The role of pulsed electric field (PEF) with thermocontrol modification as an elicitor to increase bioactive compound in local soybean

J M Maligan, M W Fitria and W D R Putri

Department Agricultural Product Technology, Agricultural Technology Faculty, Brawijaya University Jl. Veteran, Malang 65145, East Java-Indonesia

Email: maharajay@gmail.com

Abstract. Soybean is an agricultural commodity, being third in harvest amount after paddy and corn. The processing of soybean using heat causes the bioactive compounds to decrease. There is another way to increase the bioactive compounds in soybeans. Elicitation is a method that functions to increase biosynthesis of secondary metabolite formation in plants by using elicitor. This research used Factorial Randomized Block Design method with 2 factors which were temperature (25 °C, 35 °C and 45 °C) and pulsed field (3 kV, 5 kV and 7 kV). Electrical voltage test result from device obtained input voltage 0.76 V resulted 3 kV, 1.04 V resulted 5 kV, 1.4 V resulted 7 kV. Research results on soybean extract obtained total phenol as much as 2.76 mg GAE/g, total flavonoid 0.31 mg QE/g, tannin content 0.18 mg/g, IC50 as much as 626.95 ppm. Whereas for the results of extraction of soybeans in the best treatment was obtained at the pulsed field of 3 kV and temperature of 25 ºC. Total phenol result was 2.92 mg GAE/g, total flavonoid 0.37 mg QE/g, tannin content 0.19 mg/g, antioxidant activity IC50 128.84 mg/L. The voltage and temperature treatment is given gave a significant difference in effect (p < 0.05) and interaction on all parameters. The best treatment in this study was obtained at a value of 3 kV and a temperature of 25 ºC with characteristics of total phenol of 2.92 mg GAE/g, flavonoids of 0.37 mg QE/g, tannin of 0.19 mg TA/E/g and antioxidant activity IC50 of 128.84 ppm. In the results of this study, there was no elicitation process because there were no research steps that showed that the elicitation process could occur.

1. Introduction
Soybean in Indonesia are strategic food commodities after rice and corn. The average consumption of soybeans reaches 8.12 kg/capita/year [1]. Soybeans are rich in nutrients and bioactive compounds that function as antioxidants such as isoflavones and flavonoids [2]. The processing of soybeans using heat can reduce bioactive compounds in soybeans. During the boiling process the isoflavone content was reduced by 46% from its origin [3]. There is an effort made to increase bioactive compounds in soybeans. So that soybeans can be produced which still contain high bioactive compounds after processing. One method that can be used is elicitation.

Elicitation is a method that function to increase the biosynthesis of secondary metabolite formation in plants using elicitor. Elicitor is a medium that can increase the formation of certain compounds when entered in living cell systems [4]. Elicitors given to plants will be considered as foreign objects, then
plants will produce secondary metabolites used for its defense [5]. Pulse Electric Field (PEF) is a non-thermal method that uses electricity at high voltages (20–80 kV) [6].

There are several studies that have been conducted with the use of electric shock as an elicitor. In a study conducted by [7] the use of electric shock with a power of 1 kV, 3 kV and 5 kV can increase antioxidant activity in berries with an increase of about 30% and a total phenol of about 40%. The use of electric shock can increase the carotenoid content of tomato skin by about 39% at a power of 5 kV [8]. Based on these data it can be concluded that the electric shock method can increase the number of secondary metabolites in plants by providing environmental conditions that can force plants to secrete secondary metabolites.

Temperature can affect texture, nutritional value and antioxidant capacity of vegetables and allow negative effects on the quality parameters of vegetables, but in some experiments, antioxidant activity even increases after heat treatment [9]. For now there is little information related to elicitation research that combines temperature and electric shock as a medium of elicitation. This can be a method that can be applied to further research. Therefore, this study was conducted by combining the electric shock method and temperature control to increase the content of bioactive compounds in soybeans in the elicitation method.

2. Materials and Method

2.1. Materials

Anjasmoro varieties of soybeans with a diameter of about 0.5 cm from BALITKABI Malang, aquades, Folin Ciocalteau reagents, Na$_2$CO$_3$, NaOH, NaNO$_2$, AlCl$_3$, DPPH reagents (diphenyl picrylhydrazyl), 96% ethanol, gallic acid standard solution, standard tannic acid solution and standard solution quercetin.

2.2. Tools

PEF devices with temperature variations, aluminum foil, fine filter paper, suction ball, glassware (measuring cup, erlenmeyer, volume pipette, alarm cup, test tube, dropper pipette), shaker (Orbital Genie), vortex (LW scientific), blender (Miyako) and UV-Vis (Shimadzu) spectrophotometer.

2.3. Method

In this study used the Random Group Combination Method (RBD) with 2 treatment factor variables, electric shock time and temperature produced. Electric shock voltage variables are divided into 3 kV, 5 kV, and 7 kV, while for temperature variables include 25 °C, 35 °C and 45 °C using 1 minute. From the combination of treatment factors 9 treatments were obtained with each treatment repeated 3 times so that 27 treatments were produced.

2.4. Total phenol analysis

0.5 ml of sample is taken, put into a test tube. Added 2.5 ml of the Folin-Ciocalteau reagent (diluted 1:10) then incubated for 5 minutes. Added 2 ml of 7.5% Na$_2$CO$_3$ solution and vortex. Incubated for 30 minutes room temperature in dark conditions. Measured absorbance at wavelength (λ) 756 nm. Calibrated with gallic acid standard curve to obtain total phenol in μg GAE/ml. Total phenol was calculated in μg GAE/g with the equation [10].

2.5. Flavonoid analysis

The sample to be tested measured 1 ml volume and then weighed its mass. Added 2 ml of distilled water in each test tube. 0.3 ml of 5% NaNO$_2$ solution was added then vortexed and incubated for 6 minutes. Add 0.3 ml of 10% AlCl$_3$ solution and wait for 6 minutes. Added 4 ml of 1 M NaOH solution and 2.4 ml of distilled water and then vortexed. Incubated for 15 minutes at room temperature in dark conditions. Measured absorbance at wavelength (λ) 502 nm. Calibrated with the standard curve equation to obtain the total flavonoids [11].
2.6. Tannin analysis
1 ml of the filtrate sample is taken and then 0.5 ml of follin-ciaocalteau is added. At vortex is then incubated for 5 minutes. Added 1 ml of 20% Na2CO3 and 2.5 ml of distilled water at vortex is then allowed to stand for 30 minutes. Absorbance measured at a wavelength of 775 nm [12].

2.7. Antioxidant activity IC50 analysis
2 ml of DPPH 0.2 nM solution is then taken and 7 ml of methanol is added. In vortex to homogeneous. Leave for 30 minutes in a dark room. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 521 nm. The procedure for analyzing the antioxidant activity of DPPH IC50 method is: The sample weighed 0.5 gram. It is dissolved with distilled water until the volume reaches 50 ml. Diluted to reach concentrations of 7500, 5000 and 2500 ppm. 1 ml sample was taken at each concentration then reacted with 7 ml of methanol and 2 ml DPPH of 0.2 nM. In vortex to homogeneous. Leave for 30 minutes in a dark room. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 521 nm. The antioxidant activity of each sample at each dilution is expressed by the percentage of free radical inhibition calculated by the equation:

% Antioxidant: The ability of antioxidants to reduce free radicals (%)
Absorbance blank: Absorbance value of the blank solution
Sample absorbance: The absorbance value of the sample solution
Then a relationship curve is made between the concentration of the sample (x) and % antioxidant activity (y). Then the regression equation and R2 are calculated. You will get the equation

\[ y = ax + b \]

where
\[ y: \text{50 (provision)} \quad x: \text{IC}_{50} \quad (\text{mg/ml}) \]

From this equation we can find the value of IC50 (x) which shows the ability of the extract to ward off 50% of free radicals [13].

3. Result and discussion

3.1. Characteristics of raw materials
The raw material used is Anjasmoro variety soybeans obtained from Balitkabi Malang Regency. Analysis of raw materials was used to determine the bioactive content of raw materials in the literature and compared with the results of sample testing. The raw material content is presented in table 1. The results of testing total phenol in raw materials showed a difference. The difference in results can be caused by material conditions in the form of varieties, environmental conditions [14].

| Parameter              | Raw Material | Literature |
|------------------------|--------------|------------|
| Total Phenol (mg GAE/g) | 2.76 ± 0.48  | 3.49 [11]  |
| Flavonoid (mg QE/g)    | 0.31 ± 0.05  | 0.22 [24]  |
| Tanin (mg TAE/g)       | 0.18 ± 0.01  | 0.88 [11]  |
| Antioxidant IC50(ppm)  | 167.3 ± 25.31| 77.72 [15] |

3.2. Total phenol
Total phenol content in soybean extracts raw materials using a combination of electric shock and temperature have a value in the range of average 1.91–3.16 mg GAE/g. The mean value on the results of the analysis of the extracts that have been given the treatment listed in table 2.
Table 2. Average total phenol of soybean extract with the influence of voltage and temperature.

| Voltage (kV) | Temperature (°C) | Flavonoid Value (mg GAE/g) | DMRT 5% |
|--------------|------------------|---------------------------|---------|
| 3            | 25               | 2.92 ± 0.03<sup>a</sup>   | 2.08    |
|              | 35               | 2.59 ± 0.08<sup>ab</sup>   | 2.17    |
|              | 45               | 1.91 ± 0.02<sup>abc</sup> | 2.23    |
|              | 25               | 2.53 ± 0.014<sup>abc</sup>| 2.25    |
| 5            | 35               | 2.21 ± 0.03<sup>bcd</sup>| 2.47    |
|              | 45               | 1.76 ± 0.01<sup>abc</sup>| 2.54    |
|              | 25               | 2.22 ± 0.19<sup>def</sup>| 2.65    |
| 7            | 35               | 2.06 ± 0.08<sup>g</sup>   | 2.86    |
|              | 45               | 1.61 ± 0.06<sup>h</sup>   | 3.22    |

Table 2 shows the highest total phenol value obtained at the treatment voltage of 3 kV with a temperature of 25 °C which has a value of 3.32 mg GAE/g. Whereas for the lowest total phenol value is shown in the treatment of 7 kV with a temperature of 45 °C which has a value of 1.91 mg GAE/g.

The total value of phenol at 3 kV, 5 kV and 7 kV voltage continues to decrease with increasing temperature. The highest drop value is obtained at 7 kV with a temperature of 45 °C. This can be caused because the higher the voltage given can make the pores in the cell wider so that the content in the cell will come out. Cells that experience damage may be able to experience further weakening due to high temperatures. Electric shocks that is given to materials can cause the disintegration of cells or cell pores dilation so that it can issue a compound that is found in these cells [15]. The higher the voltage provided to a cell cause damage that cannot restore the state of the cells so that irreversible damage occurs [16]. The total value of phenol with a voltage of 7 kV has a higher decrease compared to the voltage of 3 kV and 5 kV. This is probably due to the higher voltage, the pores of the cell membrane getting bigger which causes the contents of the cell to come out from inside the cell to the conductive medium of electricity.

3.3. Flavonoid

The content of flavonoids on the raw materials of soybean extract using a combination of electric shock and temperature has an average value in the range of 0.344–0.383 mg QE/g. The average value of the extract analysis results that have been given treatment is listed in table 3.

Table 3. Average flavonoid of soybean extract with voltage and temperature effect.

| Voltage (kV) | Temperature (°C) | Flavonoid Value (mg GAE/g) | DMRT 5% |
|--------------|------------------|---------------------------|---------|
| 3            | 25               | 0.37 ± 0.008<sup>a</sup>| 0.873   |
|              | 35               | 0.35 ± 0.004<sup>b</sup>| 0.928   |
|              | 45               | 0.33 ± 0.01<sup>c</sup>| 0.950   |
|              | 25               | 0.36 ± 0.003<sup>d</sup>| 1.021   |
| 5            | 35               | 0.33 ± 0.003<sup>cd</sup>| 1.027   |
|              | 45               | 0.30 ± 0.003<sup>f</sup>| 1.046   |
|              | 25               | 0.34 ± 0.003<sup>ef</sup>| 1.080   |
| 7            | 35               | 0.31 ± 0.002<sup>h</sup>| 1.106   |
|              | 45               | 0.28 ± 0.001<sup>i</sup>| 1.142   |

Table 3 shows the total value of the highest phenol obtained at 3 kV voltage treatment with a temperature of 25 °C which has a value of 3.32 mg of GAE/G. As for the total value of the lowest phenol is shown in the treatment of 7 kV with a temperature of 45 °C which has a value of 1.91 mg of GAE.
Flavonoids are compounds consisting of a group of phenols so indirectly when the total measurement of phenols indicates a decrease then the likelihood of a flavonoids value indicates a decrease [17]. The increase in water temperature allows for easier release of flavonoids because the flavonoids are polar and the warming influence can reduce the content of flavonoids in the ingredients [18]. The voltage and temperature given are likely to cause the flavonoids that are inside the outgoing and dissolved cells. This is likely to be caused by weak flavonoids against several factors that affect it. At the value of flavonoids decreased as the voltage and temperature values increase. The little interaction happens and not too significant. The interactions that occur can be seen at 35 °C temperature and 5 kV voltages are experiencing a higher decline than both treatments. This can be due to the time the voltage and temperature of the cell have been in a ruptured state and causing the flavonoids from the cell out while at a voltage of 7 kV suffered fewer decreases due to the likelihood of flavonoids content has come out with a lot so that it decreases lower.

3.4. Tanin
Content of tannins in the raw materials of soybean extract using a combination of electric shock and temperature has an average value in the range of 0.104–0.190 mg TAE/G. The average value of the extract analysis results that have been given treatment is listed in table 4.

| Voltage (kV) | Temperature (°C) | Tannin Value (mg GAE/g) | DMRT 5% |
|-------------|------------------|-------------------------|---------|
| 3           | 25               | 0.19 ± 0.011<sup>a</sup> | 0.873   |
|             | 35               | 0.18 ± 0.004<sup>b</sup> | 0.928   |
|             | 45               | 0.14 ± 0.005<sup>bc</sup> | 0.950   |
| 5           | 25               | 0.21 ± 0.014<sup>bcd</sup> | 1.021   |
|             | 35               | 0.20 ± 0.005<sup>c</sup> | 1.027   |
|             | 45               | 0.19 ± 0.007<sup>ef</sup> | 1.046   |
| 7           | 25               | 0.15 ± 0.011<sup>fg</sup> | 1.080   |
|             | 35               | 0.13 ± 0.012<sup>gh</sup> | 1.106   |
|             | 45               | 0.10 ± 0.003<sup>hi</sup> | 1.142   |

In table 4, the highest tannins value obtained at 5 kV voltage treatment with a temperature of 25 °C which has a value of 0.217 mg TAE/g. As for the lowest tannins value is shown in the treatment of 7 kV with a temperature of 45 °C which has a value of 0.109 mg TAE/g. Tannins have properties that bind to other compounds allowing form complex compounds that are difficult to separate and tannins can be dissolved in the water [19]. The increase in the tannins value that occurs from the voltage of 3 kV to 5 kV may be caused due to the separation of tannins compounds with other components residing in soybeans during 5 kV voltage. The highest decrease in tannins value is at 7 kV voltage and 45 °C temperature which is likely due to higher voltage and temperature and more than 5 kV can remove the tannins content contained in the cell and given style because Two factors can cause greater tannins damage.

3.5. Antioxidant activity $IC_{50}$
The value of antioxidant activity in the raw materials of soybean extract using a combination of electric shock and temperature has an average value at the range of 122.175–381.21 ppm. The average value of the extract analysis results that have been given treatment is listed in table 5.
Table 5. Average antioxidant activity IC₅₀ of soybean extract with voltage and temperature effect.

| Voltage (kV) | Temperature (ºC) | Average Antioxidant Activity IC₅₀ (mg GAE/g) | DMRT 5% |
|--------------|------------------|---------------------------------------------|---------|
| 25           | 128.84 ± 4.53 a  | 156.368                                     |         |
| 3            | 191.96 ± 2.82 b  | 220.833                                     |         |
| 45           | 215.71 ± 10.71 bc| 257.996                                     |         |
| 25           | 197.91 ± 6.04 bcd| 245.985                                     |         |
| 5            | 226.99 ± 4.40 bcde| 248.572                                    |         |
| 45           | 295.31 ± 41.03 cdef| 257.996                                    |         |
| 25           | 286.44 ± 6.23 gh | 326.738                                     |         |
| 7            | 354.54 ± 12.79 i | 386.100                                     |         |

Table 5 describes the value of the highest IC₅₀ antioxidant activity obtained at 3 kV voltage value treatment with a temperature of 25 ºC which has a value of 128.84 ppm. As for the value of the lowest antioxidant activity IC₅₀ obtained at 7 kV voltage value treatment with a temperature of 45 ºC which has a value of 354.54 ppm.

The higher the temperature and voltage given the obtained results showed a decrease in antioxidant activity. The resulting graphs can be said to be linear with the results of the total testing of phenols and flavonoids. Due to the results of the total testing chart of phenol and flavonoids decreased as the voltage and temperature are given. Antioxidant activity is influenced by the content of phytochemical compounds in the material. The larger the phytochemical compounds of the phenol may be the higher value of its antioxidant activity [20]. In addition, the flavonoids compounds are thought to affect the value of antioxidant activity on a substance [21].

At a voltage value of 5 kV with a temperature of 25 ºC and 35 ºC have a higher increase value than the voltage value of 3 kV and 7 kV. This can be caused due to the voltage of 5 kV with a temperature value of 25 ºC and 35 ºC the cell content that comes out has undergone a saturated point so that at a voltage of 7 kV experience a lower increase than the voltage of 5 kV. At a voltage of 7 kV with 45 ºC temperature has a higher increase than the voltage 3 kV and 5 kV because it can be caused by the higher the value of voltage and the temperature value given then there are two styles (voltage and heat) that affect the condition in the cell so that it can suffer greater damage.

3.6. The best treatment

Determination of the best treatment on the soybean extract with this voltage and temperature treatment using the Zeleny method. Based on table 6, the best treatment result of soybean extract with voltage and temperature treatment found at 3 kV treatment and 25 ºC temperature. This is likely due to the occurrence of the elicitation process and there is an ability to withstand less high temperatures in the sample. When the voltage and temperature begin to increase there is a possible decline due to the weakening of the cell endurance due to both treatment given.
Table 6. Best treatment of soybean extract.

| Voltage (kV) | Temperature (°C) | Lmax    | Total   |
|-------------|------------------|---------|---------|
|              | 25               | 0.01716 | 1.013   |
| 3           | 35               | 0.17599 | 1.142   |
| 45          |                  | 0.38144 | 1.234   |
|              | 25               | 0.12488 | 1.102   |
| 5           | 35               | 0.20146 | 1.167   |
| 45          |                  | 0.29653 | 1.252   |
|              | 25               | 0.25604 | 1.212   |
| 7           | 35               | 0.34852 | 1.296   |
| 45          |                  | 0.43654 | 1.381   |

4. Conclusion
The voltage and temperature treatment gave significant differences effect (p < 0.05) and interaction on all parameters. The best treatment in this study was obtained at a value of 3 kV and a temperature of 25 °C with characteristics of total phenol of 2.92 mg GAE/g, flavonoids of 0.37 mg QE/g, tannin of 0.19 mg TAE/g and antioxidant activity IC50 of 128.84 ppm. In the results of this study, there was no elicitation process because there were no research steps that showed that the elicitation process could occur.

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