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# Modelling of Pulsed Electric Field (PEF) Pretreatment on Fresh *Moringa oleifera* Leaves Extraction Using Response Surface Methodology (RSM)

# *Pemodelan* Pre-treatment Pulsed Electric Field *(PEF) pada Ekstraksi Daun Kelor (Moringa olifera) Segar Menggunakan* Response Surface Methodology *(RSM)*

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**Abstract**

Moringa leaves are a source of high antioxidants. This study aims to find an equation model for the extraction conditions of Moringa leaves with Pulsed Electric Field (PEF) pretreatment. Fresh Moringa leaves were treated with PEF in various voltages (1,000, 1,500, and 2,000 Volts), frequency (1,000, 1,500 and 2,000 Hz), and extraction time (4, 5 and 6 hours) as the factors studied. The best results and mathematical equation models obtained were determined from the extraction process. The parameters observed were yield, total phenolic compounds, and antioxidant activity. The Response Surface Methodology (RSM) was chosen to determine the equation model for the voltage, frequency, and extraction time combination. The results of ANOVA (Sum of Squares, Lack of Fit, and p-value) show that the model is adequate to present experimental data. The analysis results showed a significant value for total phenol in a quadratic equation, extract yields in a linear equation, and antioxidant activity in a linear equation. The model is presented in a 2-D contour graph and a 3-D response surface. The equation model shows that the best treatment is a PEF voltage of 2,000 Volts, frequency of 1,000 Hz, and extraction time of 4 hours.
**Keywords**: antioxidant, equation model, moringa leaves, Pulsed Electric Field, Response Surface Method

***Abstrak***

*Daun kelor merupakan salah satu sumber antioksidan yang tinggi. Penelitian ini bertujuan untuk mencari model persamaan kondisi ekstraksi daun kelor dengan perlakuan pendahuluan* Pulsed Electric Field (PEF). *Daun kelor segar diperlakukan dengan PEF dalam berbagai tegangan (1.000, 1.500, dan 2.000 Volt), frekuensi (1.000, 1.500 dan 2.000 Hz), dan waktu ekstraksi (4, 5 dan 6 jam) sebagai faktor yang diteliti. Hasil terbaik dan model persamaan matematika ditentukan dari proses ekstraksi. Parameter yang diamati adalah rendemen, total senyawa fenol, dan aktivitas antioksidan.* Response Surface Methodology (RSM) *dipilih untuk mengetahui model persamaan kombinasi tegangan, frekuensi, dan waktu ekstraksi. Hasil ANOVA (*Sum of Squares, Lack of Fit, *dan* p-value*) menunjukkan bahwa model layak untuk mewakili data eksperimen. Hasil analisis menunjukkan nilai yang signifikan pada total fenol dalam persamaan kuadratik, hasil ekstrak dalam persamaan linier, dan aktivitas antioksidan dalam persamaan linier. Model dipresentasikan dalam grafik kontur 2-D dan permukaan respons 3-D. Model persamaan menunjukkan bahwa perlakuan terbaik adalah tegangan PEF 2.000 Volt, frekuensi 1.000 Hz dan waktu ekstraksi 4 jam.*
***Kata kunci****: antioksidan, daun kelor, model persamaan,* Pulsed Electric Field, Response Surface Method

## INTRODUCTION

Moringa (Moringa oleifera Lam.) is a plant estimated from the Agra and Oudh regions in Northwest India, the Southern Himalayan region (Santoso & Parwata, 2018). Moringa plants can grow in the lowlands to highlands up to an altitude of 1,000 meters above sea level (Sharma et al., 2012) and are known as the miracle tree because it is naturally proven as a source of medicinal nutrients (Arora et al., 2013). The leaves, fruit, seeds, and roots can be extracted for various health benefits and used as functional food products (Pandey et al., 2012). Moringa leaves have the most potential for functional food because they have the highest nutrient content compared to other parts of the Moringa tree (Gopalakrishnan et al., 2016). The Food Agriculture Organization (FAO) recommends Moringa leaves as a source of vitamin A, vitamin C, vitamin B, calcium, potassium, iron, and protein to combat cases of malnutrition (Paliwal et al., 2011). Moringa leaves have 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yogurt, 15 times more potassium than bananas, and 25 times more iron than spinach (Rockwood et al., 2013). Moringa leaves contain phytochemical compounds such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, and alkaloids. The leaves also contain anti-cancer agents such as glucosinolates, isothiocyanates, glycosides, and glycerol-1-9-octadecanoate (Berkovich et al., 2013).

If the content of phenol group compounds in a material is significant, the antioxidant activity will be more excellent (Shahwar et al., 2010). In Moringa leaves, tannins, steroids, triterpenoids, flavonoids, saponins, anthraquinones, and alkaloids are antioxidant compounds (Kasolo et al., 2010). Antioxidants are active compounds that increase the function of endogenous systems and are responsible for preventing free radical reactions in the body by donating a single electron or hydrogen atom so that free radicals are stable (Satriyani, 2021). The presence of free radicals can trigger various diseases, for example, atherosclerosis, coronary heart disease, stroke, cancer, kidney failure, and the human aging process (Satriyani, 2021). Susanty et al. (2019) stated that the high antioxidant content in Moringa leaves could help the body to neutralize and stabilize free radicals. The benefits of Moringa leaves as antioxidants and their potential for the treatment of various diseases have been widely studied (Berawi et al., 2019; Tjong et al., 2021; Yusuf & Tungadi, 2022).

Antioxidants in moringa leaves can be obtained using extraction. This study used maceration extraction with Pulsed Electric Field (PEF) pretreatment to optimize the results. PEF is a non-thermal pretreatment method using an electric field. Two electrodes are electrified with a high voltage then the sample is placed between them for a short time, usually within seconds (Gulzar & Benjakul, 2019). The transmembrane potential exists in all cell membranes due to a charged ionic gradient. PEF works by inducing cell membrane polarity and creating a dipole when the transmembrane potential is under an applied electric field. Electroporation on the cell membrane is induced when high-voltage pulses are applied to the plant tissue. Electroporation helps the extraction process by increasing the cell membrane permeability, causing the diffusion of active compounds in the cell (Gulzar & Benjakul, 2019). This study aims to determine the mathematical equation model of yield parameters, total phenolic compounds, antioxidant activity in Moringa leaf extraction with PEF pretreatment (voltage and frequency), and extraction time using the RSM method.

## METHODS

### Characteristics of Moringa Leaf Raw Material

The material used in this study was fresh Moringa leaves obtained and collected from Sukun District, Malang Regency, East Java. Fresh Moringa leaves with 80±5% moisture content were harvested at 6±0.1 months. The leaves are cleaned under running water and drained.

### Scanning Electron Microscope (SEM) Analysis

Moringa leaves morphology was observed by Scanning Electron Microscope (SEM). Changes in moringa leave cell shape were observed before and after PEF treatment. Microscopic observations were made on dry leaves. Fresh moringa leaves were dried in an oven at 50 °C for 2x24 hours. Dried moringa leaves were cut 1×1 cm and given a sputter coating. Leaves treated with PEF were dried in an oven at 50 °C for 2x24 hours and then cut into 1x1 cm. The sample was placed on a specimen holder coated with double-sided tape, and the sample position was adjusted. The mounted sample was given a 400 Å thick gold-palladium coating as an electrical conductor to support the photoshoot using the JFC-1100 ion sputter machine. The sample was inserted into the specimen chamber by photoshoot several times and magnifying 2,000 – 5,000 times. The resulting image was then analyzed for its structure.

### PEF Generator

The PEF generator is a local product but has been calibrated at the High Voltage Laboratory, Faculty of Engineering, Universitas Brawijaya. The PEF generator requires 300 W of electrical power and can operate at a voltage of 5 V–15 kV, a frequency of 5 Hz–15 kHz, and a treatment time of 5 seconds to 2 hours. The cathode-anode distance can be adjusted from 5 to 40 cm and is made of SS-316 iron with a thickness of 1 mm. The chamber is made of acrylic with a diameter of 12 cm and a thickness of 0.2 mm. The PEF Generator schematic is presented in Figure 1.

**PEF Treatment and Moringa Leaf Extraction**

100 g fresh moringa leaves were weighed (in plastic bags) and treated with PEF (DC waves), with voltages of 2,000, 1,500, and 1,000 Volts, frequencies of 2,000, 1,500, and 1,000 Hz within 3 seconds. 100 g of fresh moringa leaves treated with PEF for each sample (20 runs according to the output of the Design Expert software) were put into Erlenmeyer then 96% technical-grade ethanol was added. The ratio between leaves and solvents was 1:2. Moringa leaves were macerated on a hot plate for 4.5 to 6 hours at 50 °C. The filtrate was filtered through the Whatman-42 filter paper. Moringa leaf filtrate was then concentrated with a rotary vacuum evaporator at a temperature of 50 °C for 30 minutes at a speed of 65 rpm to obtain a concentrated moringa leaf extract. The treatment results were then analyzed. 100 g moringa leaf samples without PEF treatment were also prepared in the same way as a control.

**Extract Yield Analysis**

Extract yield is the ratio between the total filtrate extract (dry) of Moringa leaves divided by the weight of fresh Moringa leaves indicated in percent. The weight value of moringa leaf extract from fresh moringa leaf extract is divided by the weight of the raw material and multiplied by 100 to get the percent value (AOAC, 2019).

$$\%\ \text{Extract Yield} = \frac{\text{Moringa leaves dry extract (g)}}{\text{fresh moringa leaves (g)}} \times 100\% \qquad (1)$$

**Total Phenol Analysis**

The amounts of phenolic compounds in moringa leaf extract were calculated using the Folin-Ciocalteau reagent approach, and a standard curve was made in the Gallic Acid Curve. The solution was homogenized to obtain 100 ppm gallic acid. Solutions of 2, 4, 6, 8, and 10 ml were then taken from 100 ppm solution and put into a 10 ml volumetric flask. The solution was diluted with distilled water to obtain various solutions with concentrations of 20, 40, 60, 80, and 100 ppm. Solution with a volume of 0.5 ml from each concentration was put into a test tube, and 2.5 ml of Folin-Ciocalteau reagent 10% was added, homogenized in a vortex, and incubated for 5 minutes in the dark. 75% $Na_2CO_3$ with a volume of 2 ml was added to the solution, then homogenized in a vortex and incubated for 30 minutes in the dark. The solution's optical density (OD) was measured in a spectrophotometer at a wavelength of 765 nm. The results of the OD measurement were plotted on a standard gallic acid curve with the X axis as the gallic acid concentration and the Y axis as the OD value (Łukaszewska *et al.*, 2020). The total content of phenolic compounds in the test material was then calculated.

**Antioxidant Activity Analysis ($IC_{50}$)**

Dried moringa leaves extract of 0.01 grams was dissolved in 10 ml of distilled water to obtain a standard solution with 1,000 ppm antioxidant concentration. 1,000 ppm standard solution with a volume of 0.25, 0.5, 0.75, and 1 ml each was put into a 10 ml volumetric flask. The solution was diluted in distilled water to obtain 25, 50, 75, and 100 ppm concentrations of moringa leave extract. Each sample of different concentrations was taken

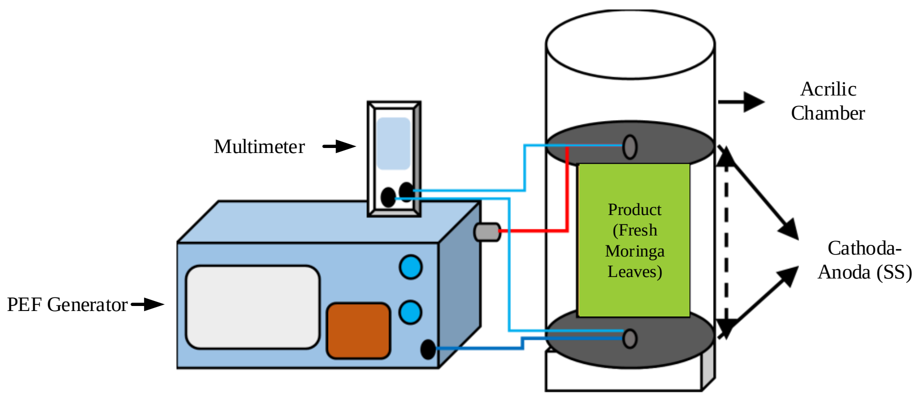



**Figure 1.** PEF Generator Schematic

using a pipette with a volume of 1 ml, then mixed with 7 ml of methanol and 2 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) 0.2 N. The mixture was homogenized by vortex and then incubated for 30 minutes. The control solution was prepared by adding 8 ml of methanol and 2 ml of DPPH. The sample OD was measured at 517 nm wavelength. Calculation of antioxidant activity is presented based on the Inhibition Concentration (IC). The equation for calculating the percent inhibition is as follows (Pinela et al., 2016):

$$IC_{50}\ (\text{ppm}) = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100\% \qquad (2)$$

### Response Analysis

Each response variable was analyzed by Design Expert (DX) software. The best model was selected according to the program recommendations. Analysis of Variance (ANOVA) was conducted to determine the significance of the variables in the linear, quadratic, special cubic, and cubic models. The model that gave significant results on ANOVA (indicated by p-value) and not significant on lack of fit was chosen to analyze the variables further. The DX program also shows the difference between the actual and predicted response values. The response value close to the normal line is a normal model, which means the actual results are close to the predicted value (Almajed *et al.*, 2021). The DX software analysis results for iterations of PEF treatment based on voltage, frequency, and extraction time factors are shown in Table 1. These results indicate that PEF treatment with a voltage of 1,500 volts, a frequency of 1,500 Hz, and an extraction time of 5 hours must be performed in 6 repetitions: R1, R2, R3, R4, R5, and R6.

### Mathematical Equation Model

The equation model of each response (yield, total phenol, and antioxidant activity) was determined in the DX program. The program provides recommendations from several equation conditions showing maximum desirability value. The desirability value is the objective function of the best value based on the final product criteria. The value ranges from 0 to 1, and a value close to 1 indicates that the desired product is perfect. The equation model's purpose is not to get the desired perfect value but to find the best conditions for all objective functions (Anihouvi *et al.*, 2011).

**Table 1.** Results of DX software analysis for iteration of PEF treatment based on voltage, frequency, and extraction time factors

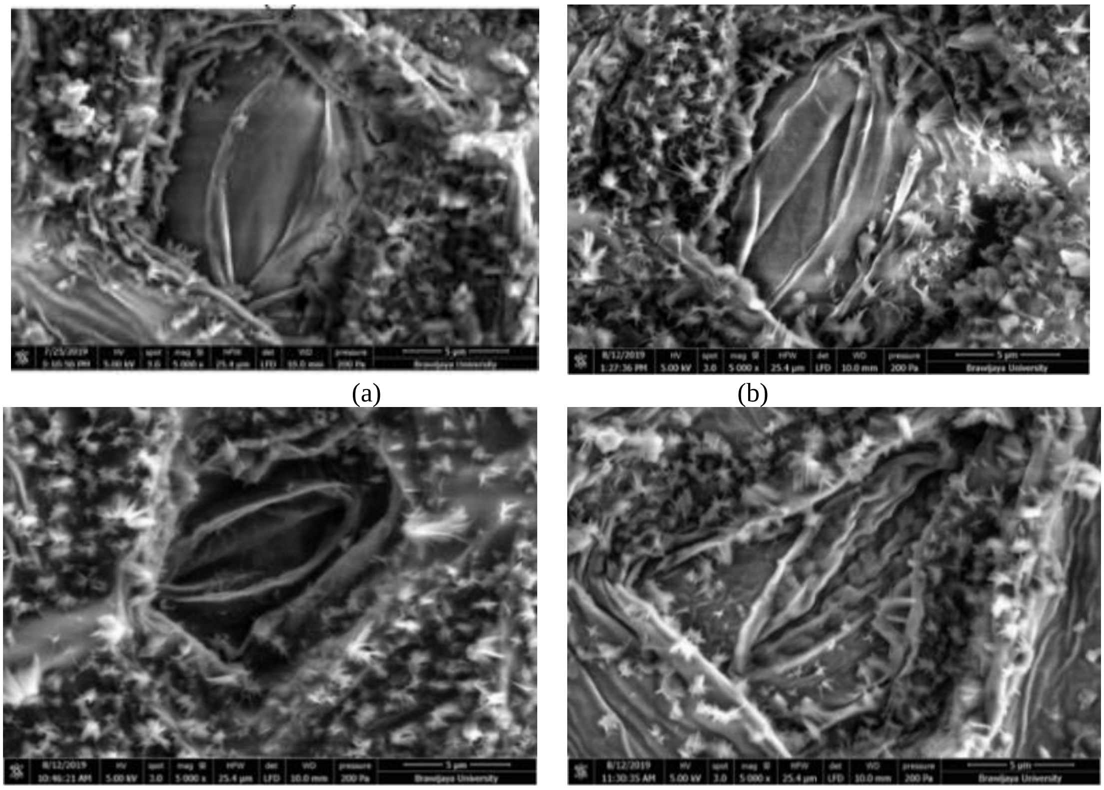
| No | Voltage (Volt) | Variable | |
|---|---|---|---|
| | | Frequency (Hz) | Extraction Time (hours) |
| 1 | 1,000 | 1,000 | 4 |
| 2 | 2,000 | 1,000 | 4 |
| 3 | 1,000 | 2,000 | 4 |
| 4 | 2,000 | 2,000 | 4 |
| 5 | 1,000 | 1,000 | 6 |
| 6 | 2,000 | 1,000 | 6 |
| 7 | 1,000 | 2,000 | 6 |
| 8 | 2,000 | 2,000 | 6 |
| 9 | 659.104 | 1,500 | 5 |
| 10 | 2,340.9 | 1,500 | 5 |
| 11 | 1,500 | 659.104 | 5 |
| 12 | 1,500 | 2,340.9 | 5 |
| 13 | 1,500 | 1,500 | 3.318 |
| 14 | 1,500 | 1,500 | 6.682 |
| 15 | 1,500 | 1,500 | 5 (R1) |
| 16 | 1,500 | 1,500 | 5 (R2) |
| 17 | 1,500 | 1,500 | 5 (R3) |
| 18 | 1,500 | 1,500 | 5 (R4) |
| 19 | 1,500 | 1,500 | 5 (R5) |
| 20 | 1,500 | 1,500 | 5 (R6) |

## RESULTS AND DISCUSSION

### Moringa Leaves Morphology

Figure 2 is the 5,000 times magnification results of the Scanning Electron Microscope (SEM) analysis used to see the morphology of fresh moringa leaves with PEF treatment and without PEF treatment. Figure 2(a) shows the surface appearance of moringa leaves without PEF treatment. The cells around the stomata appear to have solid cell walls. Figure 2 (b, c, and d) shows moringa leaves treated with PEF using voltages of 1,000, 1,500, and 2,000 Volts at a frequency of 1,000 Hz for 3 seconds. Cells and stomata look open, and the cell walls' wrinkles are also visible.

Figure 2 (b, c, and d) shows that the PEF treatment could damage the cells and tissues of Moringa leaves. Research by Ersus *et al.* (2010) stated that PEF treatment with a voltage of 500 Volts at a frequency of 1 Hz is the best treatment for onion bulbs and does not cause severe cell damage but causes cells to lyse due to electric fields. Cell damage is significant if high PEF voltage and frequency are applied to materials (including agricultural products). PEF treatment based on the formation of a conduction potential



(a) (b) (c) (d)

**Figure 2.** Morphology of (a) Moringa Leaves without PEF Treatment, (b) Moringa Leaves with 1,000 Volt PEF Treatment, (c) Moringa Leaves with 1,500 Volt PEF Treatment, and (d) Moringa Leaves with 2,000 Volt PEF Treatment, Frequency 1,000 Hz and Treatment Time 3 Seconds

difference in biological material between two electrodes can create an electric field that depends on the applied voltage, the shape of the electrodes, and the gap between the electrodes (Jäger & Knorr, 2017). PEF treatment at 500 to 5,000 Volts in less than one second can damage tissues and cells in potatoes, apples, and fish slices. Damage to these cells can facilitate the extraction of substances contained in cells (Jäger & Knorr, 2017).

**Central Composite Designs (CCD) Response Data on Yield, Total Phenol, and Antioxidant Activity**

Applying PEF for the electrolysis of biological cells will increase cell wall permeability and facilitate the release of intracellular compounds. The calculations results using Central Composite Designs (CCD) with Response Surface Methodology (RSM) showed that changes occurred in the yield, total phenol, and antioxidant activity ($IC_{50}$), as shown in Table 2.

The data in Table 2 shows that the voltage increase was followed by an increase in yield, but it had no significant effect on the frequency and extraction time. This result follows the ANOVA calculation results (Table 3). Bozinou et al. (2019) researched dried Moringa leaves treated with PEF 7 kV/cm using a pulse duration of 20 milliseconds and a pulse interval of 100 micro-seconds for 40 minutes, which gave the highest total phenol extract yield. The extract antioxidant activity test showed that its value was proportional to the total phenol content.

**Moringa Leaves Extract Yield**

ANOVA of moringa leaves extracts yield is presented in Table 3. The model obtained is significant to moringa leaves extract yield with a p-value of 0.0001, much lower than 0.05 (or $\alpha = 0.05$). Determination of the p-value of 0.05 because a validity level of 5% or a confidence level of 95% is a reasonable basis for exact science research (Bagga, 2018). All factors (voltage, frequency, and extraction time) gave a significant value to the extract yield, but the voltage had the most significant value ($p < 0.0001$). These factors are also significant based on the F value. The

**Table 2**. Data of yield response, total phenol, and $IC_{50}$ of moringa leaves extract

| No | Voltage (Volt) | Variable | | Response | | |
|---|---|---|---|---|---|---|
| | | Frequency (Hz) | Extraction Time (hours) | Yield (%) | Total Phenol (mg GAE/g) | Antioxidant Activity ($IC_{50}$) |
| 1 | 1,000 | 1,000 | 4 | 17.400 | 165.667 | 39.560 |
| 2 | 2,000 | 1,000 | 4 | 21.650 | 219.000 | 34.145 |
| 3 | 1,000 | 2,000 | 4 | 18.680 | 173.667 | 37.952 |
| 4 | 2,000 | 2,000 | 4 | 25.040 | 223.667 | 31.969 |
| 5 | 1,000 | 1,000 | 6 | 17.400 | 168.667 | 38.730 |
| 6 | 2,000 | 1,000 | 6 | 22.920 | 223.333 | 32.383 |
| 7 | 1,000 | 2,000 | 6 | 19.100 | 176.333 | 37.743 |
| 8 | 2,000 | 2,000 | 6 | 27.590 | 225.667 | 31.250 |
| 9 | 659.104 | 1,500 | 5 | 16.980 | 160.333 | 40.433 |
| 10 | 2,340.9 | 1,500 | 5 | 28.440 | 251.333 | 30.373 |
| 11 | 1,500 | 659.104 | 5 | 16.980 | 181.667 | 36.431 |
| 12 | 1,500 | 2,340.9 | 5 | 21.650 | 217.333 | 34.522 |
| 13 | 1,500 | 1,500 | 3.318 | 19.100 | 185.333 | 36.019 |
| 14 | 1,500 | 1,500 | 6.682 | 21.220 | 210.000 | 34.893 |
| 15 | 1,500 | 1,500 | 5 (R1) | 20.370 | 205.667 | 35.251 |
| 16 | 1,500 | 1,500 | 5 (R2) | 20.370 | 205.333 | 35.243 |
| 17 | 1,500 | 1,500 | 5 (R3) | 19.950 | 197.333 | 35.355 |
| 18 | 1,500 | 1,500 | 5 (R4) | 20.370 | 192.667 | 35.386 |
| 19 | 1,500 | 1,500 | 5 (R5) | 19.950 | 190.000 | 35.921 |
| 20 | 1,500 | 1,500 | 5 (R6) | 20.370 | 187.000 | 35.960 |

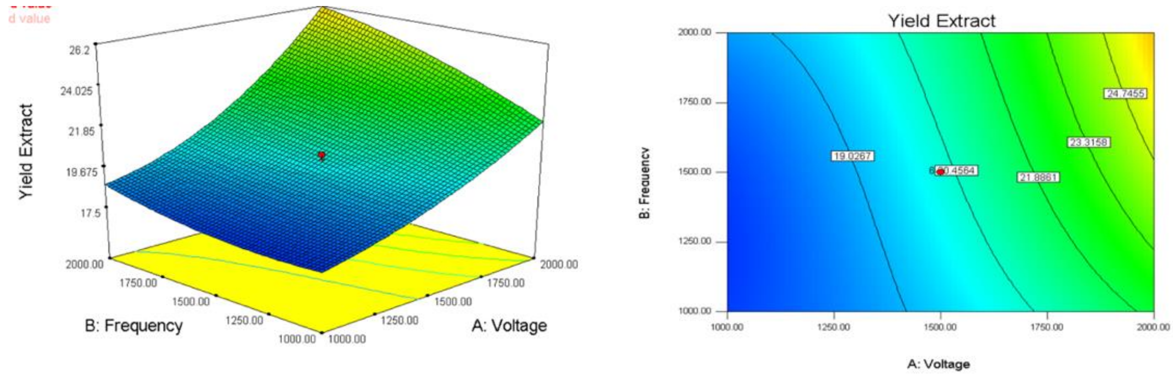
**Table 3.** ANOVA quadratic model results of moringa leaf extract yield

| Sources | Sum of Squares | Degree of Freedom | Mean of Squares | F-Value | P-Value | Significance |
|---|---|---|---|---|---|---|
| Model | 177.49 | 13 | 13.65 | 175.25 | 0.0001 | Significant |
| A-Voltages | 65.67 | 1 | 65.67 | 842.86 | <0.0001 | |
| B-Frequency | 4.41 | 1 | 4.41 | 56.61 | 0.0003 | |
| C-Extraction times | 2.25 | 1 | 2.25 | 28.84 | 0.0017 | |
| AB | 4.4 | 1 | 4.4 | 56.42 | 0.0003 | |
| AC | 0.81 | 1 | 0.81 | 10.43 | 0.0179 | |
| BC | 0.09 | 1 | 0.09 | 1.16 | 0.323 | |
| $A^2$ | 12.56 | 1 | 12.56 | 161.27 | <0.0001 | |
| $B^2$ | 0.017 | 1 | 0.017 | 0.21 | 0.6608 | |
| $C^2$ | 0.015 | 1 | 0.015 | 0.19 | 0.6772 | |
| Residual | 0.47 | 6 | 0.078 | | | |
| Lack of Fit | 0.23 | 1 | 0.23 | 4.94 | 0.0769 | Nonsignificant |
| Pure Error | 0.24 | 5 | 0.047 | | | |
| Cor Total | 177.96 | 19 | | | | |

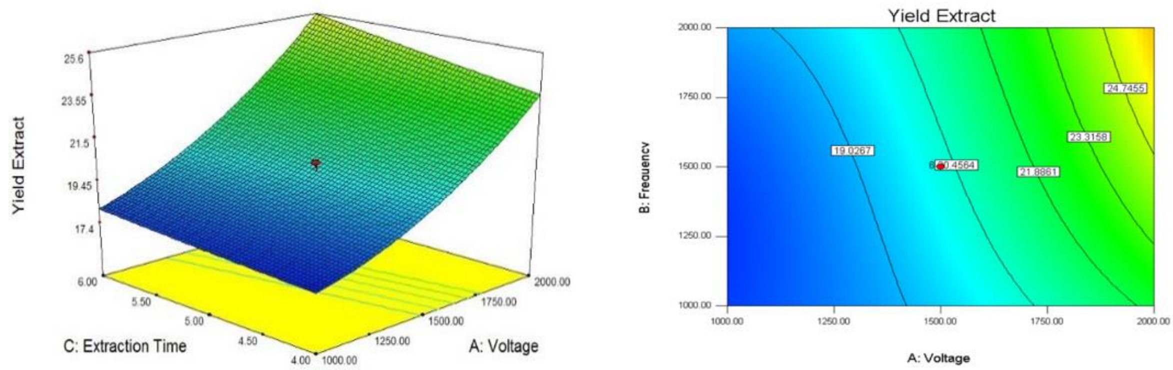
frequency and extraction time factors have F values of 56.61 and 28.84, respectively, but voltage has the most significant effect because it has an F value of 842.86. This result follows the statement of Zhong et al. (2019), that the model's high F value and low p-value (<0.0001) indicate that the model significantly represents the relationship between the response and the independent variables. The different F values are pretty significant, indicating that between treatments, there are also significant differences in moringa leaf extract yield (Winter, 2015).

PEF treatment at a voltage of 2,340.9 Volts, a frequency of 1500 Hz, and an extraction time of 5 hours gave the 28.440% extract yield, which was the highest yield (Table 2). The lowest yield was 16.980%, obtained from Moringa leaves with PEF treatment of 659.104 Volts, 1,500 Hz, and extraction for 5 hours. PEF voltage is the most influential variable on the yield of Moringa leaf extract compared to the frequency and extraction duration. This result is because the electric field treatment produces induction which can affect the permeability of cell membranes (Asavasanti *et al.*, 2011). The voltage applied to the material can increase cell membranes' electrical conductivity and permeability (Zderic *et al.*, 2013; Silve *et al.*, 2016). PEF treatment causes electroporation (Barba et al., 2015) and increases cell membrane permeability (Huang et al., 2019; Janositz et al., 2011).

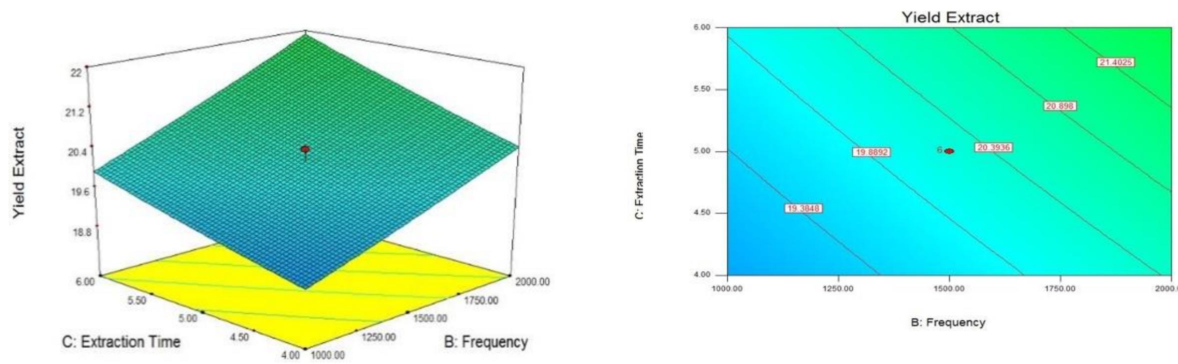
PEF treatment has been commonly used to extract bioactive compounds due to its effectiveness in increasing yields (Luengo et al., 2014). Previous research revealed that PEF treatment increased intracellular extraction yields for macromolecular compounds of sugars (Kayalvizhi et al., 2016), polysaccharides (Nowosad et al., 2021), and lipids (Gorte et al., 2020) or macromolecular compounds such as vitamins and antioxidants (Puértolas et al., 2012). Extraction of sugars from sugar cane, polysaccharides from potatoes, or lipids from legumes showed the yield and quality of extracts with PEF treatment. Extraction of vitamins and antioxidants from plant cells showed the yield and changes in vitamin function and antioxidant activity caused by PEF treatment. The effectiveness of treatment with PEF technology depends on the electric field intensity, the total energy used, and the characteristics of biological cells, such as morphology, electrical conductivity, and chemical composition (Puértolas et al., 2010)..




(a) Effect of Voltage and Frequency on Yield of Moringa Leaves Extract




(b) Effect of Voltage and Extraction Time on Yield of Moringa Leaves Extract




(c) Effect of Frequency and Extraction Time on Yield of Moringa Leaves Extract

**Figure 3.** 3-Dimensions Figure and Contour Plot Effect of Voltage, Frequency, and Extraction Time on Yield of Moringa Leaves Extract

The Lack of Fit model showed nonsignificant results with p-value = 0.0769 or 7.69%. It means that 92.31% of the model is suitable, and the voltage on the PEF treatment has the most effect on the extraction results. The next influencing factor is the frequency and extraction time. The Sum of Square (SoS) value also shows that the effect of voltage on the PEF treatment is higher than the frequency and extraction time. This value shows the variability or influence of the voltage, frequency, and extraction time factors on the extraction results (Pambi & Musonge, 2016)

The result of plotting 3-dimensional images (Figures 3a, 3b, 3c) shows the contour plot of the relationship between voltage, frequency, and extraction time on the response of the extract yield. The color on the contour shows different values in the extract yield response. Blue indicates the lowest extract yield value, and yellow to red indicates the highest extract yield value. The lines on the contour drawings represent the combination of factors and surface interactions.

Figure 3 shows a parabolic curve that opens up, the highest extract results are between the midpoints of the image, but Figure 3(c) looks relatively flat. Each image shows that the extract yield increases with the increase of PEF voltage, frequency, and extraction time. However, Figure 3(c), which is slightly squared and the color contrast is also less sharp, indicates that the frequency and extraction time have less effect on the response of the extract yield. According to Siemer et al. (2012), PEF treatment causes an electroporation process that damages the cell wall, thereby increasing the rate of diffusion and extraction, which then causes a more optimal mass transfer (Gachovska et al., 2010). The results of electro-mobilization will also increase the mass transfer of molecules and ions, the drying rate, and the extraction yield (Zderic et al., 2013; Bobinaitė et al., 2017). The polynomial equations of the 3-dimensional images and contour plots are as follows:

$$Y_1 = 20.22 + 3.41X_1 + 0.88X_2 + 0.63X_3 + 0.74X_1X_2 + 0.32X_1X_3 + 0.11X_2X_3 + 0.93\,X_1^2 + 0.034X_2^2 + 0.032X_3^2 + 0.21X_1X_2X_3 + 0.39X_1^2\,X_2 + 5.970E\text{-}0.03\,X_1^2\,X_3 - 0.44\,X_1\,X_2^2 \quad (3)$$

where:
$Y_1$: Yield, $X_1$: Voltage, $X_2$: Frequency, $X_3$: Extraction time

**Total Phenol Moringa Leaves Extract**

The total phenol content of Moringa leaves was significantly influenced by the factors used in the study. PEF treatment with a voltage of 2340.9 Volts, a frequency of 1,500 Hz, and an extraction time of 5 hours gave the highest total phenol, 251.33 mg GAE/g. The lowest total phenol was 160 mg GAE/g obtained from moringa leaf extract with PEF treatment at a temperature of 1,000 Volts, a frequency of 1,000 Hz, and an extraction time of 4 hours. Moringa leaf extract with PEF at a voltage of 1,500 Volts, a frequency of 1,500 Hz, and extracted for 5 hours produced an extract with a total phenol of 196.333 mg GAE/g from an average of 6 replicate treatments (R1 to R6 in Table 2). The total phenol of moringa leaves extract with PEF treatment at a voltage of 2,000 Volt (highest), frequency of 2,000 Hz (highest), and extraction time of 6 hours (longest) resulted in a total phenol of 217.33 mg GAE/g. The total phenolic compounds increased based on the extraction results if the voltage, frequency, and time of PEF were higher (to a certain extent). This result is proportional to the energy the material receives to break down the cell wall, resulting in compounds in the cell (including phenolic compounds) being extracted more quickly and easily (Sukardi et al., 2019). Extraction with 70% ethanol solvent performed by Nascimento et al. (2017) yielded a total phenol of 170.07 mg GAE/100 g, while the extraction in the study of Sohaimy et al. (2015) with methanol as a solvent produced a total phenol of 48.35 mg GAE/g. This result shows that the PEF pretreatment is very good for increasing the phenolic compounds obtained before extraction.

When compared with the research by Vongsak et al. (2013), who used the squeezing, boiling, maceration, and Microwave Assisted Extraction (MAE) methods on fresh moringa leaves, the research with PEF treatment prior to this extraction resulted in better results based on the total phenolic compounds and antioxidant activity obtained. The total phenol obtained from the research by Vongsak et al. (2013) was 13.23 g chlorogenic acid equivalents/100 g extract, and $IC_{50}$ was 62.94 g/ml, while the total phenol in this study was 165 to 225 mg GAE/g and $IC_{50}$ was between 30 to 40 g/g. $IC_{50}$ indicates the level of antioxidant activity, and $IC_{50}$ values below 50 g/g indicate a very strong antioxidant function. PEF treatment before extraction increased the total phenol content and better antioxidant activity.

ANOVA of the moringa leaves extracts total phenolic extract is presented in Table 4.

Total phenol examination is the basis for examining antioxidant activity because phenol compounds play a role in preventing the oxidation process (Alhakmani et al., 2013). Measurement of antioxidant activity in plant food can be performed by measuring the total phenol content using the Folin-Ciocalteau reagent. The total phenol test of moringa leaves extract was indicated by the sample changing color from yellow to blue when reacted with the Folin-Ciocaltcu reagent. Adding $Na_2CO_3$ to the phenol test aims to form an alkaline atmosphere so that the Folin-Ciocalteau reduction reaction occurs by the hydroxyl group of phenol in the sample. The sample's phenol content increases with the blue color's intensity (Sánchez-Rangel et al., 2013).

All factors significantly influence the total phenol response of moringa leaves extract, which is indicated by the p-value of each of these factors being lower than the lack of fit (0.4182), except for the interaction (Behera et al., 2018). Voltage has the most significant effect. Frequency gives the subsequent significant influence. The extraction time has a p-value of 0.1111, which is still significant, but the effect is the lowest.

Figure 4 (a, b, c) shows a slightly flat surface curve, and the highest total phenol is at the midpoint of voltage, frequency, and extraction time. The change in surface color (green to yellow or red) indicates that the greater the total phenol produced, the higher the PEF voltage and frequency and the longer the extraction time.

Table 4 and Figure 4 show that the highest treatment values did not indicate the optimum treatment for the total phenol of moringa leaf extract. The midpoint of the surface is still linearly increasing, which means that the use of frequency and extraction time has not yet reached its optimal point in producing total phenol. The Design Expert program showed that using a voltage between 1,700-2,000 Volts, a frequency of 1,500 Hz, and an extraction time of 5 hours gave the highest results for the total phenolic extract of moringa leaves. The permeability of plant cell walls after PEF treatment caused the difference in extract yields. PEF treatment helps increase cell permeability, thereby facilitating the extraction of nutrients in cells (Siddeeg et al., 2019).

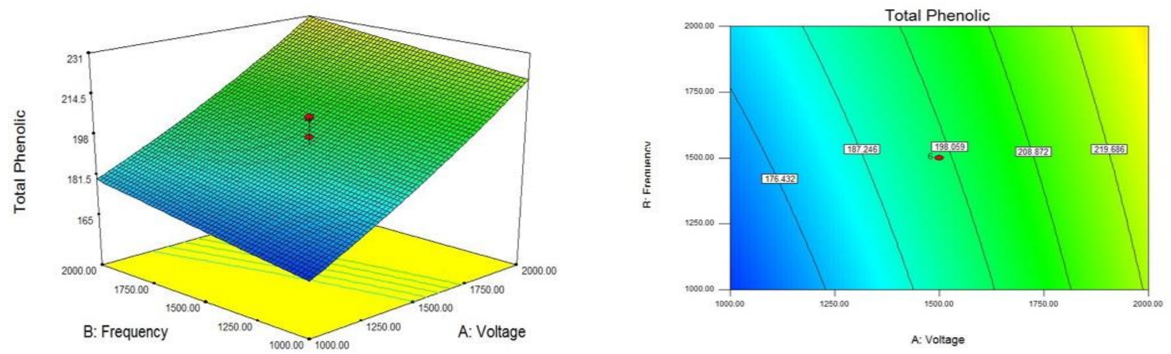
Extraction time affected the total phenol, but not significantly. Plant age, extraction method, and extraction time affect the phenolic extract in dried moringa leaves (Upadhya et al., 2015). Moringa leaf cell permeability after PEF pre-treatment in this study increased to accelerate the extraction time so that the extraction time did not significantly affect the total phenol yield of moringa leaf extract. Figure 4 shows that the graph still tends to increase linearly at the midpoint, and there is no noticeable color change. The total polynomial equation for phenol is as follows:

$$Y2 = 196.47 + 26.39X_1 + 6.05X_2 + 3.92X_3 - 1.08X_1X_2 + 0.083X_1X_3 - 0.33X_2X_3 + 2.45X_1^2 + 0.22X_2^2 - 0.43X_3^2 \quad (4)$$

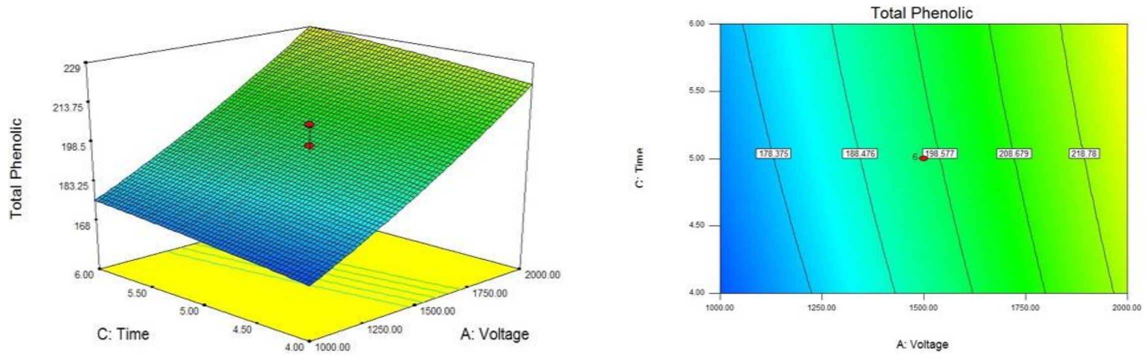
where:
$Y_2$ = Total phenol, $X_1$ = Voltage,
$X_2$ = Frequency, $X_3$ = Extraction time

**Table 4.** ANOVA quadratic model results of moringa leaf extract total phenol

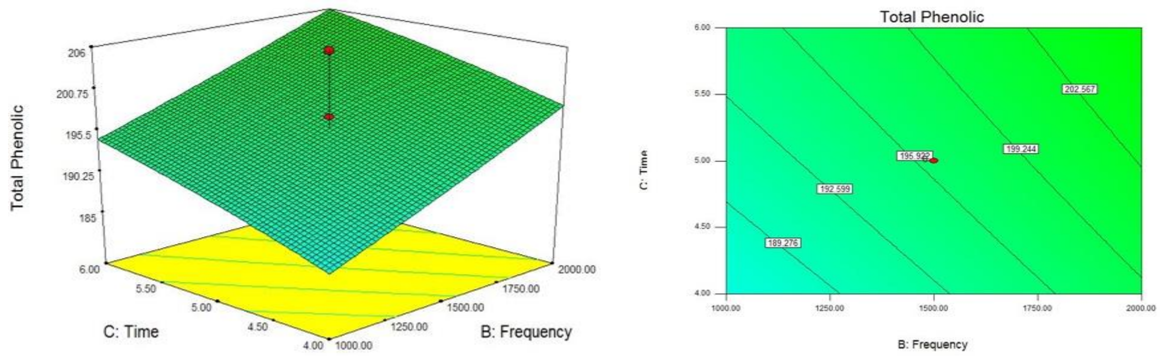
| Source of Variation | Sum of Squares | Degree of Freedom | Mean of Squares | F-Value | p-Value | Significance |
|---|---|---|---|---|---|---|
| Model | 10,323.07 | 9 | 1,147.01 | 16.72 | 0.0001 | Significant |
| A-Voltages | 9,509.58 | 1 | 9,509.58 | 138.63 | 0.0001 | |
| B-Frequency | 500.19 | 1 | 500.19 | 7.29 | 0.0223 | |
| C-Extraction times | 209.46 | 1 | 209.46 | 3.05 | 0.1111 | |
| AB | 9.39 | 1 | 9.39 | 0.14 | 0.7192 | |
| AC | 0.056 | 1 | 0.056 | 0.00081 | 0.9778 | |
| BC | 0.89 | 1 | 0.89 | 0.013 | 0.9116 | |
| $A^2$ | 86.85 | 1 | 86.85 | 1.27 | 0.2868 | |
| $B^2$ | 0.67 | 1 | 0.67 | 0.0098 | 0.9232 | |
| $C^2$ | 2.69 | 1 | 2.69 | 0.039 | 0.8469 | |
| Residual | 685.98 | 10 | 68.6 | | | |
| Lack of Fit | 376.21 | 5 | 75.24 | 1.21 | 0.4182 | Nonsignificant |
| Pure Error | 309.777 | 5 | 61.95 | | | |
| Cor Total | 11,009.06 | 19 | | | | |




(a) Effect of Voltage and Frequency on Total Phenol of Moringa Leaves Extract




(b) Effect of Voltage and Extraction Time on Total Phenol of Moringa Leaves Extract




(c) Effect of Frequency and Extraction Time on Total Phenol of Moringa Leaves Extract

**Figure 4.** 3-Dimensions Figure and Contour Plot Effect of Voltage, Frequency, and Extraction Time on Total Phenol of Moringa Leaves Extract

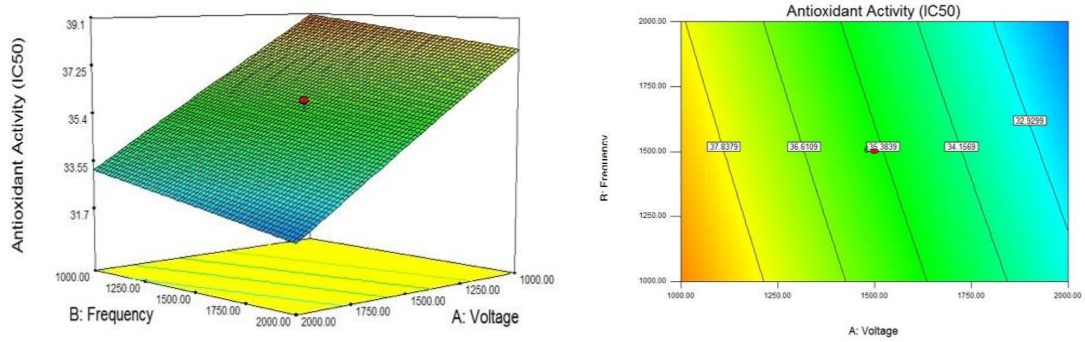
**Antioxidant Activity ($IC_{50}$) Moringa Leaves Extract**

The antioxidant activity showed the percentage of antioxidants against free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH). The concentration of antioxidants extracted from moringa leaves determines the antioxidant activity of moringa leaf extract. The $IC_{50}$ value was measured based on the absorbance of the DPPH radical scavenging indicated by a spectrophotometer. ANOVA $IC_{50}$ of moringa leaf extract is presented in Table 5. DPPH radical scavenging gets smaller the higher the $IC_{50}$ value. Samples with a low $IC_{50}$ value showed the best antioxidant activity. Table 2 shows that moringa leaf extract treated with the highest voltage of 2,340.9 Volt, frequency of 1,500 Hz, and extracted for 5 hours had the best antioxidant activity (30.373%).

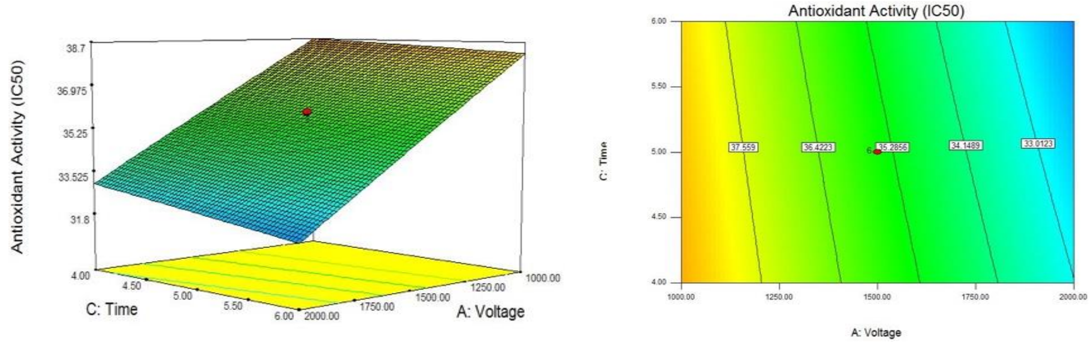
The highest $IC_{50}$ value was obtained from moringa leaf extract treated with PEF using the lowest voltage at 659.104 Volts and a frequency of 1,500 Hz, extracted for 5 hours. The same material treated with the same frequency and extraction time had a very significant yield of phenol and $IC_{50}$ when treated with different voltages. The only factor that significantly affected the total phenol and $IC_{50}$ value was the

**Table 5**. ANOVA quadratic model results of antioxidant activity moringa leaf extract

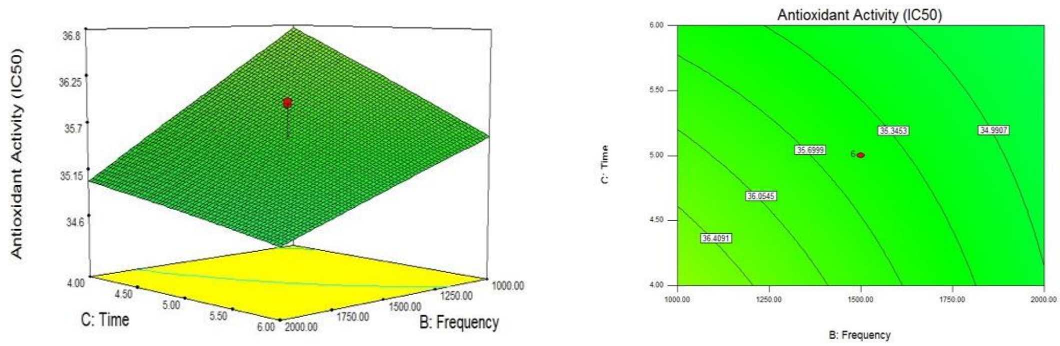
| Source of Variation | Sum of squares | Degree of freedom | Mean of squares | F-value | p-value | Significance |
|---|---|---|---|---|---|---|
| Model | 132.95 | 9 | 14.77 | 207.76 | <0.0001 | Significant |
| A-Voltages | 124.03 | 1 | 124.03 | 1744.35 | <0.0001 | |
| B-Frequency | 6.08 | 1 | 6.08 | 85.55 | <0.0001 | |
| C-Extraction times | 2.15 | 1 | 2.15 | 30.18 | 0.0003 | |
| AB | 0.064 | 1 | 0.064 | 0.9 | 0.3661 | |
| AC | 0.26 | 1 | 0.26 | 3.66 | 0.0849 | |
| BC | 0.35 | 1 | 0.35 | 4.87 | 0.0519 | |
| $A^2$ | 0.018 | 1 | 0.018 | 0.26 | 0.6225 | |
| $B^2$ | 0.00135 | 1 | 0.00135 | 0.019 | 0.893 | |
| $C^2$ | 0.00414 | 1 | 0.00414 | 0.058 | 0.8143 | |
| Residual | 0.71 | 10 | 0.071 | | | |
| Lack of Fit | 0.16 | 5 | 0.032 | 0.3 | 0.8962 | Nonsignificant |
| Pure Error | 0.55 | 5 | 0.11 | | | |
| Cor Total | 133.66 | 19 | | | | |




(a) Effect of Voltage and Frequency on Antioxidant Activity of Moringa Leaves Extract




(b) Effect of Voltage and Extraction Time on Antioxidant Activity of Moringa Leaves Extract




(c) Effect of Frequency and Extraction Time on Antioxidant Activity of Moringa Leaves Extract

**Figure 5.** 3-Dimensions Figure and Contour Plot Effect of Voltage, Frequency, and Extraction Time on Antioxidant Activity ($IC_{50}$) of Moringa Leaves Extract

PEF voltage. ANOVA results show that all factors have a very significant effect. The p-value and F-value showed a significant correlation between the factors of the moringa leaves extraction process.

The 3-dimensional figure of the surface response of antioxidant activity ($IC_{50}$) shows that the curve is relatively flat, with the midpoint as the highest recommended treatment (Figure 5). The flat curve shows that the increased extraction voltage and frequency cause the antioxidant activity not to increase significantly. The correlation between extraction time and voltage, extraction time and frequency shows different directions. This result means that the extraction time affects antioxidant activity but does not have a significant and not optimal effect in this study (Zhao & Wang, 2021). The 3D graph (Figure 5(c)), which does not change color, also shows that the extraction time does not have a significant effect on antioxidant activity (Zhong et al., 2019). Pre-treatment of freeze-dried moringa leaves with PEF of 7 kV/cm for 40 minutes had the highest total phenol and antioxidant activity (Bozinou et al., 2019). Pre-treatment using PEF in food extraction effectively increased the extract's antioxidant activity (Boussetta et al., 2015).

According to Gorobchenko et al. (2021), ion transfer in the PEF system is explained by the Nernst-Plank and Poisson equations and the material balance equation. The polynomial equation of antioxidant activity ($IC_{50}$) of moringa leaves extract is as follows:

$$Y_3 = 35.52 - 3.01X_1 - 0.67X_2 - 0.40X_3 - 0.089X_1X_2 - 0.18X_1X_3 + 0.21X_2X_3 - 0.036 X_1^2 - 9.693E - 0.03X_2^2 - 0.017X_3^2 \quad (5)$$

where:
$Y_3$ = Antioxidant activity; $X_1$ = Voltage;
$X_2$ = Frequency; $X_3$ = Extraction time

**Table 6.** Results of selected solutions

| Parameter | Standard Prediction |
|---|---|
| Voltage (volt) | 2,000 |
| Frequency (Hz) | 1,000 |
| Extraction time (jam) | 4 |
| Yield (%) | 20.22 |
| Total Phenolic (mg GAE/g) | 196.47 |
| $IC_{50}$ (ppm) | 35.52 |
| Desirability | 1 |
| Status | Selected |

This equation produces a solution from the Design Expert program for antioxidant activity. The results showed that the best treatment to produce yield response, total phenol, and antioxidant activity was PEF treatment with a voltage of 2,000 Volts and PEF frequency of 1,000 Hz. The extraction time did not provide significant progress, so 4 hours of extraction time was chosen. The results of the solutions provided by the Design Expert program are shown in Table 6.

## CONCLUSIONS

PEF used as a pretreatment to optimize yield in moringa leaf extraction significantly increased the yield, total phenol, and antioxidant activity. The PEF frequency and extraction time did not significantly increase the yield, total phenol, and antioxidant activity. The results of ANOVA (Sum of Squares, Lack of Fit, and p-value) show that the model is feasible to present experimental data. The analysis results showed a significant value of total phenol in the quadratic equation, extract yield and antioxidant activity in the linear equation. The Design Expert program with RSM produces condition formulas with PEF pretreatment presented in 2-D contour graphs and 3-D response surfaces. The equation model shows that the best treatment is a PEF voltage of 2,000 Volts, a frequency of 1,000 Hz, and an extraction time of 4 hours. The weakness in this research is that the treatment frequency and extraction time are not optimal, although the total phenol yield and antioxidant activity ($IC_{50}$) are included in the very strong category (less than 50 ppm). Therefore, further research is needed on the PEF frequency and extraction time.

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