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## Extraction of Phenol and Antioxidant Compounds from Kepok Banana Skin with PEF Pre-Treatment

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# Extraction of Phenol and Antioxidant Compounds from Kepok Banana Skin with PEF Pre-Treatment

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**Abstract.** Kepok banana (*Musa parasidiaca* L) consumption produce banana skin waste that is only used as animal feed and house waste. Kepok banana peel contains phenol and antioxidants of 12.35% and other compounds. These compounds can be extracted using maceration methods. A pre-treatment process is needed to increase phenol compounds and antioxidants using Pulsed Electric Field (PEF). The advantage of using PEF pre-treatment is that it can break down the cell membrane with the electric shock without using high temperatures. The purpose of this study is to increase the total phenol using PEF pre-treatment to obtain optimal result. PEF pre-treatment varied by electricity field strength and PEF time, where the electric field strength used was 2, 3, and 4 kv/cm and the duration of time used was 2, 3, and 4 minutes. The method used for testing the total phenol content is the Folin-Ciocalteu method and the method used for testing antioxidant activity is the DPPH (1,1-dyphenyl-2-picrylhydrazyl) method. In the study, the best value of the total phenol content was in the electric field strength variation of 4 kv/cm for 2 minutes, which was  $1.664 \pm 0.226$  mg GAE/mg dry extract. Whereas the best value on the antioxidant activity test on Kepok banana peel is on 4 kv/cm for 4 minutes, which was  $13.086 \pm 4.547$  mg/ml.

## 1. Introduction

Banana commodities in Indonesia are already familiar. Banana commodities are so popular that they occupy the first number among the types of fruit in terms of their plantations and production. In 2015, banana production in Indonesia reached 7.3 million tons with an average growth of 4.92% per year [1]. Banana fruit is very popular for consumption either directly as fresh fruit or as a processed product. Bananas consumed will produce banana peel waste that is used for animal feed or only disposed of as household or industrial waste. The discarded banana peel can be optimized because the banana skin still contains substances that are still useful. Kepok banana (*Musa parasidiaca* L) peels contain antioxidants and other nutrients such as carbohydrates, fats, proteins, calcium, phosphorus, iron, vitamin B, vitamin C, and water. Of the various contents of this banana peel, banana peels have some advantages i.e. to improve metabolic system, to increase endurance, and counteract free radicals. Banana skin is a cheap and the availability throughout the year is very high. Banana skin waste still contains antioxidant compounds such as phenol [2].

Phenol is a secondary metabolite compound that has an aromatic ring with one or more hydroxyl groups (OH-) and other concomitant groups. Phenol is one of the strongest antioxidants that has a cardio protective effect that can prevent LDL oxidants 20 times stronger than vitamin E. Antioxidants in phenols can act as inhibitors to clump blood cells, stimulate the production of nitric oxide, and can inhibit cancer cell growth. With many phenol functions that are good in the body and is one of the



secondary metabolites that are widely spread in nature, phenol compounds are widely researched and extracted using several methods. Vu et al. [3] conducted research on phenolics within banana peels have been found to possess potent antioxidant and antimicrobial properties, and linked with various health benefits. Rebello et al. [4] observed that the extracts of banana peel flour exhibited a high total phenolic content (around 29 mg/g, as GAE).

The extraction process of total phenol compounds and antioxidants from banana peels can be modified by adding a pre-treatment process using Pulsed Electric Field (PEF). PEF is a food processing process that uses the application of short pulses on high electricity field strength (20-80 kV/cm) to foodstuffs placed between 2 electrodes at room temperature for a few seconds. According to Zderic et al. [5] PEF technology applications have resulted in integration of cells or lysis of cells due to damage to cell membranes due to the high voltage electricity field being contacted to the material. Cells that rupture cause substances in the plant to diffuse easily and can increase the value of a substance. The increase occurs because the longer the extraction time with PEF, the damage to the membrane in plant cells will be greater so that the formation of pores that are widened and cannot be returned to its original shape due to the electroporation process contained in the material will be easily dissolved. The purpose of this study was to extract the total phenol compound from Kepok banana skin using PEF as a pre-treatment method. The best results compared to extraction without using pre-treatment. The extract obtained will be tested for total phenol compounds and antioxidant activity.

## 2. Materials and Methods

This research was carried out at the Laboratory of Food and Agricultural Products Processing Technology, Department of Agricultural Engineering, Basic Chemical Laboratory, Bioindustry Laboratory, Department of Agricultural Industrial Technology, Universitas Brawijaya, Indonesia. The tools used in this study are as follows: 1) PEF (Power: 0.8 kWh; capacity: 0.3-9.1 L; output voltage: 6.2-40 kV; pulse width: 70 $\mu$ s; electric field strength: 1.3- 6.45 kV / cm; Normex, Indonesia): as a source of electric shock; 2) Rotary Vacuum Evaporator (IKA RV10, Germany): to evaporate distilled water; 3) UV-Vis Spectrophotometry (Thermo scientific Genesys UV 10, United States): to measure the absorbance of materials. The materials used in this study are as follows: 1) Kepok banana skin as the main ingredient which is dried as a treatment material. Bananas are obtained from farmers in Dampit area, Malang Regency, East Java, Indonesia; 2) Aquades: as solvents; 3) gallic acid: for making a standard curve for analysis of total phenols; 4) Sodium Bicarbonate Solution ( $\text{Na}_2\text{CO}_3$ ): as an analysis of total phenol; 5) Folin Ciocalteu solution: as an analysis of total phenol; 6) DPPH: as an analysis reagent for antioxidant activity; 7) Methanol P.A (Merck): as a solvent for analysis of antioxidant activity.

The research design involved two factors i.e. PEF voltage which includes: 2, 3, and 4 kv/cm and PEF treatment time which includes: 2, 3, and 4 minutes. The total phenol content contained in the banana peel was measured using the Folin-Ciocalteu method with gallic acid as a standard curve. Total phenol measurements were carried out to determine the potential of antidote to free radicals in banana peel extract. Sample of 0.5 ml was taken and then 2.5 ml of Folin-Ciocalteu solution was diluted with distilled ratio of 1:10. Then 2 ml of 7.5%  $\text{Na}_2\text{CO}_3$  solution was added. The mixture was homogenized using vortex and allowed to stand for 30 minutes. The absorbance of the sample was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm [6]. The absorbance value that has been obtained will be used to calculate the total phenol content contained in the extract. Antioxidant activity test was carried out using DPPH (1,1-dyphenyl-2-picrylhydrazyl) method following the method used by Liu et al. [7]. The DPPH was made from 0.2 mM DPPH solution by dissolving 0.0039 g DPPH powder in a 50 ml volumetric flask using methanol. The extract sample was weighed 0.25 g which was then mixed with 25 ml of methanol to obtain a sample solution with a concentration of 10 mg/ml. Then a variation of sample concentration was made i.e. 2.5, 5, and 7.5 mg/ml by diluting 10 mg/ml sample solution. For the control solution was made from a solution of 0 mg/ml (solution without sample). The sample was taken as much as 0.5 ml in each variation of the concentration solution and put it into a test tube. Then 3.5 ml of methanol and 1 ml of DPPH 0.2 mM

solution were added to each test tube and the tube was closed until no light came in. The solution was homogenized using vortex and incubated for 30 minutes. Then the absorbance of the sample was measured using UV-Vis spectrophotometry at a wavelength of 517 nm with methanol as blank. Free radical inhibition activity by antioxidants from the sample is seen if the colour of the sample changes to light purple. Discoloration is due to the presence of stable free radicals based on the electron reserve delocalisation of the whole molecule [8].

### 3. Results and Discussion

Kepok banana peel used in this study is dry material which has been dried using oven for 30 hours until the moisture content reaches less than 15%. This banana peel will be extracted and the results of the extract will be analysed for water content, yield, total phenol, and antioxidant activity. The use of PEF with two factors, namely the electric field strength and time will be obtained for the optimal tip for the total phenol value and antioxidant activity. The dried Kepok banana skin will be seen to decrease the water content. The following is a graph of the decrease in water content which can be seen in Figure 1. It describes the decrease in water content at 50°C. The water content of the initial average Kepok banana peel in this study was 62.26%. After drying for 48 hours (until the sample mass decreases are constant), the final moisture content of Kepok banana skin is 11.44%.

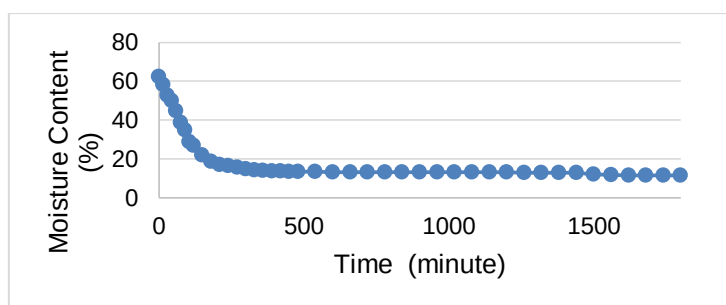


Figure 1. Decrement of the moisture content of Kepok banana skin at 50°C drying

Extract yield was calculated based on the ratio of final weight (weight of extract produced) to initial weight (weight of dry matter) multiplied by 100%. From the results obtained different yield values. The yield graph can be seen in Figure 2. In Figure 2, the electricity field strength gives a fluctuating effect on the yield. The resulting yield difference is influenced by the electric field strength and the length of time that is contacted in the material sample. The electric field strength and length of time of PEF has a linear relationship to the yield. The greater the yield value, the use of electric field strength and the longer the time will be optimal, and vice versa. An optimum electric field strength and PEF time will produce more extracts. Based on the analysis of variance, it was found that the electric field strength and PEF time in the homogeneity test table had a significant value of 0.092 ( $p > 0.05$ ) which means that the data obtained is homogeneous data.

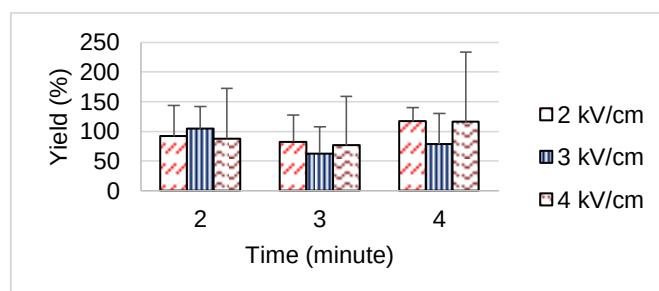


Figure 2. Effect of PEF time on the yield

Based on research data of total phenol test results, it is known that the best results with the greatest total phenol value is extract sample with a voltage of 4 kv/cm for 2 minutes with a total phenol value of  $1.664 \pm 0.226$  mg GAE/mg dry extract. While the lowest total phenol value was extract sample with a voltage of 2 kv/cm for 4 minutes in which the total phenol yield was  $1.111 \pm 0.249$  mg GAE/mg dry extract. Standard deviation is obtained with a range of 0.098 - 0.616. The graph of the effect of PEF time on the total phenol content can be seen in Figure 3. It shows the PEF time has a different effect on the total phenol in each sample.

The duration of PEF pre-treatment is one of the factors that can determine the amount of total phenol contained in an extract material. PEF which has a working principle by contacting an electric shock with a high voltage will have an impact on the material. The material given by the contact will respond by opening the pores on the cell membrane. The pores that are opened can be permanent or temporary depending on the length of time used. Open pores will cause compounds such as phenols and flavonoids in the membrane to diffuse with a solvent so that they can increase the levels of these compounds. The longer the time used in PEF, the pores on the material cell membrane will be more open and will be permanent. Whereas if the time of PEF is given only in a short time, it can cause pores that are open only temporary which means that the pores of the material will be closed and block the release of compounds in the membrane. But the content of the active compound can decrease in value when it is too long to be contacted at high stress. As a result, if the pre-treatment process takes too long to use PEF on an ingredient, the value of the active compound will decrease when the material has passed the optimal point of the desired time.

This is reinforced by the research of Zderic et al. [5] who conducted tea leaf research using PEF time. The optimal length of PEF for extraction is 1.5 seconds with an electric field strength of 0.9 kv/cm with an extraction value of 26.6%. While the extraction value decreases at 2.5 seconds. PEF application in extraction shows that PEF has an effect on cell membranes. The electric field strength given also has an effect on the total phenol content as shown in Figure 4. It can be seen the total value of phenol increases linearly. The best electric field strength is 4 kV/cm. The higher the electric field strength that is contacted, the higher the total phenol content. Electric field strength is one of the factors that influence the destruction of membrane cell tissue [9]. The electric field strength given to the material will occur electroporation process on the membrane so that the membrane cell wall will weaken and open so that the active substance in the membrane cell will diffuse with the solvent. If the electric field strength given is greater, then the cell membrane will be difficult to withstand the stress applied so that the higher the field strength is given, the cell wall will quickly open so that in a short time the substances contained in the cell membrane will diffuse. In the study of Lopez [10] who used PEF electric field strength as a variation of the research, it was obtained that with increasing electric field strength, the total value of phenol produced would also be greater. The strength of the electric field and the different length of time will cause differences in the results of the total phenol compound. The difference in results on the electric field strength depends on how high the permeability of the cell membrane electroporation is formed. If the electric field strength is used optimally, the compound that diffuses into the solvent is also more optimal and increase the level of the compound [11].

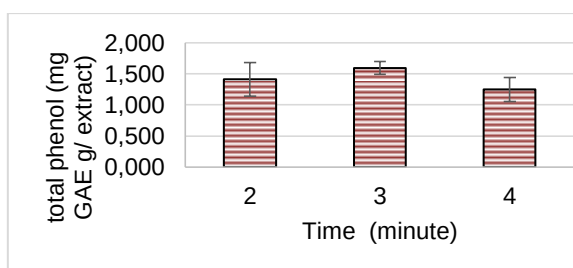


Figure 3. PEF time on total phenol content

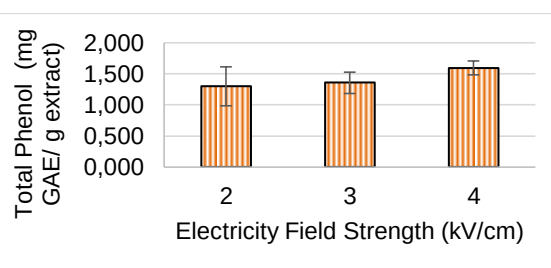


Figure 4. The Electricity fields on total phenol

The comparison between PEF result and control on total phenol can be seen in Figure 5. The results of testing the antioxidant activity of banana peel extract were further analysed by Analysis of Variance (ANOVA) at 5% confidence level. Based on the analysis of variance, it was found that the electric field strength and PEF time on the homogeneity test table had a significant value of 0.115 ( $p > 0.05$ ) which means that the data obtained is homogeneous data.

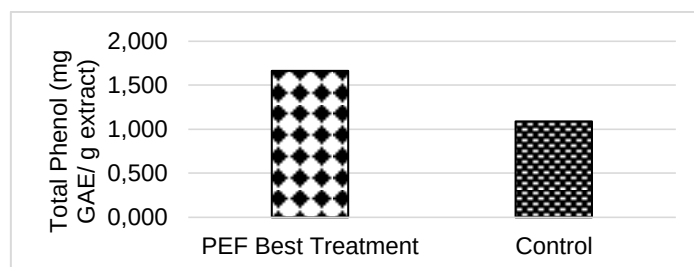


Figure 5. Comparison of best treatment (PEF) with total controlled content of phenol

The best antioxidant activity value is the result that has the lowest value of antioxidant activity ( $IC_{50}$ ). So that it can be said that the best results of antioxidant activity are extracts with a strong electric field treatment of 4 kv/cm for 4 minutes. A value of  $13.086 \pm 4.547$  mg/ml means that every  $13.086 \pm 4.547$  mg/ml of Kepok banana skin can counteract 50% free radicals. The smaller the  $IC_{50}$  value of a material, the stronger the antioxidant will be, because it only requires a small amount of Kepok banana skin to counteract 50% free radicals. The error bar value obtained in the results has a range of standard deviation values of 0.989 - 9.129. The graph of the effect of PEF time on the total phenol content can be seen in Figure 6. The longer the PEF time the  $IC_{50}$  value is higher, which means the longer the PEF time the antioxidant activity decreases due to the increase in  $IC_{50}$  value. The best  $IC_{50}$  value with a time of two minutes then decreases with increasing PEF time.

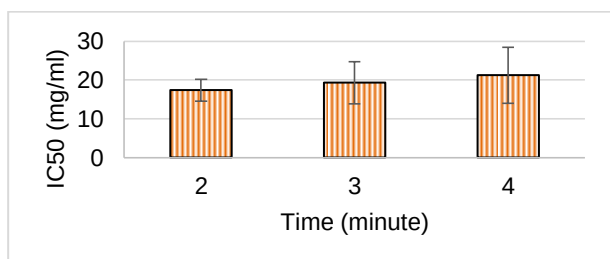


Figure 6. Effect of PEF time on  $IC_{50}$

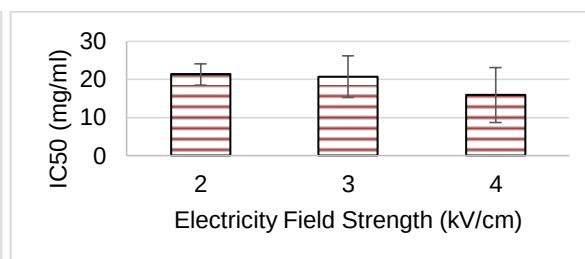


Figure 7. Effect of electric field on  $IC_{50}$

The results of antioxidant activity obtained in this study can be concluded to have antioxidant activity that is less active but still has potential as an antioxidant. Korma et al. [12] states that when treating the intensity of the time for too long, a stress reaction occurs which has the potential to increase the cell's defense mechanism by increasing the synthesis of secondary metabolites. The effects of cell membranes that are destroyed can occur permanently and cause cell death. These dead cells can reduce the levels of compounds contained in cells so that optimal time is needed when PEF to get the best  $IC_{50}$  results. The effect of electric field strength on  $IC_{50}$  value can be seen in Figure 7. The best  $IC_{50}$  value with an electric field strength of 4 kV/cm.  $IC_{50}$  values decrease linearly with increasing electric field strength. The higher the electric field strength given the  $IC_{50}$  value decreases which means that the better the antioxidant activity produced from the sample. Electric field strength and different lengths of time will cause differences in results on antioxidant activity. The difference in results on the electric field strength depends on how high the permeability of the cell membrane electroporation is formed. If the electric field strength is used optimally, the value of the compound that diffuses into the solvent is also more optimal and will increase the level of the compound [11].

Comparison of best PEF treatment and control can be seen in Figure 8. It can be said that the antioxidant activity content (IC<sub>50</sub>) in the sample using PEF (the best treatment) was better than the control sample (without PEF). Therefore, PEF pre-treatment can be used to increase antioxidant activity in Kepok banana skin compared to using maceration. This is because the electric field strength that is contacted causes electroporation on the membrane so that the compounds that come out are more. Based on the analysis of variance, it was found that the electric field strength and PEF time in the homogeneity test table had a significant value of 0.106 ( $p > 0.05$ ) which means that the data obtained is homogeneous data.

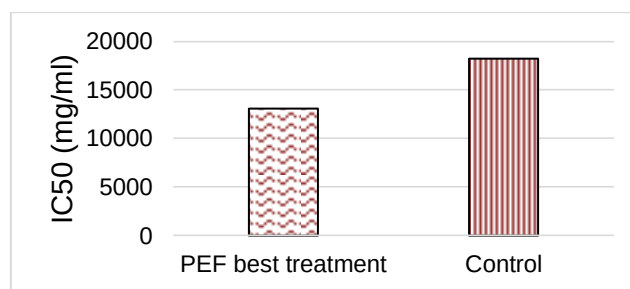


Figure 8. Comparison of IC<sub>50</sub> between best PEF treatment with control

#### 4. Conclusions

Pulsed Electric Field (PEF) method as a pre-treatment extraction process can improve the yield of Kepok banana peel extract by increasing the total phenol content and antioxidant activity in the extraction results. From the research, the best results of total phenol content with PEF were  $1.664 \pm 0.226$  mg GAE/g dry extract with antioxidant activity value of  $13.086 \pm 4.547$  mg/ml. This value is better than the control sample without PEF treatment where the total phenol content value is  $1.090 \pm 0.165$  mg GAE/g dry extract with an antioxidant activity value of  $18.231 \pm 3.392$  mg/ml. Total phenol content and antioxidant activity are better using PEF because the electric shock effect that is contacted to the material will cause electroporation on the cell membrane. The electroporation process will cause the cell membrane to open and the contents in the material will come out. The more optimal the field strength is used, the better the total phenol content and antioxidant activity.

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