Analysis of the phenol and flavonoid content from basil leaves (*Ocimum Americanum* L) extract using pulsed electric field (PEF) pre-treatment

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Abstract: Basil leaves (*Ocimum Americanum Linn*) are commonly known to contain phenol and flavonoid compounds which can act as antimicrobial and antioxidants. In general, the bioactive compound can be obtained by conventional extraction method, maceration. To increase the amount of bioactive compounds which can be extracted, it is necessary to conduct pre-treatment using pulsed electric field (PEF). PEF has an advantage in increasing the compound transfer contained in cells to be extracted from the plant tissues via electroporation mechanisms, without causing significant temperature changes. This study aims to determine the effect of electric field strength variations (2, 3, and 4 kV cm^{-1}) and duration of pre-treatment (1, 2, and 3 mins) to total phenol and flavonoid content of basil leaf (*Ocimum Americanum Linn*) extract. The result of statistical analysis using two-way analysis of variance informs that electric field strength variation, pre-treatment duration, and interaction of both variables simultaneously affect the total level of phenols and flavonoids. The combined treatment of 3 kV cm^{-1} and the two-minute pre-treatment duration results in the highest total phenol and flavonoid level, i.e. 115.203±1.115 mg gallic acid equivalents (GAE)/g extract and 75.816±0.723 mg quercetin equivalents (QE)/g extract respectively. The extraction utilizing PEF treatment also exhibits higher levels of phenol and flavonoid than those of control treatment.

Keywords: basil leaves, phenol, flavonoid, pulsed electric field

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1 Introduction

Oxidant compounds are one of the causes of oxidative damage in the body. Moreover, oxidant compounds may take form in either reactive oxygen or free radicals, having characteristics as oxidizers. The damage is caused by the low levels of antioxidants produced by the body (endogenous antioxidant), so it cannot compensate the reactivity of oxidant compounds (Alam et al., 2018; Castro et al., 2018; Nirmala et al., 2018). To overcome these negative impacts, human body needs an exogenous antioxidant system. There are two sources of exogenous antioxidant, synthetic and natural (Chand et al., 2017). However, according to the World Health Organization (WHO), currently, as many as 80% of the world's population has used the active compound extract from plants as a natural medicine (Palhares et al., 2015). This is due to the use of synthetic antioxidants beginning to be restricted. Synthetic antioxidants are known to cause some side effects such as mutagenic, toxic, and carcinogenic (Kaurinovic et al., 2011). In general, natural antioxidants are the extraction results of secondary metabolite compounds from plants. These secondary metabolite compounds are bioactive compounds which are not essential and are produced in small amounts by

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plants, animals, and microorganisms, except algae. For plants, these compounds serve to support life and defend against pests, diseases, and ultra-violet (UV) rays.

Some secondary metabolite compounds which can act as natural antioxidants are phenol and flavonoid (Berlowski et al., 2013; Khan et al., 2012). One of the plants being rich in secondary metabolite compounds such as phenol and flavonoid is Basil (*Ocimum Americanum* L.), known by its Latin name of *Ocimum Basilicum Linn* (Pandey et al., 2014). Due to its many forms (polymorphic), it often raises some difficulties in the field of taxonomy. In Indonesia, so far, the utilization of basil leaves is only focused on fresh consumption. In fact, fresh basil leaves are known to contain phenol compounds as much as 0.812±0.119 mg GAE/g fw and total flavonoid of 7.22±0.36 mg/100 g (Andarwulan et al., 2010). According to USDA (2016), basil leaves contain high amount of protein, fat, carbohydrate, calcium, iron, magnesium, vitamin A, B-6, C and E. It is considered less practical due to its non-uniform dose.

Maximizing the utilization of active compounds contained in basil leaves can be conducted through extraction process. A common method used to extract phenol and flavonoid compounds which are not heat resistant is maceration process. Maceration includes in cold extraction methods which can be performed at room temperature. However, this method has drawbacks such as requiring multiple solvents, long extraction time, and low levels of antioxidant compounds obtained (Fernández-Ronco et al., 2012; Sharifi et al., 2017). Based on these considerations, this study adds pre-treatment process before maceration, conducted by various methods such as the application of thermal treatments and the addition of enzymes or chemicals. Both methods may present drawbacks especially on heat-resistant compounds which will be damaged by heating. Meanwhile, enzyme addition requires a large operating cost to separate residues and enzymes (Cheng et al., 2018; Hou et al., 2017; Jönsson and Martín, 2016; Yu et al., 2018).

Alternatively, pre-treatment methods can be performed using pulsed electric field (PEF) technology. PEF is able to maintain sensory and nutritional properties of the material because the process does not cause significant temperature alterations, shorter treatment duration, and increasing number of extracted active compounds from plant cells. According to Zderic et al. (2013), PEF does not cause significant temperature changes, wherein the inlet and outlet elevations of the chamber treatment do not exceed 5°C. The basic principle of the PEF process is that a certain amount of energy coming from the direct current (DC) power supply is channeled through the charging resistor and stored in a series of capacitors which convert that energy into high-voltage pulses. When the switch is connected, the high-voltage pulse generated will be applied to the electrode. Further, the electrodes carry a high-intensity electrical pulse on food located between two electrodes. The food will experience a force per unit charge called the electric field being responsible for cell membrane damage. PEF applies high-voltage electrical shock (kV cm^{-1}) in the form of short pulses to break up cell membranes, called as electroporation phenomena. Electroporation results in a charge shift in the atom or molecule, so that the positively charged molecule shifts to the negative electrode and vice versa, forming a dipole. This shift increases the transmembrane potential, so the cell membrane will be thinning. The transmembrane potential exceeding the critical threshold will allow for the destruction of the cell membranes leading to the formation of pores. These pores would facilitate the rate of transfer of the compound from the cell to the solvent (EI Kantar et al., 2018; Ravishankar et al., 2008). The rupture of these cells was influenced by two main factors, electric field strength and exposure duration. Previously, there have been numerous studies on the extraction of phenol and flavonoid compounds using PEF with variations in electric field strength and pre-treatment duration (Fincan, 2015; EI Kantar et al., 2018; Lohani and Muthukumarappan, 2016; Redondo et al., 2018; Soliva-Fortuny et al., 2017). The level of the resulting compound increased with the increase of applied electric field strength, and the proper selection of duration would affect the alterations of cell structure (Bobinaitė et al., 2017). In a study conducted by Luengo et al. (2013), the polyphenol content increased by 14.14±1.52, 26.92±8.51, and 29.81±9.31 mg GAE/100 g of orange peel on the variation of electric field strength 1, 3, and 5 kV cm^{-1},

compared to control treatment by 11.76±6.05 mg GAE/100 g of orange peel. Therefore, this study utilizes electric field strength variable and PEF duration in pre-treatment process before maceration aiming to identify the effect of electric field strength and PEF pre-treatment duration to total phenol and flavonoid level of basil leaves extracted, to determine the best treatment combination which can produce the highest phenol and flavonoid levels and to recognize the balance of mass during the basil leaf extraction process.

2 Materials and methods

The research was conducted in Laboratory of Food and Agricultural Products Processing Technology, Laboratory of Bioindustry, and Laboratory of Basic Chemistry, Faculty of Agricultural Technology, Universitas Brawijaya, Indonesia. The following tools are utilized in this research: 1) Pulsed electric field (Normex): as an electric shock source for pre-treatment materials; 2) Rotary vacuum evaporator (IKA RV 10): to separate the extract from the solvent; 3) UV-Vis spectrophotometer (Biochrom Libra S12): to measure sample absorbance; 4) Oven (Binder Red Line): to dry basil leaves; 5) Blender: to minimize the size of dry basil leaves; 6) Glassware: as a container for testing total phenols and flavonoids; and 7) Dark glass bottle: as a container for maceration. The materials used in this study are as follows: 1) Basil leaves: as treatment materials; 2) Distilled water as the extraction process solvent; 3) Fine filter paper: to separate the filtrates and residues; 4) Phenolic acid: the main ingredient in the manufacture of standard curves for total phenol analysis; 5) Sodium carbonate: to analyse the total phenol; 6) Folin-Ciocalteau: to analyse the total phenol; 7) Quercetin: the main ingredient in the manufacture of standard curves for flavonoid analysis; 8) Ethanol pro-analyst: to dissolve quercetin; 9) Aluminum chloride, sodium nitrite, sodium hydroxide: as reagents for flavonoid analysis; 10) Sodium nitrite: to analyze flavonoid; and 11) Sodium hydroxide: for flavonoid analysis.

2.1 Analysis method

This research uses two factorials of random group design (RAK). The first factor is electric field strength consisting of three levels: 2, 3, and 4 kV cm^{-1}. The second factor is the duration of pretreatment consisting of three levels: 1, 2, and 3 mins. The combined treatment is presented by Table 1.

Table 1 Treatment combination on basil leaf pre-treatment

Electric field force (E)	Duration (T)		
	T1 (1 min)	T2 (2 mins)	T3 (3 mins)
E1 (2 kV cm^{-1})	E1T1	E1T2	E1T3
E2 (3 kV cm^{-1})	E2T1	E2T2	E2T3
E3 (4 kV cm^{-1})	E3T1	E3T2	E3T3

2.2 Sample preparation

Basil plants were obtained from Banjarejo village, Tumpang district, Malang regency, Indonesia and harvested two months after planting. The fresh basil leaves were separated from other parts of the plants, washed with running water, and then dried for eight hours using oven (50ºC). The dried basil leaves were blended using a blender and sieve, obtaining 40-mesh basil leaf powder.

2.3 Extraction process of basil leaves

A 40 mesh basil leaf powder as much as 20 grams and distilled water as much as 300 mL were homogenized in a beaker glass using spatula. After reaching homogeneous state, mixed basil leaves were treated using PEF with electric field strength of 2, 3, and 4 kV cm^{-1} and different pre-treatment durations of 1, 2, and 3 mins. The mixture of basil leaves-distilled water was extracted by maceration method for three hours at room temperature. Afterwards, the result was filtered using fine filter paper. The obtained macerate was evaporated using a rotary vacuum evaporator until dense extract is obtained.

2.4 Temperature observation

Temperature observation aims to determine the existence of temperature alterations during the process which can affect the results of the analysis. The observations and temperature measurements are carried out in the following processes: 1) Observing the temperature on the oven display before reaching a temperature of 50ºC; 2) Observing oven temperature every 15 minutes during drying; 3) Measuring temperature using thermometers before and after PEF pre-treatment; and 4) Measuring temperature using thermometer before and after maceration.

2.5 Measurement of simplicial moisture content

The analysis of simplicial moisture content is based

on the gravimetric method, utilizing an oven. The moisture content of the material is calculated as the percentage of sample weight loss after drying. The moisture content measurements take place until a constant weight is obtained and calculated on a wet basis using the following equation (AOAC, 1995):

$$\text{Moisture content } (\%) = \frac{(\text{initial weight} - \text{final weight})}{\text{initial weight}} \times 100 \quad (1)$$

2.6 Yield calculation of the extract

The yield of the extract was calculated by comparing the weight of the extract with the initial weight of the simplicial. The yield of the extract was determined based on the following calculation method (Ahmad et al., 2009; Seggiani et al., 2009):

$$\text{Yield} = \frac{\text{weight of extract)}}{\text{initial weight of simplicial}} \times 100\% \quad (2)$$

2.7 Analysis of total phenol content

The total phenol content was determined by Folin-Ciocalteau test. The test was conducted by adding Folin-Ciocalteau reagent of 1:10, 2.5 mL into the diluted extract of 0.5 mL. Afterwards, the mixture was incubated for 5 mins. Thereafter, 7.5% (2 mL) of sodium carbonate was added, and the mixture was incubated in the dark room for 30 minutes, then measuring the absorbance of the solution at a wavelength of 756 nm using a UV-Vis spectrophotometer. The concentrations of gallic acid of 0, 20, 40, 60, 80, and 100 ppm are used in the production of calibration curve. The measurement results are expressed in mg GAE/g extract (Lee et al., 2003).

2.8 Analysis of flavonoid content

The total flavonoid content of dense extract was determined by colorimetric method. The diluted extract of 1 mL was mixed with distilled water of 2 mL. Afterwards, 5% (0.3 mL) of sodium carbonate solution was added. The mixture was homogenized and incubated for 6 mins, and then 10% (0.3 mL) of aluminum chloride solution was added and incubated for 6 mins. Subsequently, the mixture was added with 1 M (4 mL) of sodium hydroxide solution and 2.4 mL of distilled water, and it was homogenized and incubated in the dark room for 15 minutes. The solution was measured its absorbance using a UV-Vis spectrophotometer at a wavelength of 502 nm. Quercetin concentrations of 0, 40, 80, 120, 160, and 200 ppm were used in the manufacture of calibration curves. The measurement results are expressed in mg of QE/g extract (Atanassova et al., 2011).

3 Results and discussion

3.1 Moisture content

In this study, basil leaves were dried by oven at a temperature of 50°C. However, in practice, the drying temperature used reached 52.27°C±2.03°C. To obtain simplicial according to quality requirement, the researchers offer a graph of measurement of moisture content of withered basil leaves as presented in Figure 1. Figure 1 shows the final moisture content of withered basil leaves reaching 6.790%±0.052%. Meanwhile, the moisture content initially reached 66.248%±3.347%. This final moisture content has met the requirements of simplicial quality, less than 10%, which is suitable for storage because it is not possible for the enzyme to alter the chemical content in the simplicial. At this stage, fresh basil leaves as much as 2199.77 g will be dried to obtain 204.02 g basil leaf powder. The mass balance in the sample preparation is shown in Table 2.

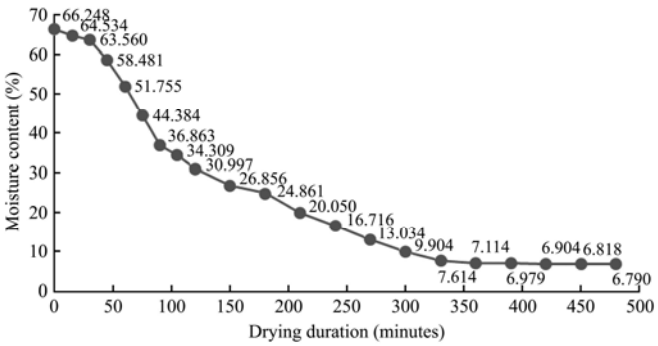

Figure 1 Moisture content increase on withered basil leaves

Table 2 Mass balance on the sample preparation

No.	Process	Input mass (g)	Loss of mass (g)	Output mass (g)
1	Sorting	4200	2000.23	2199.77
2	Washing	2199.77	72.11	2271.88
3	Whitering	2271.88	1283.23	988.65
4	Drying	988.65	767.80	220.85
5	Grinding	220.85	8.87	211.98
6	Sifting	211.98	7.96	204.02

3.2 Temperature

Dried basil leaf powder is used for PEF pre-treatment. In the pre-treatment process, there was no temperature alteration when measured using a thermometer. This could be due to the occurring low temperature changes. Therefore, it cannot be measured by a thermometer. The

results of the temperature measurements are in accordance with research conducted by Zderic et al. (2013) stating that the PEF treatment did not significantly affect the temperature of the material. Increased inlet and outlet temperature of chamber treatment did not exceed 5°C.

3.3 Yield extract

After the evaporation process is completed to reduce the solvent, the result of the calculation of the yield of the extract could be achieved as shown in Table 3. Table 3 presents the value of yield extract ranged from 23.70% to 33.15% with a standard deviation range of 1.472-2.484. The yield on treatment of 2 kV cm^{-1} for 1, 2, and 3 mins has a higher value than other electric field strength treatments. The highest yield of 33.15%±2.484% is obtained in a combination of 2 kV cm^{-1} for 1 minute while the lowest yield of 20.33%±0.029% is obtained at 3 kV cm^{-1} for 2 mins. The discrepancies in the yield values of each treatment can be influenced by several factors, the sample volume before evaporation, the presence of the powder which passes in the screening process and weighted in the extract; thus, affecting the yield value, and the mass of the material left in the treatment chamber PEF; thus, affecting mass input for maceration. The results of the calculation of yield were statistically analyzed using two-way analysis of variance (ANOVA). The result of analysis shows that electric field strength, pre-treatment duration, and interaction of both variables significantly influence the value of yield (sig. <0.05). Due to an existing real influence, then the analysis continued using Tukey HSD and Bonferroni to recognize the variation of the electric field strength and the pre-treatment duration which is different significantly to the value of yield. Based on the previous analysis, there is a significant difference between the electric field strength of 2 kV cm^{-1} and 3 kV cm^{-1} and the treatment of 2 kV cm^{-1} and 4 kV cm^{-1} (sig. <0.05). Meanwhile, according to treatment duration, there are significant differences on the average yield value between durations of one and two minutes and the durations of two and three minutes (sig. <0.05), whereas there is no real difference between durations of one and three minutes (sig. >0.05). In the extraction process, dense extract as much as 4 mL or 4.07 g of the initial sample as much as 320.82 g is obtained. The mass balance of the extraction process is shown in Table 4.

Table 3 Yield of basil leaf extract using PEF treatment

Treatment		Average yield (%)	Standard deviation
Electric field strength (kV cm^{-1})	Time (minute)		
2	1	33.15	2.484
2	2	23.70	1.472
2	3	29.05	2.295
3	1	24.52	1.488
3	2	20.33	0.029
3	3	23.90	1.303
4	1	23.85	1.297
4	2	23.83	1.447
4	3	26.70	2.948

Table 4 Mass balance on extraction process

No.	Process	Input mass (g)	Loss of mass (g)	Output mass (g)
1	PEF pre-treatment	320.82	7.25	313.57
2	Maceration	313.57	0	313.57
3	Filtration	313.57	58.52	255.05
4	Evaporation	255.05	4.07	250.98

3.4 Analysis of total phenol

The determination of the total phenolic content of basil leaf extracts is initiated with the manufacture of standard curves with gallic acid as the standard. The standard curve of gallic acid presented in Figure 2 has a good accuracy based on the R^2 value of 0.9983 produced. The equation is used to determine extracted phenolic content of basil leaves using absorbance data.

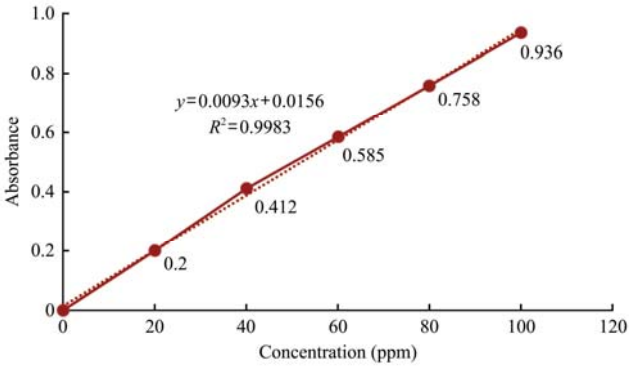

Figure 2 Standard curve of gallic acid

The sample absorbance measurements were carried out at a wavelength of 756 nm and obtained the results as presented in Table 5. According to Table 5, the highest total phenol content of 115.203±1.115 mg GAE/g extract was obtained from electric field strength treatment of three kV cm^{-1} for two minutes while the lowest total phenol content of 23.507±1.656 mg GAE/g extract was obtained at treatment 2 kV cm^{-1} for 1 min. The total

phenol content resulting from the three variations in electric field strength has the same trend, which increased in the duration of 2 mins and decreased during pre-treatment for 3 mins. This proves that the optimum duration of pre-treatment of basil leaves using PEF is 2 mins, so the addition beyond that duration cannot increase the total phenol content of basil leaf extract. The longer the PEF duration means the greater the likelihood of cytoplasm cell membrane damage to form irreversible pores due to high-voltage pulse exposure in the long run (Saldaña et al., 2017). After passing the optimum time, the levels of antioxidant compounds decreased. The decreasing levels were suspected because antioxidant compounds were damaged due to long exposed to high-voltage electric current. While in the electric field strength treatment, the total phenol content produced by 2 kV cm^{-1} treatment is lower than 3 kV cm^{-1} treatment. This can be explained by the electroporation mechanism by PEF, when biological cells were exposed to an electric field, the charge would accumulate along the plasma membrane. As a result, there was a potential difference in transmembrane in the cell which caused porosity. Such an increase in transmembrane potential would result in leakage of the cell membrane. In a lower electric field, it is believed that the pores formed were much smaller, allowing the ions to pass through the pores, but large molecules could not get out of the cell. Furthermore, in the intensity of the higher electric field (3 kV cm^{-1}), irreversible membrane rupture occurred, and wider pores formed on the cell membrane (Ersus et al., 2010; Joannes et al., 2015). However, when given a higher electric field strength, which was 4 kV cm^{-1}, the total content of phenol decreased. This shows that the electric field strength of 3 kV cm^{-1} was the optimum point in the pre-treatment of basil leaves using PEF in this study. Decreased levels were suspected because antioxidant compounds were damaged due to long exposure to high-voltage electric current. The high intensity of the electric field led to the formation of irreversible pores on the cell membrane. The formation of permanent pores caused the mass transfer from within the cell to the solvent to be faster, allowing for oxygen-induced oxidation reactions (Comuzzo et al., 2018; Janositz et al., 2011; Platonova et al., 2018). The results of two-way analysis show that the variables of electric field strength, duration, and interaction of both variables simultaneously significantly influence the total content of phenol produced (sig. <0.05). Because there is a significant influence, a further analysis (Post Hoc Test) is conducted using Tukey HSD and Bonferroni to recognize which electric field strength variations and duration differ significantly to phenol levels. Based on the results of further analysis, there is a significant difference in mean phenol levels in all variations in electric field strength (2, 3, and 4 kV cm^{-1}) and pre-treatment durations (1, 2, and 3 mins).

Table 5 Total phenol content of basil leaf extract using PEF treatment

Treatment		Average phenol (mg GAE/g extract)	Standard deviation
Electric field strength (kV cm^{-1})	Time (minute)		
2	1	23.507	1.656
2	2	68.149	1.908
2	3	59.196	2.215
3	1	87.172	0.857
3	2	115.203	1.115
3	3	87.848	0.274
4	1	76.087	1.891
4	2	82.959	0.844
4	3	79.477	1.246

3.5 Comparison with other pre-treatment methods

The best treatment was determined based on the highest phenol level obtained from a combination of 3 kV cm^{-1} electric field strength treatment and a 2-minute pre-treatment duration of 115.203±1.115 mg GAE/g extract as compared to the result of control treatment and other extraction methods performed by Ghasemzadeh et al. (2016). The control treatment sample was obtained by maceration for 3 hours with phenol level of 16.680± 2.653 mg GAE/g extract. Meanwhile, the phenol level in the study of Ghasemzadeh et al. (2016) of 55.79 ± 2.31 mg GAE/g extract was obtained through UV-B pre-treatment at radiation intensity of 3.60 W m^{-2} for 8 hours and followed by reflux using 95% ethanol solvent. The comparison of best treatment with control samples and other extraction methods can be seen in Figure 3. Figure 3 presents the highest phenol content obtained from a combination of pre-treatment using PEF and maceration for three-hour duration compared with control treatment and pre-treatment of UV-B radiation. The differences in phenol content can be influenced by several factors such as

solvent type, extraction method, pre-treatment, analytical methods, and differences in raw materials obtained including growth site, harvest time, and post-harvest handling. The maceration method is suitable for extracting phenol compounds which are sensitive to high temperatures because maceration is a method which does not utilize heat energy in the process, whereas PEF is considered effective in increasing the amount of phenol compounds extracted out of the cells. In addition, PEF has advantages as the process does not cause significant temperature alterations, shorter duration of pre-treatment, and increasing rate of product diffusion out of the plant tissue. PEF pre-treatment utilizes a high-voltage electric field to form a hole in the cell membrane, in which the intensity of the applied electric field is directly proportional to the potential difference across the cell membrane. If the transmembrane potential exceeds the threshold, membrane permeability (electropermeabilization) will be formed leading to the formation of temporary or permanent pores. Thus, the PEF process helps the release of compounds out of the cells without any significant temperature increase (Galván-D'Alessandro and Carciochi, 2018; Cemazar et al., 2018; Pataro et al., 2018).

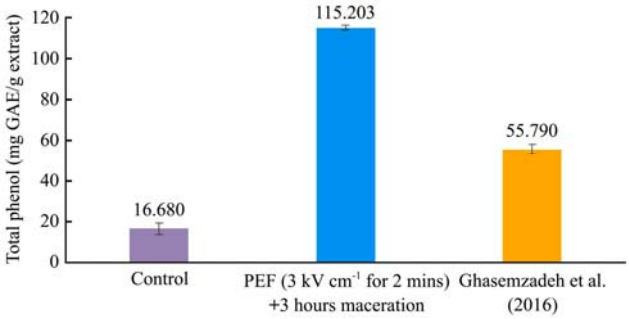

Figure 3 Total phenol content of basil leaf extract using best treatment comparison with sample control and other extraction methods

3.6 Analysis of total flavonoid

Determination of flavonoid levels in basil leaf extract began with the manufacture of standard quercetin curves. The standard quercetin curve in Figure 4 shows a good equation based on the value of R^2 close to 1. The equation is used to determine the total flavonoid content of basil leaf extract using absorbance data.

The total flavonoid content was determined according to the method of colorimetry with aluminum chloride as reactant and the UV-Vis spectrophotometer was used to measure the absorbance of the sample solution. The principle of analysis of total flavonoid content based on the method of colorimetry with aluminum chloride is the formation of complex compounds between aluminum chloride with the existing clusters in the extract. The addition of aluminum chloride into the sample caused a reaction between flavonoid with aluminum chloride, so that the yellow-color complexes are formed. The dense intensity of the color indicates higher level of flavonoid. Based on the total flavonoid analysis data, the total flavonoid content of basil leaf extract was obtained by electric field strength treatment and pre-treatment duration using PEF as in Figure 5. The highest total flavonoid content of 75.816 ± 0.723 mg QE/g extract is recognized in a combination of 3 kV cm^{-1} treatment for 2 mins. Based on the data obtained, 2, 3, and 4 kV cm^{-1} treatments have the same trend that flavonoid levels increase in pre-treatment for 2 mins and decrease in the duration of 3 mins. Similarly, in the electric field strength treatment, where there are an increase and decrease in the levels of flavonoid compounds at 3 kV cm^{-1} and 4 kV cm^{-1} electric field strength respectively. This shows that the PEF duration of 2 mins and electric field strength of 3 kV cm^{-1} are the optimum points for the pre-treatment of basil leaves, thus, the addition of the values of the two variables cannot increase the flavonoid levels. The exposure duration which is too long and exceeds the optimum point can result in the destruction of the active compound. The damage to the active compound is suspected to be due to oxidation reactions in flavonoid compounds, where flavonoids are derivatives of phenol compounds which are easily oxidized. This oxidation reaction can occur due to the presence of airborne oxygen and is characterized by a decrease in the number of flavonoid compounds. However, the occurrence of oxidation in this phenol class does not affect its antioxidant properties (Atala et al., 2017). The total flavonoid content obtained is directly proportional to total phenol content of basil leaf extract. This is because flavonoid compounds are one of the largest groups making up phenol compounds. In this study, the highest total phenol and total flavonoid levels are obtained at 3 kV cm^{-1} electric field power treatment within 2 mins.

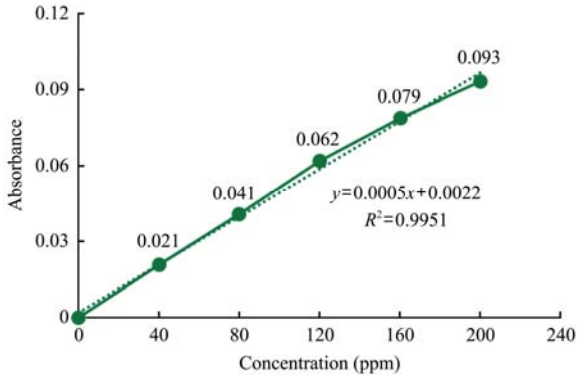

Figure 4 Quercetin standard curve

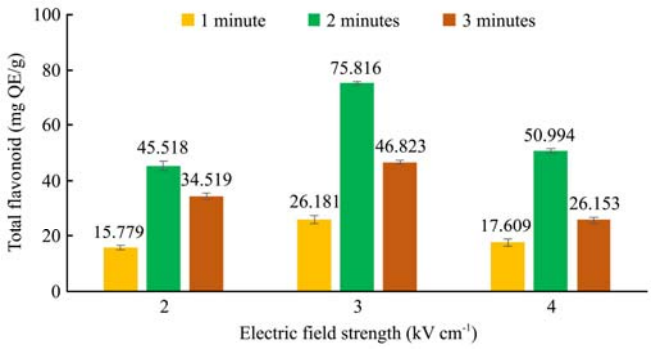

Figure 5 Total flavonoid content of basil leaf extract using the treatment of electric field strength and the durations of PFE pre-treatment

The analysis of variance results show that electric field strength, pre-treatment duration, and interaction of both variables significantly influence the total flavonoid content (sig. <0.05). Because there is a real effect, the analysis is continued (Post Hoc Test) using Tukey HSD and Bonferroni to determine the variations of electric field strength and the duration which differ significantly to flavonoid levels. Thus, there are significant differences in all variations in electric field strength (2, 3, and 4 kV cm^{-1}). In the pre-treatment duration variables, there is a significant difference of flavonoids between durations of 1 and 2 mins and durations of 2 and 3 mins (sig. <0.05).

3.7 Comparison with other pre-treatment method

The best treatment is determined based on the highest flavonoid levels obtained from a combination of 3 kV cm^{-1} electric field strength treatment and 2 minute PEF duration of 75.816±0.723 mg QE/g extract. This best treatment is compared to the control treatment sample and the extraction method of the study conducted by Ghasemzadeh et al. (2016). In the control treatment, flavonoids were obtained 12.270±1.746 mg QE/g extract through maceration for 3 hours using distilled water solvent and absorbance measurements at 502 nm wavelength. In the study of Ghasemzadeh et al. (2016), the flavonoid levels of 41.19±1.460 mg QE/g extract were obtained by UV-B radiation pre-treatment method to fresh basil leaves at radiation intensity of 3.60 W m^{-2} within 8 h. The sample absorbance measurements were carried out at a wavelength of 510 nm. The comparison graph of total flavonoid contents of basil leaf extract with various treatments can be seen in Figure 6.

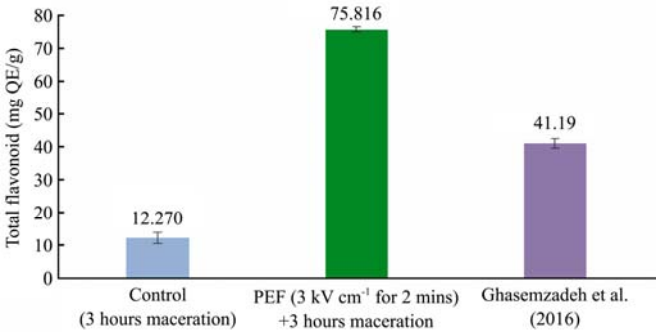

Figure 6 Comparisons of total flavonoid content of basil leaf extract using various treatments

In Figure 6, it is known that PEF and maceration combinations for three hours are capable of producing the maximum total flavonoid levels compared to control treatment and UV-B radiation combined with reflux. There are several factors which can influence the difference of flavonoid levels from the three methods, which is raw material related to the growth site, storage, harvest age, type of solvent, extraction and pre-treatment method, determining method of flavonoid level, and pretreatment process like drying method using oven, air-dried, freeze drying, and direct sunlight.

4 Conclusion

Based on the results of the research after going through various stages, concentrated extract is found as much as 4.07 g or 4 mL. 2 kV cm^{-1} treatment for 1-minute duration results in the highest yield of 33.15% ± 2.484% while 3 kV cm^{-1} combination for 2 mins presents the lowest yield of 20.33%±0.029%. PEF pre-treatment can increase total phenol and flavonoid contents compared with control treatment (without PEF). The levels of phenol and flavonoid in the control treatment are 16.680±2.653 mg GAE/g extract and 12.270±1.746 mg QE/g extract. The strong interaction of the electric field strength and the duration of pre-treatment simultaneously

had a significant effect on total phenol content and total flavonoid produced. The highest total phenol and flavonoid levels are achieved in a combination of 3 kV/cm electric field strength treatment and PEF duration of 2 mins, 115.203±1.115 mg GAE/g extract and 75.816±0.723 mg QE/g of the extract respectively.

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