Extraction of phenolic compounds from basil (*Ocimum americanum* L.) leaves with pretreatment using pulsed electric field (PEF)

Sukardi, M H Pulungan, I Purwaningsih and P F Sita

Department of Agro-industrial Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia

E-mail: sukardi@ub.ac.id

Abstract. The study was conducted to determine the role of the pulsed electric field (PEF) as a pretreatment on the extraction of phenolic compounds from basil (*Ocimum americanum*, L.) leaves. The research used Randomized Block Design (RBD) with two factors, factor 1: exposure time (10, 15, and 20 seconds) and factor 2: voltage (1000, 1500, and 2000 Volts). Extraction was done by maceration using 80% ethanol at 50°C. The results showed that PEF treatment had a significant effect on total phenolic compounds (TPC) and antioxidant activity (IC₅₀) of basil extract. The best treatment at exposure time of 10 seconds and a voltage of 1500 Volts obtain TPC of 260.50 mg GAE/g and IC₅₀ of 35.96 ppm. Whereas the untreated sample (without PEF pre-treatment) obtains TPC of 175.75 mg GAE/g and IC₅₀ of 116.25 ppm. The data showed that PEF treatment before the extraction of basil leaves increase TPC by 1.5 times and antioxidant activity by 3.2 times. FTIR spectra showed that PEF pre-treatment increases absorption or decreases transmittance, which indicates the increase of extract concentration. Scanning electron microscopy (SEM) imaging showed that PEF treatment causes pore formation on the cell membrane, which might facilitate the release of phenolic compounds.

1. Introduction

Antioxidants have several benefits, such as reducing the risk of heart disease, cancer, cataracts, and other degenerative diseases [1]. Based on their sources, antioxidants can be divided into natural antioxidants and synthesis antioxidants. Synthesis antioxidants are carcinogenic because of the harmful effect on the body if consumed over, so people switch to use natural antioxidants that are safer to consume in the long term [2]. The natural antioxidants were found in fruits, leaves, and seeds in the form of phenolic compounds such as tocopherols, flavonoids, and phenolic acids [3]. Natural antioxidants are also found in medicinal plants, one of which is basil (*Ocimum americanum* L.).

Recently, basil is consumed freshly as a complement to food and flavourings, so it has not been used optimally. Basil leaves contain phenolic compounds, flavonoids, saponins, tannins, and alkaloids [4]. Taking phenolic compounds through an extraction process can increase the economic value of basil leaves. The most common method used for the extraction of a phenolic compound of basil leaves is maceration. Maceration has disadvantages such as long processing times, many solvents, and low yields [5]. The increase of yield can be done by applying a pre-treatment to raw material.

One of the pre-treatments being developed currently is pulsed electric field (PEF). PEF treatment proved to be effective in increasing yields during the extraction, as indicated by several previous studies.
Extraction of patchouli leaves oil was optimal with PEF pre-treatment at the voltage of 2,000 Volts and a frequency of 1,874 Hz, with patchouli oil yield is 1.5 times higher than the untreated sample [6]. PEF treatment was also shown can increase polyphenol compounds and antioxidant activity of black grape extract up to 1.41 times and 1.05 times respectively compared to untreated sample, with a maximum increase in field strength of 10 kV/cm, pulses number of 15, pulse duration of 100 μs and pulse interval of 100 ms [7].

PEF treatment causes the formation of pores in the cell membrane, thereby facilitating the release of intracellular substances [8]. The formation of pores depends on the exposure time and the electric field strength or voltage per cathode-anode distance [9]. Research of PEF as a pre-treatment on the extraction of basil leaves has never been done. Therefore, the purpose of this study was to determine the effect of exposure time, and voltage of PEF on total phenolic compounds (TPC), antioxidant activity (IC₅₀) and functional groups of basil leave extract.

2. Materials and Method
2.1. Materials
The main material used in this study was basil leaves (Ocimum americanum L.) obtained from the Kebalen market in Malang, East Java. The chemicals used were 80% ethanol, gallic acid, aquadest, Folin-Ciocalteau, Na₂CO₃, DPPH 0.2 mM, and methanol. These chemicals were obtained from the Laboratory of Biochemistry and Food Analysis, Universitas Brawijaya, Malang.

2.2. Experimental design
The experimental design in this study used a randomized block design (RBD) with 2 factors: exposure time of PEF and voltage of PEF. The exposure time factor consists of 3 levels (10, 15 and 20 seconds), while the voltage factor consists of 3 levels (1000, 1500, and 2000 Volts). Each treatment was carried out in duplicate.

2.3. PEF pre-treatment and extraction of Ocimum americanum, L.
Basil was separated from the stem, flowers and rotten leaves, and then washed with clean water to remove dirt. Basil leaves were dried using cabinet dryer at 45 ± 5°C for 3 hours. Dried basil leaves were crushed using a grinder and sieved using 20-mesh sieves. Basil leaves powder (40 g) was pre-treated by PEF with a voltage of 1000, 1500, and 2000 Volts, exposure time of 10, 15 and 20 seconds, frequency of 1500 Hz, with cathode-anode distance of 10 cm. Treated basil leaves powder was macerated using 80% ethanol with a ratio of 1:10 (w/v). Maceration was carried out for 90 minutes on hotplate at 50°C. The solution was filtered using fine filter paper to obtain macerate. Macerate was concentrated using rotary vacuum evaporator for 30 minutes at 50°C, 65 rpm and 100 mBar. The condensed extract was dried using cabinet dryer at 50 ± 5°C for 24 hours. The dried extract was analysed for total phenolic compounds and antioxidant activity.

2.4. Total phenolic compounds (TPC) analysis
Total phenolic compounds (TPC) of basil leaves extract were analyzed quantitatively using Folin-Ciocalteau method using UV-Vis Spectrophotometer [10]. TPC analysis was started by making standard gallic acid. 1 ml of sample, 5 ml of Folin-Ciocalteau reagent (10%) and 2 ml of Na₂CO₃ (7.5%) were introduced into test tubes and then incubated for 30 minutes. The absorbance of the solution was measured at 765 nm.

2.5. Antioxidant activity analysis
Antioxidant activity was analysed using 2,2-diphenyl-l-picrylhydrazyl (DPPH) method [11]. Basil leaves extract were dissolved with distilled water to obtain an aliquot 1000 ppm. The aliquot was diluted to obtain 25, 50, 75, and 100 ppm. Samples of each concentration were piped as much as 1 ml and put into test tube. Then, 7 ml of methanol and 2 ml of DPPH 0.2 mM were added. Control was 8 ml of ethanol and 2 ml of DPPH 0.2 mM. The absorbance of sample and control were measured at wavelength of 517
nm. Calculation of antioxidant activity represented as inhibition concentration (IC), based on the absorbance of the control and absorbance of the sample. The following is the formula for calculating inhibition concentration (IC):

\[
IC(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{absorbance of control}} \times 100\%
\]  

(1)

The percent value of inhibition is plotted on the x and y axes respectively, so that the linear regression equation is obtained:

\[
y = ax + b
\]  

(2)

where y is 50 (determination), and x is an antioxidant activity (IC50)

2.6. Statistical analysis
Data analysis was carried out to find out the significant effect of factors on the parameters. Data were analyzed by Analysis of Variance (ANOVA) using SPSS 16.0 software. If the results of ANOVA showed there are interactions between factors, then proceed using Duncan Multiple Range Test (DMRT) with the confidence level of 95% or \( \alpha < 0.05 \).

2.7. Determination of the best treatment
The best combination of factors was selected using Multiple Attribute based on the Zeleny method [12]. The parameters used to determine the best treatment consisted of total phenolic compounds and antioxidant activity. The best treatment was chosen based on the minimum L1, L2, and \( L_{\infty} \) values.

2.8. Fourier transform infra-red (FTIR) analysis
The best treatment was analyzed for its functional groups using the FTIR method. Functional group analysis aims to ensure that basil leaves extract contains phenolic compounds seen from the functional group components. This analysis was carried out by Unit of Analysis and Measurement of Chemistry Department, Universitas Brawijaya, Malang.

2.9. Scanning electron microscopy (SEM)
Micromorphology of fresh, dried, and 20 mesh basil leaves powder with and without PEF treatment was analyzed using SEM. A leaf sheet is cut (10x10 mm) and placed on carbon. The analysis was done at voltage of 5 kV and pressure of 200 Pa.

3. Results and Discussion
3.1. Total phenolic compounds (TPC)
The study found that TPC of basil leaves extract was ranged from 219-260.5 mg GAE/g. The graph of the TPC average of basil leaves extract due to exposure time and voltage is shown in Figure 1. Based on Figure 1, it can be seen that the highest TPC average was obtained at the combination of 10 seconds and 1500 Volts with the value of 260.5 mg GAE/g, while the lowest TPC average was found in the combination of 20 seconds and 2000 Volts. The results showed that the longer exposure time obtains the lower TPC, while the higher voltage obtains the higher TPC up to 1500 Volts.
Exposure time and voltage correlate negatively with TPC, which means that the longer exposure time and the higher voltage cause the decrease of TPC. Thus, the suggestion for the next study is to use exposure time under 10 seconds and voltage below 1500 Volts. This is consistent with Raso and Heinz's study [13] that the longer exposure time will cause damage to the cytoplasmic membrane which is characterized by the formation of irreversible pores due to exposure of high-voltage pulses for a long time. Increasing the electric field (voltage per distance of the anode-cathode) will cause membrane rupture to form wider pores [14]. If the given voltage exceeds the optimal point, permanent pores decreases the active compound.

The results of ANOVA showed that exposure time significantly influence TPC of basil leaves extract, indicated by the value of Sig. 0.000 (Sig. <0.05). The voltage has a significant effect on TPC of basil leaves extract, indicated by the value of Sig. 0.001 (Sig. <0.05). The interaction between exposure time and voltage also significantly affected TPC of basil leaves extract, indicated by the value of Sig. 0.016 (Sig. <0.05). The result of DMRT showed that the exposure time of 10 seconds and 15 seconds did not show a significant difference in TPC, but there was a significant difference in TPC compared to the exposure time of 20 seconds. The voltage of 1000 Volts and 2000 Volts did not show a significant difference in TPC, but there is a significant difference in TPC compared to 1500 Volts. The highest TPC was produced at 10 seconds and 1500 Volts.

3.2. Antioxidant activity (IC50)
IC50 value of basil leaves extracts was in the range of 35.94-51.31 ppm. The graph of IC50 average of basil leaves extract due to exposure time and voltage is illustrated in Figure 2. The lower IC50 value is the higher antioxidant activity [15]. Based on Figure 2, the highest antioxidant activity of 35.94 ppm is produced at exposure time of 10 seconds and voltage of 1000 Volts, while the lowest antioxidant activity of 51.31 ppm was produced at exposure time of 20 seconds and voltage of 2000 Volts.
Exposure time and voltage correlated negatively with antioxidant activity, which means that the longer exposure time and the higher voltage caused the decrease of antioxidant activity so that the suggestion for the next study is to use exposure time under 10 seconds and voltage below 1000 Volts. This is consistent with Putranto’s study that the increase of electric field strength (voltage per distance of the anode-cathode) decreases antioxidant activity [16]. The greater electric field strength and the longer exposure time cause more pores in the cell membrane to form irreversible pores [17]. The formation of permanent pore cells with a relatively large amount causes mass transfer on the cell wall was occurred quickly. This condition causes the non-antioxidant compounds contained in cells to also dissolve into solvents, which causes a decrease in antioxidant activity.

The results of ANOVA showed that exposure time had a significant effect on the antioxidant activity of basil leaves extract, indicated by the Sig. 0.000 (Sig. <0.05). The voltage has a significant effect on the antioxidant activity of basil leaves extract, indicated by the value of Sig. 0.000 (Sig. <0.05). The interaction between exposure time and voltage also significantly affected the antioxidant activity of basil leaves extract, indicated by the Sig. 0.002 (Sig. <0.05). The result of DMRT showed that differences in the level of exposure time and voltage produce IC₅₀ that was significantly different, indicated by the differences of notation at each level of exposure time and voltage. The highest antioxidant activity was obtained at 10 seconds and 1000 Volts.

3.3. Best treatment

The best treatment was obtained at an exposure time of 10 seconds and the voltage of 1500 Volts. The best treatment results were compared with the untreated sample to determine the difference of parameters, thus the influence of PEF treatment can be determined. A comparison of the sample treated with PEF and untreated sample can be seen in Table 1.

| Parameter | Sample treated with PEF | Untreated Sample |
|-----------|-------------------------|------------------|
| TPC       | 260.5 mg GAE/g          | 175.75 mg GAE/g  |
| IC₅₀      | 35.96 ppm               | 116.25 ppm       |

Table 1 shows that sample treated with PEF contains TPC of 260.5 mg GAE/g, while the untreated sample contains TPC of 175.75 mg GAE/g. It showed that PEF treatment could increase TPC up to 1.5 times. The sample treated with PEF has IC₅₀ of 35.96 ppm, while the untreated sample has IC₅₀ of 116.25 ppm. It showed that PEF treatment could increase antioxidant activity up to 3.2 times. Antioxidant activity is divided into four categories: very strong (IC₅₀ values <50 ppm), strong (IC₅₀ values ranging from 50-100 ppm), medium (IC₅₀ values ranging from 100-150 ppm), and weak (IC₅₀ values ranging from 151-200 ppm) [15]. Based on IC₅₀ values, the sample treated with PEF has very strong antioxidant activity, while the untreated sample has moderate antioxidant activity.

3.4. FTIR spectra

The functional groups of basil leaves extract treated with PEF (10 seconds and 1500 Volts) were analyzed using Fourier Transform Infra-Red (FTIR). FTIR spectra of basil leaves can be seen in Figure 3. FTIR spectra result showed the absorption at wavenumber 3421.48; 2937.38; 1627.81; 1521.73; 1384.79; 1269.07; 1155.28; 1076.21; and 617.18. Absorption at wavenumber 324.48 cm⁻¹ indicates the presence of O-H group (phenol). Absorption at wavenumber 2937.38 cm⁻¹ indicates the presence of –C-H- (alkane) groups. Absorption at wavenumber 1627.81 cm⁻¹ shows the presence of -C=C- (alkene) group. Absorption at wavenumber 1521.73 cm⁻¹ showed the presence of -C=C- (aromatic) group. Absorption at wavenumber 1384.79 cm⁻¹ indicates the presence of –C-H (alkane) groups. Absorption at wavenumber 1269.07 cm⁻¹ indicates the presence of C-O-C (ether) groups. Absorption at wavenumber 1155.28 and 1076.21 cm⁻¹ indicates the presence of –C-O group (alcohol). Absorption at wavenumber 617.18 cm⁻¹ indicates the presence of C-Cl group (alkyl halide).
Each absorption showed a different intensity or transmittance. Transmittance is the amount of light reflected by the sample. The higher concentration of the sample obtains more absorbed light and lower transmittance value [18]. Absorption at a wavenumber of 324.48 cm\(^{-1}\) shows the lowest percent transmittance, which is 10%. It indicates that the O-H group (phenol) is a functional group with the highest concentration, indicating that the main component of basil leaves extract is phenol compounds.

Basil extracts that were treated by PEF compared to without PEF (Table 2) aimed to determine the effect of PEF pre-treatment at the functional group components obtained. The number of waves indicates the type of functional group compounds using the FTIR method. The treated basil leaves extract did not have -C=O (carbonyl) group, whereas the untreated basil leaves extract contains -C=O (carbonyl) group. Literary basil leaves extract did not contain functional groups –C-H (alkane), -C=C- (aromatic), C-O-C (ether), and C-Cl (alkyl halide). It showed that PEF pre-treatment increased the component of the functional group so that the compounds of the extract are more diverse. Also, absorption of basil leaves extract treated with PEF was formed at higher wavenumber than untreated basil leaves extract. It showed that PEF treatment increases the absorption or decreases the transmittance, which indicates the increase of extract concentration.

| No. | Basil Leaves Extract Treated with PEF | Byzantinum Basil Leaves Extract[19] | Wavenumber (cm\(^{-1}\)) | Type of Functional Groups |
|-----|-------------------------------------|------------------------------------|--------------------------|---------------------------|
| 1   | 3421.48                             | 3393.69                            | 2000-3600                | O-H (Phenol)              |
| 2   | 2937.38                             | -                                  | 2850-2960                | -C-H (Alkane)             |
| 3   | -                                   | 1735.07                            | 1690-1760                | -C-O (Carbonyl)          |
| 4   | 1627.81                             | 1637.37                            | 1620-1680                | -C=C- (Alkene)           |
| 5   | 1521.73                             | -                                  | 1500-1600                | -C=C- (Aromatic)         |
| 6   | 1384.79                             | 1355.14                            | 1350-1470                | -C-H (Alkane)            |
| 7   | 1269.07                             | -                                  | 1230-1270                | C-O-C (Ether)            |
| 8   | 1155.28; 1076.21                    | 1217.22                            | 1080-1300                | -C-O (Alcohol)           |
| 9   | 617.18                              | -                                  | 500-680                  | C-Cl (Alkyl halide)      |
3.5. **Scanning electron microscopy (SEM)**

SEM was carried out on the Glandular Trichomes (GT) cells of fresh basil leaves before and after PEF treatment, dried leaf (cabinet dryer) before and after PEF treatment, and 20 mesh basil leaf powder before and after PEF treatment. PEF treatment showed GT cell differences, as shown in Figure 4.

Untreated fresh basil leaf (Figure 4-a1) can maintain cell integrity and a relatively smooth cell surface. Fresh basil leaf treated with PEF shows the cell shrinks, and the cell surface is slightly rough (Figure 4-a2). Untreated dried basil leaf shows shrinkage of the cell, which causes the cell surface to be wavy and rough, but shrinking cells look smooth (Figure 4-b1). Cell shrinkage is caused by loss of water during drying, which causes cell shrinkage and distortion [20]. In contrast to dried leaf treated with PEF, cell shrinkage looks irregular (Figure 4-b2). Based on these differences, it can be known that PEF treatment can damage the cell membrane more than conventional thermal dehydration [10]. Untreated 20 mesh basil leaf powder did not show any pores on the cell surface (Figure 4-c1), while 20 mesh basil leaf powder with PEF treatment showed pore formation on the surface of the tissue. The formation of pores in cell membranes can increase the ethanol penetration capacity into cells. It facilitates the diffusion of phenolic compounds through cell membranes so that phenolic compounds increased.

![Figure 4. SEM images of untreated fresh basil leaf (a1), fresh basil leaf treated with PEF (a2), untreated dried basil leaf (b1), dried basil leaf treated with PEF (b2), untreated basil leaf powder (c1), basil leaf powder treated with PEF (c2)](image)

4. **Conclusions**

The results showed that exposure time and voltage had a significant effect on TPC and antioxidant activity. The best treatment in this study was obtained at 10 seconds and 1500 Volts with a TPC value of 260.5 mg GAE/g and IC$_{50}$ of 35.96 ppm. The untreated sample had TPC of 175.75 mg GAE/g and IC$_{50}$ of 116.25 ppm. These results indicated that PEF pre-treatment increases TPC by 1.5 times and antioxidant activity by 3.2 times. Based on the results of FTIR analysis, PEF pre-treatment increases the concentration of basil leaves extract, seen from the number of the functional groups component formed and the decrease of transmittance. SEM imaging showed that PEF pre-treatment causes damage to cells and pore formation, thus facilitating the diffusion process of phenolic compounds.
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