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# Antioxidant Extraction from Pedada (*Sonneratia caseolaris*) with Pulsed Electric Field Pretreatment

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**ABSTRACT**

*Pedada (Sonneratia caseolaris) has great potential as a source of natural antioxidants. The high antioxidant content in pedada fruit requires an appropriate extraction method so that the extracted antioxidant levels in pedada fruit can be maximally extracted. However, the extraction of active compounds from pedada fruit is often limited by the dense cell structure. Pedada fruit extraction can be done using a modified maceration method with Pulsed Electric Field (PEF) pretreatment, so as to shorten the extraction time and require less solvent in the process. It is expected that PEF can damage the cell structure and increase the solubility of antioxidant compounds in the solvent. The quality of the resulting extracts will be evaluated based on total phenol content, total flavonoids, free radical scavenging activity, and antioxidant compound profile. The best treatment was obtained based on antioxidant analysis of the extraction results, namely at a PEF time of 2 minutes with $IC_{50}$ DPPH values of 18 ppm, $IC_{50}$ FRAP 37 ppm, total phenol content of 434 mgGAE/L, and total flavonoid content of 120 mgQE/L.*

## 1. INTRODUCTION

The Pedada (*Sonneratia caseolaris*) is often consumed by coastal communities in Indonesia as one of the food ingredients that can be processed into various processed food products. In addition, pedada fruit is also rich in antioxidants (Jariyah *et al*., 2014). Processed food products such as pedada peel steeping are considered to have potential as functional beverages because they show a percent inhibition of antioxidant activity of 12.10%. In addition, the Pedada fruit jelly candy product has a percentage inhibition of antioxidant activity of 67.34% (Ramadani *et al*., 2020). Bioactive components contained in pedada fruit include alkaloid compounds, flavonoids, glycosides, saponins, and phenols (Prastiwi *et al*., 2023).

The pedada, with its naturally sour taste due to the presence of ascorbic acid, can be considered a valuable natural antioxidant for food products. Antioxidant substances are compounds that can protect a product, especially fatty food products, from oxidation reactions, such as oxidative rancidity. The high water content of up to 79% causes the pedada fruit to rot easily (Febrianti, 2010), so it needs to be processed so that the pedada fruit can be utilised properly and can be used as a food source.

Free radicals are foreign compounds that enter the body. The immune system can be damaged by free radicals, which are harmful substances affected by radiation, pollution, chemicals, and toxins, as well as foods fried at high temperatures and fast foods (Amin, 2013). Excessive levels of free radicals can damage proteins and lipids, two fragile components, and lead to degenerative diseases. Therefore, antioxidants are essential to prevent and treat these diseases because they protect the body's cells from the damage that free radicals can cause to macromolecules such as proteins, lipids, and nucleic acids (Helmi *et al*., 2021).

To extract the antioxidant levels and bioactive compounds from pedada fruit to their maximum potential, the right extraction method is crucial. One method that is quite often used is the maceration extraction method. However, maceration has the disadvantages of a long extraction time, a lot of solvent, and producing low yields. Pedada fruit can be extracted faster and with less solvent by using a modified maceration method that includes a Pulsed Electric Field (PEF) pretreatment (Dewi *et al*., 2019). Pulsed Electric Field (PEF) extraction is one of the modern extraction techniques that can be done. Breaking down plant cell walls can increase their porosity and produce active chemicals. The application of Pulsed Electric Field (PEF) can be used to break down plant tissue or cell walls with relatively minimal damage (Rahman *et al*., 2023).

The application of the use of the PEF method of extraction shows a comparison of specific antioxidant activity values, such as in Dewi *et al*. (2019), which revealed that the highest increase in antioxidant value in the extraction of tobangun leaves with the PEF method with a voltage of 3 kV/cm showed the lowest $IC_{50}$ value of 0.98 µg/ml compared to only using the maceration method, which showed an $IC_{50}$ value of 151.21 µg/ml. The PEF technique can effectively extract phenolic compounds from Malacca fruit juice. When using a voltage of 18-24 Kv/cm along with heating, it achieved an antioxidant capacity of 94.83%. This is better than the method without PEF, which had an antioxidant capacity of 89.42% (Bansal *et al*., 2014). The application of PEF with heat treatment specifically enhances the extracted products' physical, chemical, and microbiological properties (Priyanto *et al*., 2022).

Based on this background, it is necessary to research the determination of pulsed electric field time used as a pretreatment for pedada fruit extraction that produces the best antioxidant activity.

## 2. METHODS

### 2.1. Materials and Tools

The materials used in this research include pedada obtained from Wonorejo Mangrove Farmers in Surabaya City and distilled water. Materials used for analysis are DPPH, distilled water, ethanol, Folin-Ciocalteu reagent, $Na_2CO_3$, gallic acid, quercetin, $NaNO_2$, $AlCl_3$, NaOH, 2,2-diphenyl-2-picrylhydrazyl (DPPH) reagent, methanol, TPTZ, $FeCl_3.6H_2O$, and acetate buffer (sodium acetate and acetic acid).

The tools used in the extraction of pedada are pulsed electric field (PEF) extraction tools, digital scales, and hot plates. The tools used for analysis are an analytical balance, test tubes, volumetric pipettes, a vortex, a 100 ml volumetric flask, a UV-Vis spectrophotometer, a pipette, an Erlenmeyer, filter paper, and a beaker glass.

### 2.2. Research Design

The preparation of pedada fruit extract with Pulsed Electric Field (PEF) pretreatment was carried out to determine the best treatment by collecting data on antioxidant parameters in the equation of Fua'ida (2016) on PEF pretreatment modelling antioxidant levels, namely, the analysis of antioxidant activity DPPH and FRAP methods, the analysis of total phenol content, and total flavonoid content (Dewi *et al*., 2019).

This research uses a simple, completely randomised design with one factor of PEF pretreatment, which has three levels: 1, 2, and 3 min, along with a control (boiled) sample that does not undergo PEF pretreatment. This results in a total of five different treatments. We repeated each treatment three times, resulting in 15 experimental units.

### 2.3. Research Procedure

The pedada fruits were sorted to remove any impurities or rotten fruits, and were then peeled and mashed to expand its surface. The PEF machine chamber was cleaned and sterilised. Pedada fruit of about 200 g was put into the PEF generator chamber, and was added with distilled water solvent with a predetermined ratio 1:4 (b/v).

The research variables dictated the use of an electric shock with a voltage of 18 kV/cm, a frequency of 8.197 kHz, and a pulse width of 66 µs at certain times. The research variables dictated the use of an electric shock with a voltage of 18 kV/cm, a frequency of 8.197 kHz, and a pulse width of 66 µs through increased cell membrane permeability, prevent the degradation of bioactive compounds due to heat or oxidative stress that may occur at too high a voltage,

and balance extraction efficiency without diluting the extract excessively so that it remains economical and effective. We further macerated the PEF extraction results by heating them on a hot plate for 30 minutes at 50°C. We filtered the pedada fruit filtrate using filter paper. We conducted antioxidant testing of the extraction results using control samples without any treatment, without PEF pretreatment, and with boiling pretreatment. The whole research procedures were presented in Figure 1.

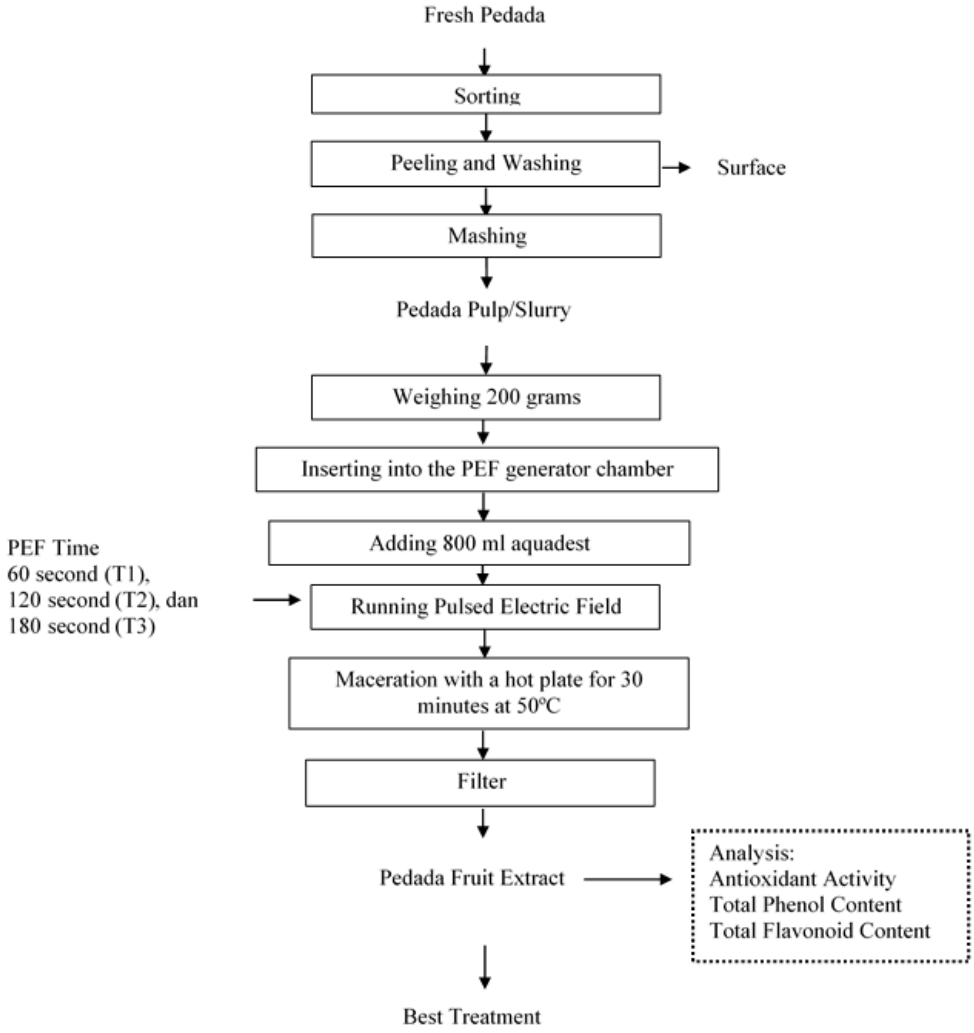


Figure 1. Flowchart of extract preparation process pedada fruit

### 2.4. Analysis of Pedada Fruit Extract with PEF Pretreatment

Analysis of pedada fruit extracts with PEF pretreatment was carried out in a time range fairly short, namely 0 minutes, 1 min, 2 min, and 3 min. Longer Pulsed Electric Field (PEF) treatments can make cell membranes more permeable. This helps release important compounds like phenolics and flavonoids faster. However, if the treatment duration is too long, these compounds are at risk of degradation. This is caused by the accumulation of electrical energy, which can raise the temperature and potentially damage sensitive molecules due to therm The research conducted by Nury *et al.* (2024) on the ultrasonic extraction of binahong leaves revealed that the final result is influenced by the combination of power and extraction time.although the ultrasonication method is used, the principle of parameter optimisation to prevent the degradation of bioactive compounds is similar to the approach applied in PEF.

Antioxidant Activity and flavonoid compounds are the main contributors to the antioxidant properties of pedada fruit (Avenido & Serrano, 2012). Pedada contains active compounds in the form of high phenols. This compound serves as the foundation for the bioactivity of pedada fruit, specifically its antioxidant capacity. Phenol and flavonoid compounds are the main contributors to the antioxidant properties of pedada fruit (Avenido & Serrano, 2012) activity test in this research used the determination of $IC_{50}$ DPPH (1.1-diphenyl-2 picrylhydrazyl) method (Fatminati *et al*., 2022) and $IC_{50}$ FRAP (Ferric Reducing Antioxidant Power) (Raharjo & Haryoto, 2019).

The difference between DPPH and FRAP tests for measuring antioxidant activity is based on the specific types of antioxidants present in the sample. In the FRAP test, the antioxidant activity results increase if the sample contains a high concentration of secondary antioxidant compounds. In the DPPH test, the active compounds work by grabbing free radicals, stopping the reaction chain by donating hydrogen (Nur *et al*., 2019). The analysis carried out on raw materials includes the determination of total phenol, total flavonoid, and antioxidant activity. The total phenol analysis used the Folin-Ciocalteu method (Routray & Orsat, 2014), while the total flavonoids by the spectrophotometric method (Chang *et al*., 2002).

### 2.5. Research Data Analysis

Antioxidant activity data were analyzed by linear regression using Analysis of Variance (ANOVA) when data showing differences were continued using Tukey and IBM SPSS Statistical 22.

## 3. RESULTS AND DISCUSSION

### 3.1. Raw Material Characteristics

We analyzed their antioxidant levels, total phenol levels, and total flavonoid levels. The analysis continued on antioxidant testing of pedada fruit extract with Pulsed Electric Field (PEF) pre-treatment to determine the best pre-treatment time obtained from the analysis of antioxidant activity levels, total phenol content, and total flavonoid content. The best results of pre-treatment (PEF) were used in the raw material for making effervescent powder. The results of pedada fruit analysis can be seen in Table 1.

Based on Table 1, antioxidant activity, total phenolics, and total flavonoids in fresh pedada fruit were analyzed. Measurement of antioxidant activity using the DPPH method showed an $IC_{50}$ value of 44.6±0.49 ppm. This value is higher than the results reported in the literature by Yulianis *et al*. (2015) which amounted to 24.59 ppm. According to Furi *et al*. (2020), plants with levels of total compounds higher phenolic have stronger antioxidant effects. flavonoid and This is due to the hydroxyl groups in flavonoid and phenolic compounds bound to carbon with conjugated double bonds, which makes it easier for hydroxyl groups to donate hydrogen atoms to free radicals. Therefore, the antioxidant activity of a plant increases with the amount of flavonoid and phenolic chemicals it contains.

Tabel 1. Pedada fruit analysis results at pedada to water ratio of 1:4

| **Parameters** | $IC_{50}$ DPPH (ppm) | $IC_{50}$ FRAP (ppm) | TPC (mgGAE/L) | TFC (mgEQ/L) |
|---|---|---|---|---|
| **Value** | 44.6±0.49 | 49.7±0.39 | 288.2±0.6 | 91.33±0.95 |

Notes: Data from the analysis is the mean of 3 replicates with ± standard deviation

The difference in $IC_{50}$ value in the results of antioxidant activity analysis of DPPH method is due to the literature using samples of pedada fruit extract with ethyl acetate fraction, so that the concentration of the material will be more concentrated and obtained more maximum analysis results. However, the results of the analysis on the pedada fruit that has been done still show a high level of antioxidant activity ability to inhibit free radicals, namely the $IC_{50}$ value of less than 50 ppm. In line with the statement of Rahmi (2017) that the compound $IC_{50} < 50$ ppm has a very strong antioxidant activity ability in counteracting the influence of free radicals.

In FRAP analysis with the determination of antioxidant content based on the reduction of $Fe^{3+}$-TPTZ compounds are represented as oxidizing compounds in the body that can damage or reduce the function of organs, while the pedada sample contains antioxidants that are read through spectrophotometric waves. Wang *et al*. (2014), say that the

$IC_{50}$ value shows the concentration of a sample needed to reduce free radical activity by 50%. The smaller the $IC_{50}$ value, the higher the antioxidant activity of the compound. This is because the less effective sample concentration used to counteract free radicals, the stronger the antioxidant activity. The antioxidant activity of a sample is said to be very strong if the $IC_{50}$ value is <50 µg/mL, strong if the $IC_{50}$ value is between 50-100 µg/mL, moderate if the $IC_{50}$ value is between 100-150 µg/mL, and is said to be weak if the $IC_{50}$ value is between 150-200 µg/mL (Molyneux, 2003). The results obtained $IC_{50}$ value of 49.72 ppm. So pedada fruit can be concluded that pedada fruit has a very strong antioxidant capacity.

Phenolic compounds are chemical components that affect the antioxidant activity of a material (Rupadani *et al*., 2013). Secondary metabolites contained in pedada fruit such as flavonoids can be used as a source of antioxidants (Indrawati & Blegur, 2021). In the analysis of pedada fruit raw materials, the total phenol content and total flavonoid content of pedada fruit were tested, the total phenol content was 288.2 mgGAE/L and the total flavonoid content was 91.33 mgEQ/L. This proves that pedada fruit is one of the plants that has the potential as an antioxidant, because this plant contains flavonoids and phenols (Avenido & Serrano, 2012).

### 3.2. Properties of Pedada Extract

Table 2 summarizes the results on the properties of pedada extract in terms of $IC_{50}$ DPPH, $IC_{50}$ FRAP, total phenolic, and total flavonoids. The table also inserts properties of pedada extract using only maceration followed by boiling.

Tabel 2. Analysis data of pedada extract under

| Treatment | $IC_{50}$ DPPH (ppm) | $IC_{50}$ FRAP (ppm) | Total Phenolic (mgGAE/ml) | Total Flavonoids (mgQE/ml) |
|---|---|---|---|---|
| **Boiling** | 48.40±1.0 | 77.94±0.14 | 4.31±1.19 | 15.08±1.57 |
| **0 min (T0)** | 44.22±0.14 $^{d}$ | 64.64±0.11 $^{c}$ | 294.53±0.34 $^{b}$ | 95.5±0.6 $^{b}$ |
| **1 min (T1)** | 20.52±0.47 $^{b}$ | 47.45±0.52 $^{b}$ | 370.37±0.91 $^{c}$ | 120±0.72 $^{c}$ |
| **2 min (T2)** | 18.66±0.15 $^{a}$ | 37.05±0.71 $^{a}$ | 434.05±1.24 $^{d}$ | 94.04±1.3 $^{d}$ |
| **3 min (T3)** | 21.47±1.8 $^{c}$ | 69.18±0.12 $^{d}$ | 255.609±1.5 $^{a}$ | 75±1.25 $^{a}$ |

Notes: Values followed by different letter notations in the same column indicate significant differences ($p<0.05$).

#### 3.2.1. Antioxidant Activity $IC_{50}$ DPPH

The working principle of DPPH testing is that the solvent extract becomes a donor of hydrogen atoms or electrons in the transformation of the DPPH reagent, so that the extract, as a donor, will destabilize the DPPH solution by converting the purple DPPH radical into yellow DPPH-H (Xiang *et al*., 2020). The working principle of FRAP testing is the ability of antioxidant compounds to reduce Fe ions, then the more $Fe^{3+}$ ion concentration is reduced, the greater the antioxidant activity (Kurniasari *et al*., 2022).

Antioxidant activity is expressed as percent inhibition ($IC_{50}$) or absorbance value compared to the antioxidant power of ascorbic acid ($IC_{50}$). Table 2 displays the average DPPH antioxidant activity of the pedada extract after PEF pretreatment. It can be seen in Figure 2, the increase in antioxidant activity is due to the extraction process using PEF. Based on Table 2, the average $IC_{50}$ values of DPPH antioxidant activity ranged from 18.66 - 48.40 ppm. The PEF pretreatment extract had better antioxidant activity on average than that of maceration and boiling method. Using PEF pretreatment to extract pedada fruit resulted in a higher average antioxidant activity than using maceration or boiling methods. The real effect was observed, along with an increase in the antioxidant activity of the pedada extract. This is in line with Calleja-Gomez *et al*. (2022), that the application of areca nut extraction using PEF showed an increase in the production of extracted bioactive components on antioxidant activity and total phenols.

Based on Table 2, the antioxidant activity value shows that the longer the exposure time to PEF will increase the antioxidant activity value of pedada fruit extract, which is indicated by a decrease in the inhibition value ($IC_{50}$). Statistical analysis of the $IC_{50}$ data produced an F-count value of 5740.22, higher than the F-table of 4.066, which implies that the PEF treatment resulted in a significant difference in the $IC_{50}$ value of the pedada fruit extract. The increase in antioxidant activity is directly proportional to the total phenol and flavonoid values (Table 2), which shows

that the optimal PEF exposure time is at 2 min, because the levels of phenol and flavonoid components decrease if the exposure time is increased. Dewi *et al*. (2019), found that the antioxidant activity level ($IC_{50}$) in torbangun leaf extract is influenced by the amounts of phenol and flavonoid compounds it contains. The higher the content of phenol and flavonoid compounds, the higher the antioxidant activity value and the smaller the $IC_{50}$ value.

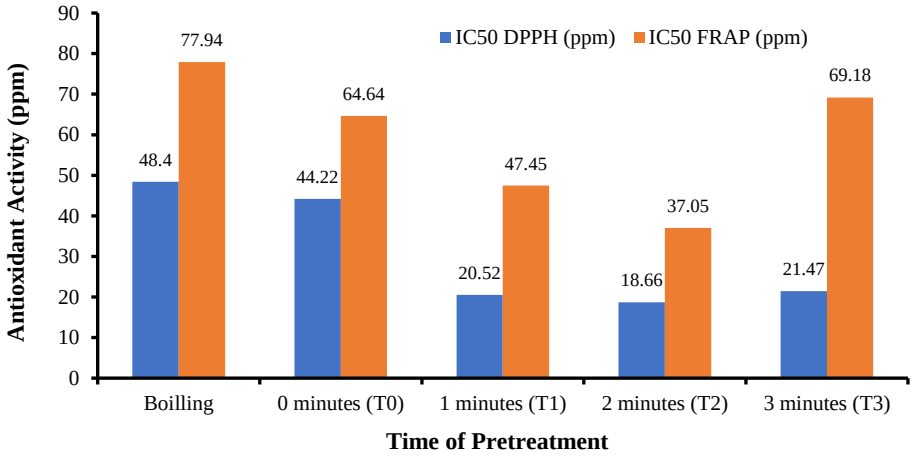


Figure 2. Antioxidant activity of pedada fruit extract under PEF pretreatment.

According to Ribeiro *et al*. (2009), the greater the PEF time imposed on the material, the higher the electric field strength produced. The increase in PEF exposure time given to the material results in an increase in specific input energy. This causes the energy received by the material to be greater, so the resulting membrane damage is also greater, causing a decrease in the $IC_{50}$ value, which means that the antioxidant activity of pedada extract with PEF pretreatment can increase compared to the analysis of fresh pedada fruit. Phenolic compounds obtained during the PEF pretreatment influence the lower $IC_{50}$ value or higher antioxidant activity (Dewi *et al*., 2019). The difference is clear when look at Table 2. In the study by Fatminati *et al*. (2022), they used a multistage maceration method for extraction. However, in this study, the extraction was done using a modified maceration method with PEF pretreatment. This shows that PEF pretreatment is able to increase the antioxidant activity better as seen from the lower $IC_{50}$ value when compared to the control treatment. Wang *et al*. (2014), explained that bioactive compounds in the extract can function as electron donors that convert free radicals into more stable ones.

The increase in $IC_{50}$ value or the decrease in antioxidant activity after 180 seconds of PEF pretreatment may be because the nutrients were damaged during the long extraction process, which reduced their antioxidant activity. This is because the phenol compounds that have been extracted are damaged for a long time so that it has an impact on the decrease in antioxidant activity. Dewi *et al*. (2019) mentioned that extraction using an electric field with an increasingly long time will produce a total phenol that decreases due to the strong electric field given can damage the phenol compounds that have been extracted. The graph of the relationship between PEF pretreatment treatment and antioxidant analysis in pedada fruit extract can be seen in Figure 2.

### 3.2.2. Antioxidant Activity $IC_{50}$ FRAP

The FRAP value is expressed in absorbance which is compared with the antioxidant power of ascorbic acid ($IC_{50}$). The average value of FRAP antioxidant activity of pedada extract with PEF pretreatment can be seen in Table 2 that demonstrates an increase in antioxidant activity as a result of the PEF pretreatment. The results (Table 2) show the average reducing power $IC_{50}$ value of the extracts ranged from 37.05 – 77.94 ppm. Statistically, the PEF extraction method gives a significant effect with F-count 3295.74, greater than F-table 4.066 at 5%. In general, reducing power of PEF increases the antioxidant activity of pedada fruit extract. Based on statistical calculations, the reducing power value of the PEF pretreatment treatment resulted in a higher $IC_{50}$ value compared to the DPPH method, which means that the lower antioxidant activity value is absorbed by Fe. The suspected influencing factor is that the voltage applied

is not suffic. The voltage applied is not sufficient to extract secondary metabolite compounds in pedada that act as metal chelators.

Phenolic compounds, which function as antioxidants, influence this difference.epancy. High flavonoid values show a correlation to the antioxidant activity of the DPPH method, because flavonoids can donate hydrogen atoms that can reduce the DPPH reagent. The compounds that play a role in FRAP antioxidants are phenols that can chelate metals. Fatminati *et al*. (2022), stated that the content of active substances in the most dominant pedada as an antioxidant is flavonoids. Therefore, measuring the value of antioxidant activity in pedada is more effective using the DPPH method.

The FRAP method's antioxidant activity value is associated with secondary metabolite compounds that function as antioxidants in plants. Poli *et al*. (2022), stated that the metabolite compounds have a major role, namely phenol compounds in pedada fruit. A'yunin *et al*. (2019), stated that the FRAP value is related to the presence of antioxidant compounds, such as total phenol compounds and total flavonoid components. Poli *et al*. (2022), stated that antioxidants have two types of antioxidant compounds based on their function, namely primary and secondary antioxidants. Djasibani *et al*. (2013) stated that the principle of secondary antioxidants is to reduce the rate of lipid autooxidation by binding metal ions, oxygen capture, decomposing hydroperoxides into non-radical products. Based on the results, it shows that the value of antioxidant activity reducing power $IC_{50}$ is efficient in the T2 treatment.

### 3.2.3. Total Phenolic

The increase in total phenol content (Figure 3) in the PEF pretreatment extraction treatment of pedada fruit compared to before extraction. This occurs due to the ability of compounds in contact with the solvent so that it is able to conduct electrical voltage as a result of which the substances contained are able to be extracted maximally. In line with the statement Izza *et al*. (2016), that the solvent will penetrate the cell wall and enter the cell cavity containing the active substance, so that the active substance will dissolve and will be pulled out along with the solvent. Pashazadeh *et al*. (2019), stated that the treatment of electric voltage 4 kV/cm on the pretreatment of cinnamon extraction showed the best value of phenol 518.60 mg/kg compared to the treatment of 2 kV/cm and 6 kV/cm on the value of phenol, with 409.20 mg/kg and 405.80 mg/kg. So that with the use of 18 kV/cm in this study produced higher phenol levels in the pretreatment of pedada extraction.

The total phenol value dropped during the 3-minute extraction process because the high electric voltage damaged the phenol compounds. In addition, the length of extraction time can also reduce the total phenol value due to oxidation reactions. Dewi *et al*. (2019), mentioned that extraction using an electric field with an increasingly long time will produce a total phenol that decreases due to the strong electric field given can damage the phenol compounds that have been extracted. Komala & Husni (2021), stated that the nature of phenol compounds, apart from being thermosensitive, is also easily oxidized. Long extraction time increase the chance of oxidation reactions of phenolic compounds due to the contact of phenol compounds with environmental oxygen. According to Asshidiqy *et al*. (2020), giving a

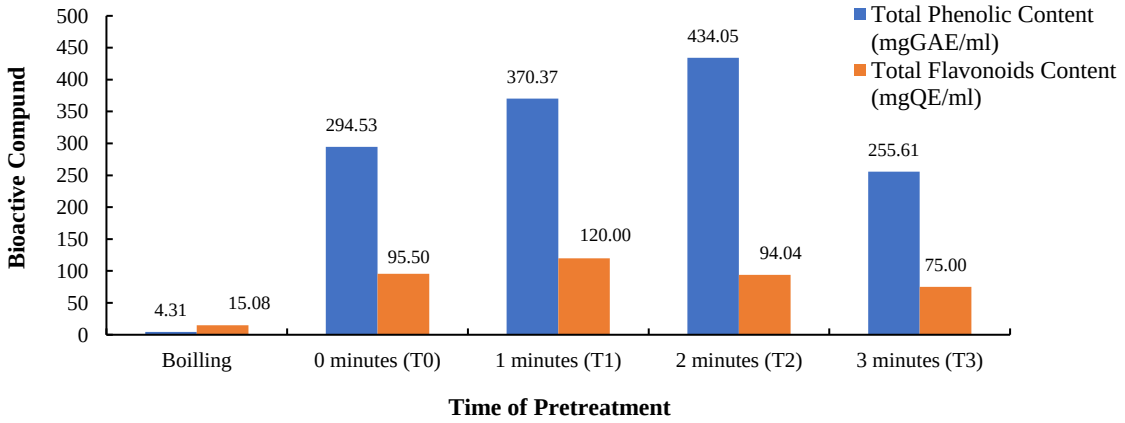


Figure 3. Graph of bioactive components in pedada fruit extraction with PEF pretreatment.

long extraction time and high electric shock treatment will disrupt the process of cell self-defense mechanisms in the material.

The increase in total phenol content can be caused by the destruction of the cell wall in the pedada which causes mass transfer from the material to the solvent. The results show that the PEF treatment produces more phenol than the traditional squeezing method. We can conclude that the PEF extraction method efficiently extracts pedada into total phenol. Siemer (2014), stated that the PEF method to increase the diffusion rate of target compounds out of plant tissue when extracted by exposure to high electrical voltage on the tissue which can result in rupture of the plant tissue cell membrane. Safithri *et al*. (2020) noted that the tissue pores will become larger because of the electroporation process on the cell membrane when an electric charge is applied. The larger the pore in the cell makes the solvent can easily enter the deepest tissue and the compound can also be dissolved in the solvent.

The results show that the PEF method can have an effect on increasing the total phenol content of pedada extraction. Analysis of variance (ANOVA) on the phenolic content data produced an F-count value of 15,855.40, higher than the F-table of 4.066, which implies that the PEF treatment resulted in a significant difference in the phenolic cotent of the pedada fruit extract. The highest total phenol content of 434 mgGAE/ml is resulted with PEF treatment for 2 min. This is in line with the research of Liu *et al*. (2014), that using the PEF method is used as a pretreatment of shallot extraction of phenol components followed by extraction by distillation method. The extraction results using pretreatment show the value of the phenol component 102.86 mg GAE/100g is higher than without PEF pre-treatment treatment of the total phenol component 32.13 mg GAE/100g. Calleja-gomez *et al*. (2022), reported that the results of pre-treatment using PEF showed an increase in phenol and mineral components of mushroom material. The total phenol value increased by 96.86% compared to the conventional method (constant stirring).

### 3.2.4. Total Flavonoids

In the research results, the highest value of phenol is directly proportional to the highest value of flavonoids. In line with the statement of Dewi *et al*. (2019), revealed that the higher the flavonoid content, the higher the extracted phenol content. This is due to the optimal extraction of flavonoid compounds, which occurs when the extraction time is longer. The higher the voltage and the longer the extraction time, the more active the movement of ions will be, so that the pores of the cell wall will be wider and cause the tannin compounds to be obtained in greater quantity and quality.

Analysis of variance (ANOVA) on the flavonoids content data produced an F-count value of 15.948, higher than the F-table of 4.066, which implies that the PEF treatment resulted in a significant difference in the flavonoids content of the pedada fruit extract. It can be seen that the highest total flavonoid content of 120 mgQE/ml is in PEF treatment for 1 minute. In line with Putranto *et al*. (2022), that Pulsed Electric Field is able to put pressure on cell membranes which results in the transfer of bioactive compounds contained in pedada fruit to the solvent. However, there was a decrease in value at a PEF time of 2 minutes, this is because at that time the antioxidants extracted out of the cells began to be damaged as well as flavonoid compounds. This is in accordance with the research of Góngora-Nieto *et al*. (2003), where the increase in PEF exposure time is too large, the energy received by the material exceeds the threshold so that some of the content is lost or degraded.

## 4. CONCLUSION

Pulsed Electric Field (PEF) pretreatment was shown to affect the $IC_{50}$ value of antioxidant activity, total phenol content, and total flavonoid content of pedada fruit extract. PEF treatment as a pretreatment was able to increase antioxidant activity better as seen from the lower $IC_{50}$ value when compared to the control treatment because the bioactive compounds in the extract can function as electron donors that convert free radicals into more stable ones so that there is an increase in the production of extracted bioactive components towards antioxidant activity and total phenols. The best treatment obtained from this study is the 2-minute PEF pretreatment time (T2) which is the best antioxidant treatment with $IC_{50}$ value of DPPH method of 18.66 ppm, $IC_{50}$ value of FRAP method of 37.05 ppm, total phenol content of 434.05 mgGAE/mL, while the 1-minute PEF pretreatment time treatment (T1) produces the highest total flavonoid content of 120 mgQE/mL. Further research needs to be done on the analysis of antioxidant activity in mangrove fruit with other types so that it can be used for alternative and commercialized in the future food.

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