Analysis of pulsed electric field (PEF) specific input energy and its effect to the tannin content of Areca (*Areca catechu* L.) seed powder extract

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Abstract. *Areca (Areca catechu L.)* is one of the plants that contain phenolic compounds as tannin as antioxidant. Pre-treatment prior plant extraction using Pulsed Electric Field (PEF) has been reported to improve the yield and the quality of the extracts. The specific input energy of PEF, counted an energy needed during the PEF process, was found to be influenced by voltage and duration of PEF. In this study, the relationship between specific input energy using variation voltage and duration of PEF was studied on extraction of areca seed. This research examined tannin levels and changes in areca cell structure before and after PEF. This research used Factorial Randomized Block Design with two factors. The first factor was PEF voltage with three levels of 3, 4 and 5 kV and the second factor was PEF duration with three levels of 3, 5 and 7 minutes. The obtained data was analyzed using one-way ANOVA. The results revealed that specific input energy of PEF in the range of 1.92-12.44 kJ/cm³ did not have significant effects on tannin content of areca seeds extract. However, when it compared with control (without PEF pretreatment), better results was obtained on the tannin content. Scanning Electron Microscopy-Energy Dispersive X-ray (SEM-EDX) analysis of areca seed powder demonstrated significane differences in structure and element concentration of samples with and without PEF pretreatment. The findings confirmed that PEF pretreatment offers great promise in tannin extraction.

1. Introduction

*Areca (Areca catechu L.)* is one of the palm plants that can grow naturally and is widely distributed in Indonesia. Indonesia is third largest country in areca nut production after India and Burma. In 2017, the number of Areca nut Indonesia production was 136,837 tonnes, while India was 1,337,115 tonnes and Burma was 147,524 tonnes [1]. Areca are commonly used in the treatment of various diseases. The seeds of areca nut has 59 compounds that can be isolated and identified that the majority of its compounds are is the alkaloid type piridine and condensed tannins as its identifier [2].

One of the secondary metabolites found in areca seed is tannins as antioxidant. The level of tannin in areca seed can reach 6.45-8.53% depending on climatic factors and the environment where the plant grows [3]. The removal of tannin in areca seed is generally carried out by extraction method. One extraction technique that can be used is maceration. Extraction of ripe and raw areca seed after
Maceration using water solvent for 72 hours at room temperature resulted in yields of 0.982% and 2.522% [4]. However, this yield value is still relatively low. Therefore, pretreatment of the material is needed to increase the extract yield and to reduce the extraction time. Various pretreatments are potential, such as particle size reduction and application of pulsed electric field (PEF) method.

PEF is the provision of electrical voltage to materials intended to damage the cell wall of materials without damaging the bioactive components in the cell. PEF applies a high electric field which is contacted with the material between two electrodes in a short time [5]. Treatment with PEF can increase the production of secondary metabolites associated with regulation of cell life defenses. It is because the presence of electric fields, accumulation, and pulling of charged particles in non-conductive cell membranes causing a reduction in thickness or damage to cell membranes [6]. Damage or rupture of the membrane will cause the rate of diffusion of the outgoing product to increase during extraction, resulting in a higher yield [7]. This shows that electric shock (i.e., PEF) has the potential to optimise extraction processing procedures and reduce energy.

Damage or rupture of the cell membrane material occurred due to the energy given during PEF pretreatment. PEF is an alternative technology with low heat energy used which is between 9-12 °C (non-thermal pretreatment) [8]. In the process, PEF uses 220-240 V and 300 W of power which allows voltage output from PEF devices to increase to 10,000 V, so that electrical energy can also increase. Electrical energy resulted from PEF pretreatment can be analyzed using the calculation of specific input energy. The amount of specific input energy of PEF depends on several things such as the anode-cathode distance, the dimensions of the room, the voltage (electric field strength), and the treatment time [5].

The use of PEF with the right voltage and exposure time can affect the number of specific input energy so that changes in cell structure of a material such as damage to the cell's cytoplasmic membrane. It relates with channel ion K in plant cell. In general, plant cells find cations such as K⁺, Na⁺, Ca²⁺, and H⁻ which play an important role in plant metabolism. On the inside of the cell membrane there are potassium ions (K⁺) and on the outer side of the cell membrane there are sodium ions (Na⁺). The concentration of Na⁺ ions on the outer side of the cell membrane is 10 times higher than inside. This causes the inner side of the cell to be more negative compared to the outer side of the cell. This indicates the presence of a membrane potential caused by the electrical potential energy possessed by each cell in general [9]. Different PEF specific input energy applied to areca seed cells is expected to affect the opening of ion channels so that it forces pore formation [5]. The presence of these pores allows diffusion and transfers more tannins to the solvent. Thus, it is important to conduct research on PEF pretreatment aiming to increase tannin content of areca seed extracts.

2. Materials and Method
2.1. Materials
The raw materials of areca (Areca catechu L.) used in the study were 9 weeks old. These obtained from sellers in Malang, East Java. The areca was peeled and the seeds were taken. Areca seed was crushed using a knife and grinder. After that, it was dried for 24 hours at 50 °C. The dried areca seeds were refined using a disc mill and sieved to a size of 60 mesh. This areca seed powder was used for further extraction. Supporting materials include Folin Ciocalteu reagent, Na₂CO₃, distilled water, and gallic acid.

PEF generator was used in this research (Figure 1). Specification of PEF generation was as follows: input voltage 150 W, electrical field output 0.5 - 5000 V/cm, frequency 10 - 10,000 Hz, pulse width 1 until 200 mS, PEF time 1 until 2 hours, voltage output bipolar and monopolar, PEF diameter chamber 11.5 cm and height 25 cm.
2.2. Method

2.2.1. Extraction procedure

Areca seed powder was weighed 50 g, packaged using clear plastic and prepared for 27 samples at the same weight. These samples prepared for this experiment was based on $3 \times 3$ factorial design, randomised block and replicated 3 times for further preliminary treatment using PEF and extraction. The PEF voltage (3, 4, and 5 kV) and exposure time (3, 5, and 7 minutes) were two factors used in this experimental design. The preliminary treatment of PEF was carried out with a frequency of 6,000 Hz; cathode anode distance of 10 cm; voltage of 3, 4, and 5 kV; exposure time using 3, 5, and 7 minutes. Before extraction process, the samples were analysed using SEM-EDX. The areca nut powder which had been given pretreatment was put into erlenmeyer and added with a distilled water (1:4), covered with aluminium foil, macerated for 120 minutes at 60 °C using a water bath shaker (100 rpm). The maceration solution was filtered using a double filter: cloth and Whatmann filter paper no. 40 to obtain areca seed filtrate. Furthermore, the areca nut filtrate was dried in an oven for 24 hours at 50 °C to obtain dried areca seed extract. The results of extracts were analysed for tannin content.

2.2.2 Tannin content

Tannin content analysis was conducted according to Tambe et al. [10]. Prepared 100 ppm gallic acid stock solution, then diluted to 80, 60, 40 and 20 ppm, then pipetted 0.1 mL of these gallic acid solution into a 10 mL measuring flask. Added 7.5 mL of distilled water, 0.5 mL of Folin Ciocalteu reagent, and 1 mL of $\text{Na}_2\text{CO}_3$ 35%. Added distilled water to the boundary mark and stored at room temperature. Then, measured the absorbance of the prepared samples at 725 nm. A standard curve was made with the x axis (concentration of gallic acid) and the y axis (absorbance). The blank was made of 8.5 mL of distilled water, 0.5 mL of Folin Ciocalteu reagent, and 1 mL of $\text{Na}_2\text{CO}_3$ 35%. To measure the tannin level of a sample, the procedure was carried out in the same way as making a standard curve, but gallic acid was replaced with a 0.1 mL of sample of 0.1 mL 1000 ppm.

2.2.3 Scanning electron microscope – energy dispersive X-rays analysis (SEM-EDX)

Microscopic observation of the appearance of cell structure of areca seed powder was carried out using SEM analysis at 1000x magnification. According to Jelin et al [18], SEM is one of the types of electron microscopes that can be used to identify the shape of nanoparticles of a material. At this magnification, cell structure of areca seed powder can be seen before and after PEF treatment. According Michalak et al. [15], SEM-EDX was equipped with energy dispersive X-ray system in order to obtain a distribution of elemental composition of the surface of plant cell wall.
2.2.4 Input energy calculation

The amount of input energy of PEF in kJ/kg (Q) can be calculated using equation (1). It calculated by multiplication of voltage in volt (U), electric current in A (I), number of pulses (n), and time of pulses in ms (tp), then divided by mass flow rate in kg (M) and duration of modality in ms (tm) [11]. The minimum energy needed to damage the material per unit sample weight can be known during the process.

\[ Q = \frac{U \times I \times n \times t_p}{M \times t_m} \] (1)

2.3. Statistical analysis

Observation data were analysed by using ANOVA and continued with the Duncan test with a 5% confidence interval.

3. Results and Discussion

3.1. Relation of PEF specific input energy (W PEF) to the tannin content

According to Figure 2, it can be seen that the amount of specific input energy of PEF increases with increasing voltage and time of PEF given. Specific input energy requirements for PEF in the areca nut pretreatment ranged from 1.92 - 12.44 kJ/cm³. The lowest PEF specific input energy is found in the treatment of PEF 3 kV voltage and PEF time of 3 minutes which is 1.92 kJ/cm³, while the highest specific input energy was found in the treatment of PEF 5 kV and PEF 7 minutes which is 12.44 kJ/cm³. This is in accordance with the opinion of Srisuhartatk [12] which states that the increase in PEF shock time was parallel to the increase in the amount of energy needs. The amount of energy can be varied by adjusting the time or voltage of PEF.

According to Nieto et al. [13], increasing voltage passed resulted in an increase in, the electric field produced and in the energy received by the material. The longer the application of PEF, the greater the energy of PEF is produced [14]. However, based on the ANOVA results, it is found that PEF specific input energy does not significantly affect the tannin content of areca seed extract (Table 1), as indicated by a sig value greater than 0.05. It is assumed that the PEF specific input energy from 1.92 - 12.44 kJ/cm³ have the same ability to form pores in areca nut wall cell so that it have ability to diffuse tannins into solvents.

In this study, it is also assumed that the PEF specific input energy was higher, thus enable to widely open the membrane pores to enhance the quantity and quality of tannin from areca nut extract. In Table 1, it can be seen that the specific input energy of PEF increased with an increase in PEF time and
voltage. But, the value of tannin content produced from areca seed extract was found to be constant, which possibly due to the condition of the raw material used. Similarly, previous research by Putri et al. [16], reported that increasing the exposure time of PEF was parallel to an increase in the specific input energy. According to Srisuhartatik [12], specific input energy needed for PEF pretreatment prior citrus fruit juice pasteurization was ranged from 2.65 - 166.73 kJ/cm³, where an increase in shock time was followed by an increase in the amount of energy required.

| Table 1. Relation of PEF specific input energy to the characterization of areca seed tanin |
|-----------------------------|-------------------------------|-----------------|-----------------------------|
| PEF voltage (kV) | PEF time exposure (min) | WPEF (kJ/cm³) | Tannin content (mg GAE/g) | Increasing tannin content compared with control* % |
|----------------|-----------------|----------------|-----------------------------|---------------------------------|
| 3 | 3 | 1.92 | 529.42 ± 70.11 | 5.77 |
| 3 | 5 | 3.20 | 571.34 ± 83.17 | 14.14 |
| 3 | 7 | 4.48 | 537.66 ± 71.20 | 7.41 |
| 4 | 3 | 3.41 | 554.85 ± 91.21 | 10.85 |
| 4 | 5 | 5.69 | 557.59 ± 67.46 | 11.40 |
| 4 | 7 | 7.96 | 522.89 ± 80.19 | 4.46 |
| 5 | 3 | 5.33 | 519.11 ± 33.59 | 3.71 |
| 5 | 5 | 8.88 | 504.33 ± 35.45 | 0.76 |
| 5 | 7 | 12.44 | 516.36 ± 48.37 | 3.16 |
| 0 | 0 | 0 | 500.55±42.27* | |

However, Table 1 shows that there was an improvement in the tannin content after pretreated with PEF. Without PEF pretreatment, the tannin content was 500.55 mg GAE/g. After PEF treatments, the tannin content increased by 3.16% - 14.14%, giving the value in the range of 504.33 – 571.44 mg GAE/g. The results indicate that the specific energy ranged from 1.92 - 12.44 kJ/cm³ can facilitate the diffusion process during extraction. The electric shock was also found to increase the permeability of plant cell membranes. The effects of the electric shocks can accelerate mass transfer, thus increasing compound extraction and resulting in lower energy consumption [17].

3.2 Structural changes of areca seed cells

The structural images of areca seed cell powder before and after PEF pretreatment at 1000x magnification are shown in Figure 3.

![Figure 3. Areca nut cell structure without PEF pretreatment (control) (a); the structure of areca nut cells with PEF pretreatment at 3 kV and 3 minutes (b)](attachment)
ANOVA results indicate that all treatments were not significantly influenced the tannin content. Therefore, in this study, PEF pretreatment using the lowest voltage (3 kV) and time of PEF (3 minutes) was analyzed using SEM-EDX and compared with control (Figure 3). The cell structure in untreated areca nut powder was still intact (Figure 3a), whereas after PEF pretreatment showed a broken cell structure and smaller particle size of areca seed powder (Figure 3b). This is in accordance with Jelin et al. [18] who states that the electrical voltage applied to cells can fracture the cell wall due to the flow of electricity.

According to Sumitro et al. [20], plant cell size ranges from 10 µm - 100 µm. The SEM results (Figure 3b) identified that areca seed cell powder has particles size of 66.6 µm, 71.3 µm and 25.4 µm. The control treatment (Figure 3a), however, has higher particles size with the value of 118 µm, 104 µm and 39 µm. This findings indicate that one or several cells was found in areca seed powder. PEF pretreatment resulted in structural changes of areca seed powder, as shown by more smaller particles were produced than that of without PEF pretreatment.

The EDX analysis results identified the potassium (K) compound in the areca seed powder. The number of potassium (assumed as ions K+) contained in cells of the control and PEF pretreatment samples were 0.588 and 0.383, respectively. This incidates that PEF pretreatment at 3 kV voltage and 3 minutes was not only damage the plant’s cell wall, but also damage the potassium ion channel contained in the cell, causing a great reduction in the number of potassium ions.

According to Schow et al. [21], increasing both the voltage and the exposure time causes a more active ion movement, indicating an increase in the pore size of the cell wall. Such conditions contribute to enhancing the cell membrane permeability. Furthermore, the cell membrane permeability continues to increase as a result of continuous exposure to the electric field. This causes the pores in the cell membrane to be irreversible [22]. In addition, the solvent becomes more easily reaching the inside part of the cell’s tissue such as the vacuole where the tannins were stored. Therefore, a better quality of the tannins can be produced. In this study, the tannin content was higher than 504.33 mg GAE/g, which was superior to the previous PEF pretreatment experimentat 4.5 kV and 20 seconds with the value of < 500 mg GAE/g [23].

4. Conclusions

The PEF specific input energy from 1.92-12.44 kJ/cm³ has no significant effect on tannin content of areca seeds extract. However, compared with control (without PEF preteatment), sample with PEF pretreatment has better results in the tannin content. SEM-EDX analysis from areca seed powder showed structural changes after PEF pretreatment. EDX results indicate the low number of K ions in PEF pretreatment was caused by a damage to K ion channels in areca seed powder cell membranes. The findings also confirms that PEF pretreatment offers great promise in enhancing tannin extraction.

Acknowledgments

Authors would like to thanks for the financial support from UniversitasBrawijaya through HPP Research Grant in 2017.

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