsinski et al., 2017; Gong et al., 2019). Recent research shows that FA plays a unique role in the prevention and control of human and animal diseases (Sherry et al., 2013), control of heavy metal pollution in water and soil (Gunsolus et al., 2015; North et al., 2017), agriculture modern (Kumar et al., 2020), and other fields. The potential of FA has attracted the interest of many researchers. Efficient and environmentally friendly FA extraction processes and identifying their practical components have been at the center of research.

Coal-based extraction methods involve the precipitation of alkali-dissolved acids and extraction with strong acids. Recently, a combination of FA extraction methods using sulfuric acid and ethanol, ion exchange resin, and organic solvents has been developed (Shihua et al., 2014; Swidsinski et al., 2017). However, these extraction methods cause corrosion and environmental pollution. In addition, large amounts of acids and bases remained in the solid residue of the FA extract. Moreover, the quality of FA obtained using conventional extraction methods is also mixed with salts, some of which even contain inorganic salts, resulting in low yields of pure FA. As for the ion exchange resin method, the extraction speed is slow, the selection of suitable resin greatly determines the success of the extraction process, many FA components will dissolve and destroy the resin, and maintenance costs are also high. These factors limit the application of the method in industry. Extraction of FA using organic solvents is a method that is widely used, but the yield obtained is low. Some organic solvents are also toxic. This is why an effective way to separate organic solvents from FA must be developed.

Javed et al. (2013) extracted FA from Shilajit using a microwave extractor, while Zhang et al. (2020) and Gong et al. (2020) extracted FA from coal using a microwave extractor. However, FA extraction from renewable biomass (OPEFB) using a microwave extractor in combination with H_2O_2 has never been reported. Hydrogen peroxide can destroy the reticular structure of macromolecules in OPEFB and oxidize macromolecules into small molecules containing oxygen molecules, thereby increasing the FA yield. In this research, FA extraction was carried out from OPEFB biomass oxidized by microwaves with hydrogen peroxide. Optimization will be carried out by varying the volume and concentration of H_2O_2 as solvent, the reaction time and the power of the microwave extractor.

This research assessed variations in extraction time, volume, and concentration of solvent, as well as the power of the microwave extractor that influences the extraction of FA from biomass. The extracted FA was purified by freeze drying and characterized using FTIR, H-NMR, and Fluorescence spectroscopy.

Materials and Methods

Materials

The equipment used in this research were a microwave extractor, condenser, pump, separating funnel, beaker glass, Erlenmeyer, and measuring cup. The materials used for this research were dry-chopped OPEFB 1-2 cm in size that contains 27.78% lignin and 95% purity H_2O_2.

Experimental design and data analysis

Extraction formulation of FA uses a Box Behnken Design (BBD) with Response Surface Methodology (RSM). The Box Behnken Design consists of four (4) independent variables (K=4), namely: A (H_2O_2 concentration (%)), B (volume (mL)), C (time (minutes)), and D (microwave extractor power (W)). The upper and lower limits were obtained from preliminary trials by modifying the Zhang et al. (2020) method on raw material use, concentration, and volume. The response or dependent variable observed was FA content (Y%). The experimental design is presented in Table 1.

Table 1. Variables and levels are given to the FA extraction process in OPEFB with a Box Behnken Design

	Treatments	Levels	
		Lower limits	Upper limits
A	H_2O_2 concentration (%)	18	24
B	H_2O_2 volume (mL)	25	45
C	Time (minutes)	8	12
D	Microwave extractor power (W)	200	400

Replication of the optimum value (center point) is based on the number of factors used. For k=4, the center point is repeated five times with a running number of 29. There are 29 test points in the BBD with four factors. All points in the table will be tested in the laboratory to determine the concentration of each treatment variation given by Response Surface Methodology (RSM). Then, RSM will provide several optimum conditions for a process. These optimum conditions will be validated to prove how good the recommended design is.

Extraction of FA from OPEFB

The extraction process of FA uses a microwave extractor based on the method of Gong et al. (2020) with modifications starting by preparing chopped OPEFB with a size of 1-2 cm. A sample of OPEFB is washed and dried in an oven at 60ºC for 24 hours. The dried OPEFB was then added to an H_2O_2 solution with varying concentrations and volumes (Figure 1). Then, the final step was placed in the microwave extractor (Power 200-400W) for the specified time (8-12 minutes). After that, filtration was done to separate the OPEFB and the filtrate. The filtrate was then characterized for its FA content.

The fulvic acid content is determined based on the internal method (Syamsiah et al., 2019). The sample was weighed 1 g into the extract bottle, and 25 mL of 0.1N Na-pyrophosphate solution was added and then shaken for 30 minutes. Then, the sample was left for approximately one night and filtered using filter paper into a collection bottle to obtain the filtrate. Determination of total humic + FA was as follows: pipette 1 mL or 1 g of filtrate into a 100 mL volumetric flask + 5 ml 2N $K_2Cr_2O_7$ + 7.5 mL H_2SO_4 (concentrated) and measure with a spectrophotometer with a wavelength of 561 nm (Syamsiah et al., 2019).

Purification of FA extract

The extract of FA was purified using a freeze dryer (Labconco Freeze Dry 4.5L). Extract of FA in liquid form was placed in a 50 ml falcon bottle and frozen at -80 ℃. Freeze drying was carried out for 72 hours at a temperature of -84 ℃ and a vacuum pressure of 0.34 Mbar. Once purified, FA powder can be characterized.

Characterization of FA using FTIR (Fourier-Transform Infrared Spectroscopy)

Powdered FA extracted from OPEFB, a commercial FA fertilizer, and Shilajit was characterized using FTIR to compare FA extract from OPEFB with the commercial product–(fertilizer and Shilajit). Approximately 1.5 mg and 400 mg KBr were pressed into pellet form, then placed in a sample holder for analysis using a Bruker VERTEX 80v spectrophotometer (Bruker, Germany). Characterization of FA using FTIR is carried out by applying gentle force to the sample using a knob that can be adjusted to the sample holder. Infrared data was collected by scanning samples at 4000-400 cm^{-1} waves. Apart from OPEFB, a comparative study was also conducted on commercial samples, fertilizer of FA and Shilajit; different concentrations of acid peroxide solvent, microwave power, and volume were used. FTIR analysis uses FA powder from OPEFB, Shilajit, and fertilizer samples. The extraction results were then analyzed qualitatively using FTIR at the Test Laboratory of the Chemical Engineering Department, University of Indonesia.

Characterization of FA using H-NMR

The extract of FA was characterized using H-NMR nuclear magnetic resonance, using an Agilent 500 MHz NMR spectrometer with a DD2 console system, at a chemical shift of -1 to 4 ppm.

Characterization of FA using fluorescence spectroscopy

The fluorescence index of the FA extract was measured using a Hitachi F4600 fluorescence spectrophotometer, which has an excitation wavelength of 370 nm.

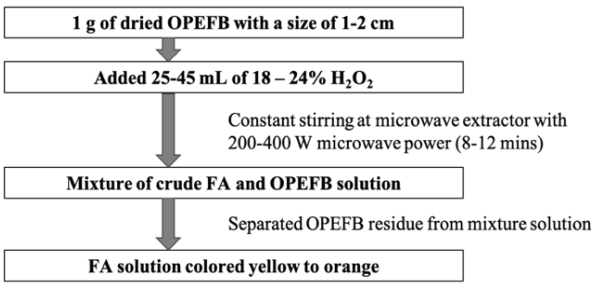

Figure 1. Flow chart showing all the steps of FA extraction using microwave extractor

Result and Discussion

Response Surface Methodology (RSM) result for FA extraction

Extraction was optimized using expert design to obtain optimal conditions based on predetermined variations. This research was designed using the Box Behnken Design (BBD) model with a variety of factors 1-4 (Table 1), and the observed response variables can be seen in Table 2.

Based on Table 2 and Table 3, the total experimental units are 29 consisting of 24 units of combinations of the lower limit, midpoint, and upper limit (-1, 0, +1), as well as five repetitions at the midpoint of the experiment (5 experimental units at the middle of each factor (0). Based on data processing

Table 2. Variation of FA extraction optimization factors based on BBD

Run	Variable				Response
	H_2O_2 concentration (%)	Volume (mL)	Time (minute)	Microwave power (W)	Content of FA (%)
1	21	35	8	200	23.15
2	24	45	10	300	19.22
3	21	45	10	200	22.47
4	24	35	12	300	21.42
5	21	35	12	200	21.50
6	21	35	12	300	19.20
7	18	25	12	300	20.43
8	21	35	10	300	24.54
9	18	35	10	300	21.35
10	21	25	10	400	22.10
11	21	35	8	400	23.30
12	21	35	10	300	25.19
13	21	25	8	300	20.54
14	21	35	12	400	22.10
15	18	35	10	200	20.59
16	21	25	10	200	21.00
17	24	25	10	300	21.05
18	21	45	12	300	20.23
19	18	45	10	300	23.18
20	24	35	10	200	24.52
21	21	35	10	300	23.16
22	21	45	8	300	22.50
23	21	35	10	300	26.31
24	18	35	8	300	22.45
25	24	45	10	400	22.04
26	21	35	10	400	24.55
27	21	35	10	300	25.63
28	24	35	8	300	24.31
29	18	35	10	400	22.10

using RSM, a data distribution graph between H_2O_2 concentration and FA concentration was obtained with the highest value of 26.31% (Run 9) and the lowest value of 19.2% (Run 3). The highest value of FA concentration of 26.31% was obtained from an H_2O_2 concentration of 21%, microwave power of 300 W, reaction time of 10 minutes, and H_2O_2 volume of 35 ml.

Analysis of the selected model based on ANOVA which had the lowest seq p-value, highest LoF p-value, adj r-square, and highest pred r-square with the details described in Table 3. Based on the analysis results of the selected model, this research model is a quadratic model with ANOVA details described in Table 4.

The model can be accepted if the model is significant (p-val < 0.05) and LoF is not significant (p-val > 0.05). The selected model is quadratic, suitable for describing the response data and whether the factors are influential (p-val < 0.05). Influencing factors: quadratic time, quadratic H_2O_2 concentration, quadratic power, and quadratic volume H_2O_2. Based on the analysis results, the R-square value is 0.7153 with the equation:

$$Y = -177.01 + 9.67\,A + 2.27\,B + 9.17\,C + 0.09\,D - 0.03\,AC - 0.033\,AD - 0.011\,BC + 2.45\,BD + 5.62\,CD - 0.17\,A^2 - 0.02\,B^2 - 0.43\,C^2 - 6.93\,D^2$$

This equation interprets that factors A, B, C, and D have positively affect Y. Factors AB, AC, AD, BC, A^2, B^2, C^2, and D^2 negatively affect Y. In addition to graphs of the distribution of FA concentrations against the factors that have been determined, graphs of each factor against the FA concentrations obtained can also be seen in Figure 2.

Table 3. Analysis of the selected model

	Sequential	***Lack of Fit***	***Adjusted***	***Predicted***	
Source	***p-value***	***p-value***	***R-Squared***	***R-Squared***	
Linear	0.2672	0.2777	0.0528	-0.1031	
Two Factor Interaction	0.8727	0.2065	-0.1161	-0.6127	
Quadratic	0.0081	0.5092	0.4305	-0.3193	*Suggested*
Cubic	0.4470	0.4599	0.4740	-4.3432	*Aliased*

Table 4. The ANOVA for response surface quadratic model

	Some of		***Mean***	***F***	***p-value***	
Source of variables	***Squares***	***df***	***Square***	***Value***	***Prob > F***	
Model	72.70	14	5.19	3.10	0.0212	*Significant*
A- H_2O_2 conc	0.50	1	0.50	0.30	0.5918	
B - H_2O_2 vol	3.98	1	3.98	2.38	0.1455	
C - Time	10.77	1	10.77	6.43	0.0237	
D - Microwave	0.73	1	0.73	0.44	0.5198	
AB	3.35	1	3.35	2.00	0.1792	
AC	0.19	1	0.19	0.11	0.7417	
AD	3.98	1	3.98	2.38	0.1455	
BC	0.22	1	0.22	0.13	0.7247	
BD	0.24	1	0.24	0.14	0.7106	
CD	0.051	1	0.05	0.03	0.8645	
A^2	15.34	1	15.34	9.16	0.0091	
B^2	31.30	1	31.30	18.69	0.0007	
C^2	19.40	1	19.40	11.58	0.0043	
D^2	3.12	1	3.12	1.86	0.1941	
Residual	23.44	14	1.67			
Lack of Fit (LoF)	17.70	10	1.77	1.23	0.4538	*not significant*
Pure Error	5.74	4	1.44			
Cor Total	96.15	28				

Based on Figure 2, all factors configure a quadratic curve. Factor A at the midpoint is the product with the highest FA value, factor B at the midpoint is the product with the highest FA , factor C at the midpoint is not the highest FA value, factor D at the midpoint is the product with the highest FA, or it could also be shifted slightly to the right at around 35<B<37.5. The interaction between factors on the response to FA concentration can be seen in Figure 3.

The darker an area is, the higher the response value. The darker the blue area is, the lower the response value. In the research carried out, the midpoint of the experiment was in the high FA area (yellow) but not yet optimal (dark red). Apart from that, the graph formed is quadratic, as shown in Figure 3. Then, the interaction of AC on the response to FA concentration can be seen in Figure 3. The midpoint of the experiment is already in the high FA area (yellow) but not yet maximal (dark red).

Higher FA values can also be achieved when 300<D<350 and the B combination is between 35<B<37.5. Then, the interaction of BC on the response to FA concentration can be seen in Figure 4. Higher FA values can also be achieved when 9<C<10 and the B combination is 35<B<37.5. The RSM optimization value is obtained after the correlation between the factors and the response.

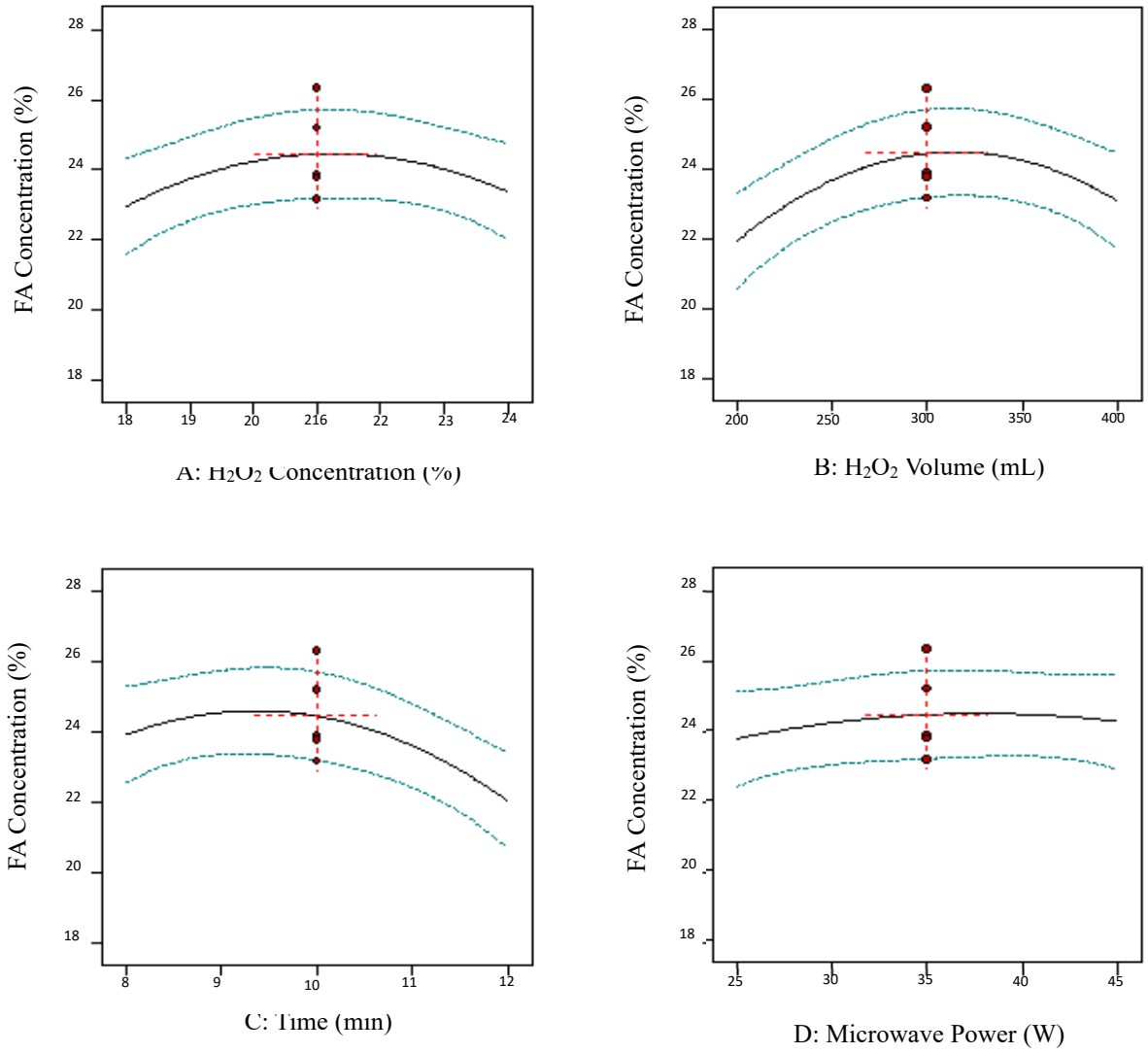

Figure 2. Graph of each factor against FA concentration

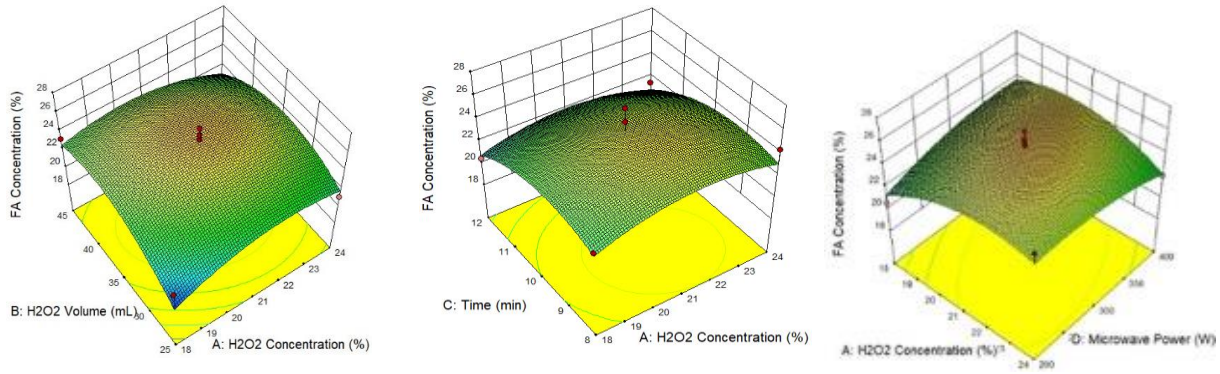

Figure 3. Interaction of AB, AC, and AD on the concentration-response of FA

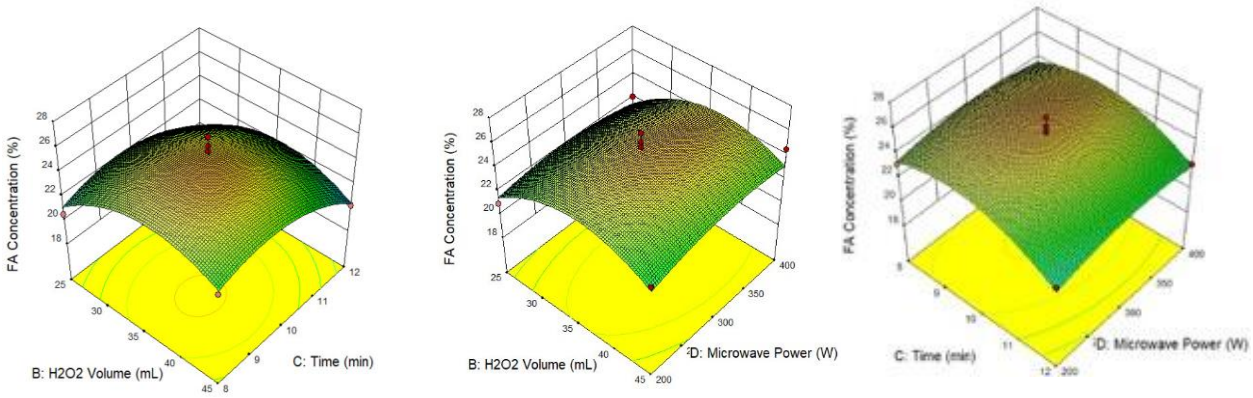

Figure 4. Interaction of BC, BD, and CD on the response to FA concentration

The search for optimum point recommendations is carried out to find the highest (maximum) point of FA concentration response with variations in factors A, B, C, and D within the predetermined lower and upper limits. Based on the results of this analysis, the Design Expert recommends two solutions with different desirability values. The solution that will be taken as the alternative solution with the highest desirability value is alternative solution one, which has a desirability value of 0.776 or 77.6%. The program's ability to make the desired product becomes perfect when the desirability value obtained is close to one. Based on this research, the product is predicted to have an FA concentration of 24.716% resulting from treatment A 20.462%, B 37.4193mL, C 9.384 minutes, and D 351.397W. Research by Gong et al. (2020) and Zhang et al. (2020) showed that FA extraction from coal using H_2O_2 resulted in a purity of 23-30%. The FA was extracted from renewable biomass (OPEFB), while the previous method uses coal and lignite samples, which are non-renewable natural resources. Using OPEFB is the advantage of this method, although the yield obtained is not as optimal as coal. However, the optimal value of FA extraction from OPEFB will depend on the lignin content of OPEFB. The lignin content in OPEFB is the precursor for the formation of FA (Tan, 2014).

Fourier-Transform Infrared Spectrometer (FTIR) characterization result of FA extract using microwave extractor method

Fourier-Transform Infrared Spectrometer (FTIR) analysis was performed to determine the functional groups formed in the FA extract from OPEFB using a microwave extractor. The results obtained are in the form of a spectrum, as seen in Figure 5. The wave number used is 4000 – 400 cm^{-1} (fingerprint area). The three samples have the five strongest peaks in the wave number areas 3200 cm^{-1}, 2900 cm^{-1}, 1600 cm^{-1}, 1380 cm^{-1}, and 1100 cm^{-1}, with strong vibrations possibly from alkene C-H bonds or aldehyde H-C=O bonds at a wave number of 2900 cm^{-1} in each sample.

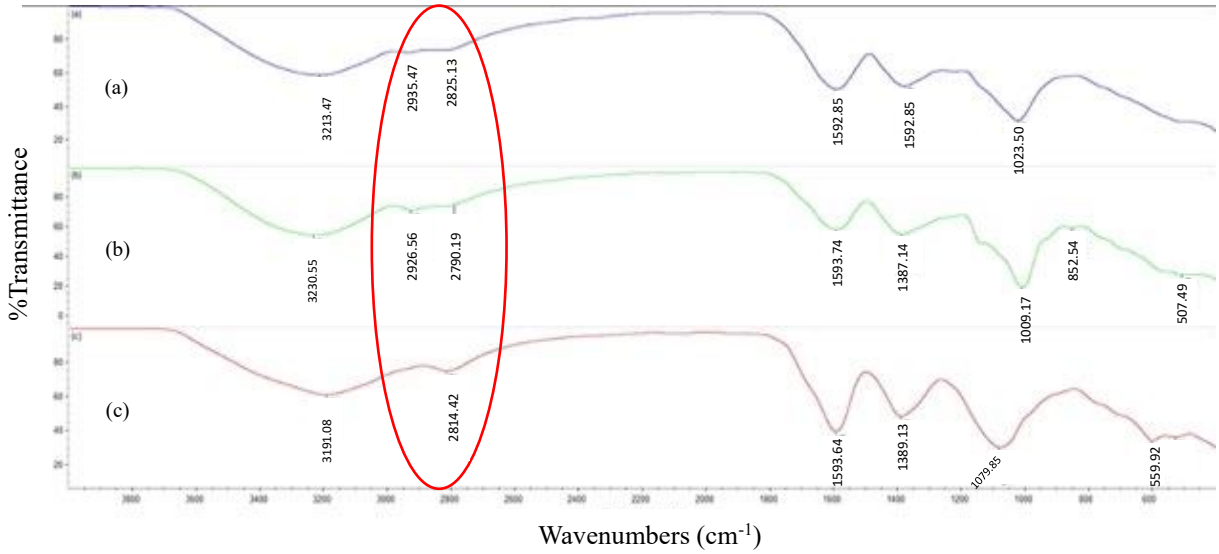

Figure 5. FTIR analyses results on FA powder from the samples of (a) OPEFB, (b) Shilajit, and (c) fertilizer

According to Gong (2020), vibrations in the wave number area of 2800 cm^{-1} are alkyl (C-H) from aliphatic hydrocarbons (open chains). As confirmed previously by Jordaan et al. (2019), fulvic acid vibrates at wave numbers 2940 – 2840 cm^{-1}, originating from C–H bonds from aliphatic hydrocarbon chains. The three samples show peaks but don't have high intensity. There are 2935.47 and 2825.13 cm^{-1} in the OPEFB FA sample, a peak of 2926 and 2790 cm^{-1} in the shilajit FA sample, and 2814 cm^{-1} in the Fertilizer FA sample.

The next peak is at 1600 cm^{-1}, present in all three samples, and may come from the vibration of the C=C bond in the aromatic group (Asemani, 2020). This vibration also appeared in Jordaan's 2019 research, where a peak appeared at wave numbers 1620-1600 cm^{-1}, which came from the aromatic ring of C=C bonds or H-C=O bonds found in conjugated ketones. According to Gong et al. (2020), the wave number 1900-1000 cm^{-1} is the vibration of functional groups containing oxygen. Zhang et al. (2020) also report vibrations at the wave number 1646 cm^{-1} originating from the C=C bond. Then, there is a peak at wave number 1380 cm^{-1} in samples (b) and (c), which may experience vibrations from methyl or methylene groups (Zhang et al., 2020).

Strong vibrations in the wave number range of 3500 cm^{-1} in each sample indicate the presence of the O-H group. According to Zhang et al. (2020), there is also a strong peak from the O-H group at wave number 3423 cm^{-1} and a strong peak at wave number 3400-3220 cm^{-1} (Gong et al., 2020; Jordan, 2019). There are peaks at 3213 cm^{-1} in the OPEFB-FA sample, 3230 cm^{-1} in the Shilajit-FA sample, and 3191 cm^{-1} in the Fertilizer- FA sample. The strong peak of OPEFB-FA occurs due to using H_2O_2 as a solvent for the noise reduction factor produced by the sample solution.

Result of HNMR characterization of FA extract using the microwave extractor method

The ^{1}H NMR analysis method is used to elucidate the structure of a compound (Gunawan & Nandiyanto, 2020). This method can be used to characterize the presence of FA as a result of extraction (Zhang et al., 2020). The results of the analysis using H-NMR can be seen in Figure 6. There are single peaks at 2.5 ppm and around 3.4-3.5 ppm in the three samples, which are DMSO solvent and water bound to the samples (Zhang et al., 2020). Apart from that, there is also a singlet peak at 10.1-10.2 ppm in the three samples. This signal likely comes from protons from the carboxylic group (CO_2H) detected at 10-13 ppm (Gunawan & Nandiyanto, 2020). This signal arises because FA's number of carboxylate groups (CO2H) is relatively abundant based on elemental analyzer characterization (Zhang et al., 2020). The OPEFB and fertilizer samples did not have other peaks, and this needs to be evaluated further to optimize the extraction process so that the extract results are correct FA.

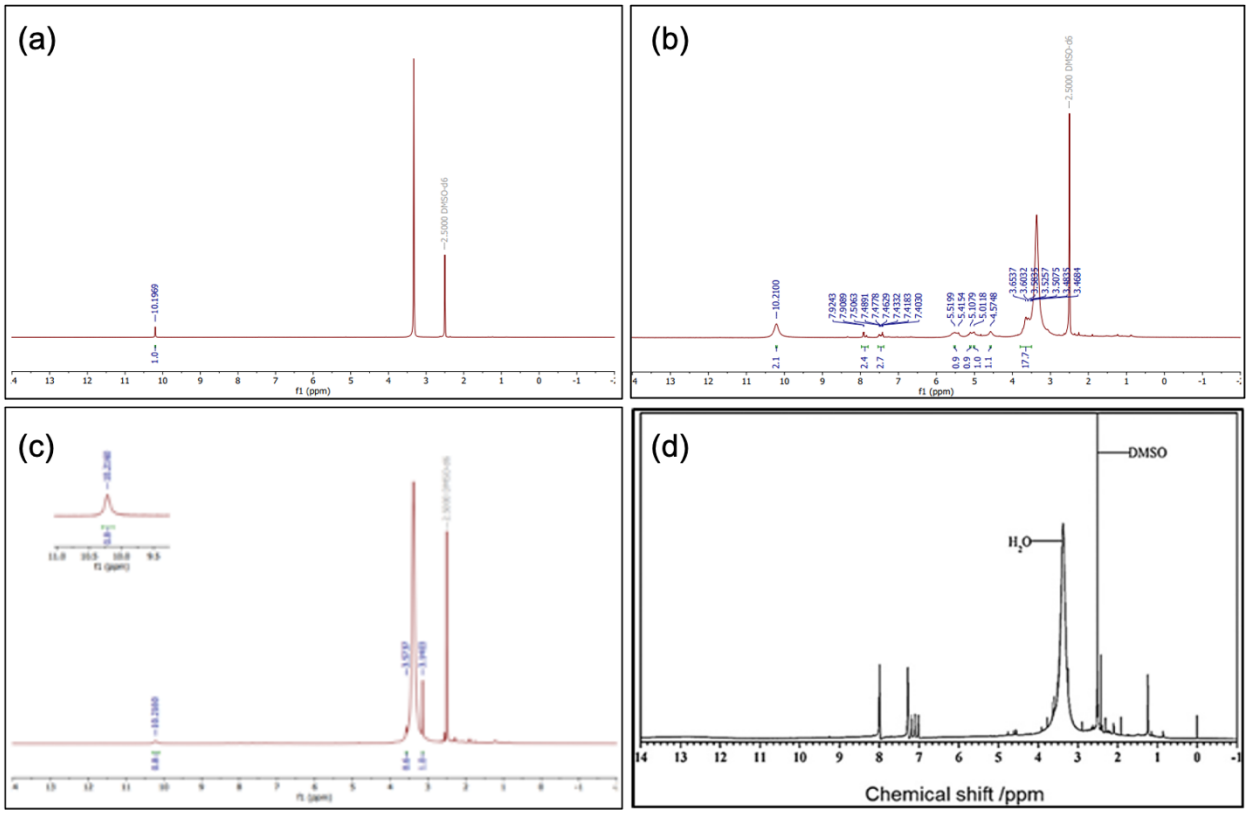

Figure 6. H-NMR Spectrum on FA powder from the sample: (a) OPEFB; (b) Shilajit ; (c) Fertilizer; and (d) FA (Zhang *et al.,* 2020)

Shilajit has multiple peaks at 3.4-3.6, 4.5-5.5 ppm, and 7.4-7.9 ppm, as seen in Figure 6. The 4.5-5.5 ppm signal likely comes from protons from alcohol groups (-OH), which appear in the 0.5-5 ppm range (Gunawan & Nandiyanto, 2020). There are various alcohol compounds characterized by GC-MS that allow the appearance of signals detected in NMR (Zhang et al., 2020). The 3.4-3.6 signal probably comes from the methoxy group, which appears at 3.8 ppm, or comes from the alcohol group, which appears in the 0.5-5 ppm range (Gunawan & Nandiyanto, 2020). Besides the alcohol group, several ester compounds have been characterized using GC-MS (Zhang et al., 2020). That is what allows the presence of a methoxy group signal. The 7.4-7.9 ppm signal probably comes from aromatic phenolic groups (Ar-H) and (Ar-OH) (Gunawan & Nandiyanto, 2020). Based on the results of the elemental analyzer and FTIR characterization, hydroxyl groups from phenol and aromatic structures allow the presence of this signal (Zhang et al., 2020). Based on the 1H-NMR spectrum, the characterization of extracts from shilajit has results that follow the suitability of the functional groups found in FA.

Spectrofluorescence characterization results of FA extraction using the microwave extractor method

Monodimensional fluorescence spectroscopy (spectrofluorometry) is one of the techniques used to characterize and differentiate HA and FA, which can provide comparative information about the structural and functional similarities and differences of the materials analyzed (Bertoncini et al., 2005). Analysis with a spectrofluorometer was carried out on the emission spectrum 220 – 790 nm at an excitation wavelength of 370 nm according to the method of Gong et al. (2020). Based on the overlay of the fluorescent spectrum results of FA extract samples from OPEFB, shilajit, and fertilizer samples, similarities can be observed, namely that there are peaks that appear in the emission spectrum at 360 – 380 nm, 380 – 700 nm, and 730 – 760 nm (Figure 7).

Spectrum at 380–700 nm was observed following Gong et al. (2020), so comparatively, there are the same compounds, namely FA compounds. According to Bertoncini et al. (2005), fluorescence emission at these wavelengths is caused by the presence of simple structural components of small molecular size that

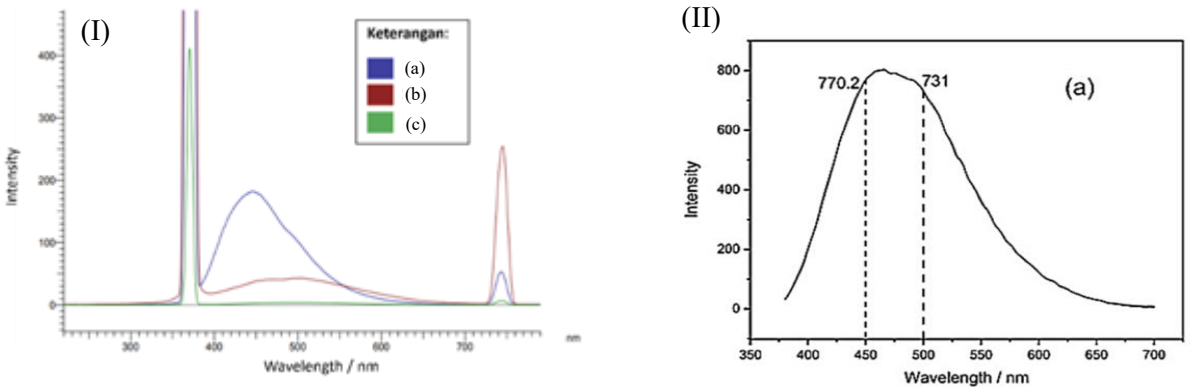

Figure 7. Spectrofluorescence result on FA powder: (I) The three samples (a) Fertilizer; (b) Shilajit; (c) OPEFB; (II) FA (Gong et al., 2020)

Table 5. The ratio of fluorescent emission intensity between wavelengths of 450 and 500 nm

Sample	Emission λ_{450}	Emission λ_{500}	$f_{450/500}$
Fertilizer	180.2	101.9	1.768
Shilajit	38.29	42.74	0.896
OPEFB	2.501	3.478	0.719

carry electron-accepting substituents such as hydroxyl groups (-OH), methoxy (-OMe), and aromatic polycondensation and conjugate chromophores. Based on these three samples, the emission intensity of the OPEFB sample is the smallest among all. This indicates that the presence of FA compounds is low in the sample, so further optimization is needed in the extraction and purification method for FA from OPEFB.

Another parameter shown to determine the presence of FA is the ratio of fluorescent emission intensity between wavelengths of 450 and 500 nm to the excitation wavelength of 370 nm. The aim is to determine aromaticity or the presence of aromatic groups that are inversely proportional to the ratio (Gong et al., 2020). Based on Table 5, it shows that only fertilizer samples have a ratio of >1.05. According to Gong et al. (2020), a ratio of 1.05 indicates that the compound contains few aromatic compounds. Therefore, it can be concluded that the FA extraction process in fertilizer samples is more efficient than that in shilajit and OPEFB.

Conclusion

Optimizing FA extraction using expert design with the Response Surface Methodology 4 factors (H_2O_2 concentration, H_2O_2 volume, microwave power, and reaction time) obtained the highest FA concentration namely 24.716%, at H_2O_2 concentration and volume of 20.462% and 37.419 ml respectively, reaction time 9.384 minutes, and microwave power 351.397W. Analysis using FTIR, NMR and fluorescence spectroscopy shows that FA is contained in OPEFB-FA extracted.

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