UDC 631.9

EFFECT OF FREQUENCY AND DURATION OF PULSED ELECTRIC FIELD PRE-TREATMENT ON ANTIOXIDANT COMPOUNDS EXTRACTION OF DRY ARECA NUT (ARECA CATECHU)

Rahmah Nur Lailatul*, **Sukardi**, **Dewanti Beauty Suestining Diyah**, **Wulandari Hanun Ari**
Agro-industrial Technology Department, Faculty of Agricultural Technology,
University of Brawijaya, Indonesia
*E-mail: nur_laila@ub.ac.id

ABSTRACT
The production of Areca palm plantations in 2012-2015 increased from year to year, i.e. 42.0, 42.8, 47.0, and 47.1 thousand tons, respectively. Areca palm *(Areca catechu*) is one of the plantation products with many benefits due to its antibacterial, antifungal, anti-inflammatory, anti-allergic, analgesic, and blood sugar and fat regulators activities. Areca nut contains phenolic compounds which function as antioxidants. The extraction process of areca nut is required to obtain these compounds. Pre-treatment before extraction using Pulsed Electric Field (PEF) has been shown to improve the yield and quality of antioxidant in the extracts. Therefore, a research was conducted on the extraction of areca nut using PEF pre-treatment with a variation of frequency and duration of PEF application. The experimental design of this study was a Randomized Block Design with two factorial groups. The first factor was the PEF frequency with three levels of 1.2, 1.4, and 1.6 kHz and the second factor was the PEF duration with three levels of 30, 35, and 40 seconds. The extract of areca nut from PEF pre-treatment with a combination of PEF frequency and duration did not result in significantly different yield and quality. The best frequency and duration of PEF on yield and antioxidant quality of areca nut extract were at 1.2 kHz and 30 seconds with yield value of 4.63%; water content of 9.18%; the IC_{50} value of 162.23 ppm; and tannin content of 507.42 mg GAE/g. The best treated infrared spectra showed that the extracts were strongly believed to contain tannins.

KEY WORDS
Areca catechu, pulsed electric field, yield, quality, tannins, IR spectra.

Indonesia is agrarian country that has considerable plantation potential. One of the plantations that have benefits for treatment is the areca palm. Areca palm (*Areca catechu*) contains pharmacological activities which include antiparasitic, antioxidant, antibacterial, antifungal, anti-inflammatory, and anti-allergic activities; nervous and digestive system influencer; and blood sugar and fat regulator (Gilani et al., 2004). Areca nut has 59 isolated and identifiable compounds whose majorities of the compounds are pyridine-type alkynes and condensed tannins (Peng et al., 2015). Water extract and methanol from areca nut have higher antioxidant and phenolic activity than root extract (Hamsar et al., 2011).

Antioxidant is able to act as hydrogen radical contributor or act as free radical acceptor, enabling it to delay the initiation stage. Antioxidants stabilize free radicals by supplementing electron deficiencies possessed by free radicals and inhibit the occurrence of a chain reaction of free radical formation (Winarsi, 2007). Extraction process by maceration method is required to obtain the antioxidant content in the areca nut.

PEF is also called electroporation which is the electrical voltage applied to cells, so that the cell suffers pore damage without damaging the biochemical components present in the cell (Sukardi, 2016), such as antioxidant compounds and secondary metabolites. Palm seeds such as Palma Swamp (*Mauritia flexuosa*) are composed of endosperm cells. Vacuoles in endosperm cells contain phenolic, alkaloid, and terpenoid compounds (Silva, 2014). Similarly, antioxidant compounds and secondary metabolites in areca nuts are also stored in vacuoles of endosperm cells.

With PEF pre-treatment, the cell membrane pores have been damaged so that the bioactive components can be easily extracted with the solvent and the time required for a maceration process is faster. This study aims to determine the best PEF frequency and period of time on the areca nut extract.

MATERIALS AND METHODS OF RESEARCH

Materials. The main ingredients used were unripe to half-ripe areca nuts (green until orange in color) obtained from Malang, East Java. Materials used included distilled water, ethanol p.a., methanol p.a., tannic acid, gallic acid, Na2CO3, Folin Denis, and DPPH. Tools used included a set of PEF generators and chamber, ovens, UV-Vis spectrophotometers, analytical balance, vortex, and micropipettes.

Experimental design. This study used factorial Randomized Block Design with two factors. The first factor was PEF pulse frequency factor with 3 levels (1.2, 1.4, and 1.6 kHz). The second factor was PEF period factor with 3 levels (30, 35, and 40 seconds). Therefore, there were 9 combination treatments. Each treatment was repeated 3 times to get 27 units of the experiment. Table 1 presents the combination of the experimental treatment.

Table 1 – Combination treatment

No.	Frequency PEF (Hz)	Time of PEF		
		T_1 (30 s)	T_2 (35 s)	T_3 (40 s)
1.	F_1 (1,2 kHz)	F_1T_1	F_1T_2	F_1T_3
2.	F_2 (1,4 kHz)	F_2T_1	F_2T_2	F_2T_3
3.	F_3 (1,6 kHz)	F_3T_1	F_3T_2	F_3T_3

Implementation of Research. Antioxidant extraction of areca nut with PEF pre-treatment process was described as follows: areca fruits were sorted to remove dirt or rotten fruit. Areca fruits were then peeled and the nuts were removed. The areca nuts were crushed and dried in the oven for 24 hours at 50^{o}C. Dry areca nuts were mashed and sifted on 60 mesh. The powder of areca nut was weighed 50 g each. It was inserted into the chamber on PEF generator, then electric shock pre-treatment with 4.5 kV/cm voltage and with each frequency 1.2, 1.4, and 1.6 kHz respectively at 30, 35, 40 seconds according to the treatment combination in Table 1 were performed. Pre-treated areca nuts then were added by distilled water (1: 4), were covered with aluminum foil, and were macerated kinetically for 120 minutes at 60°C. It was filtered with filter paper to obtain the areca nut filtrate. It was roasted for 24 hours at 60ºC. Yield calculation, water content, antioxidant activity, and tannin content analysis were then performed.

Antioxidant extraction of areca nut without PEF pre-treatment process (control) was described as follows: areca fruits were sorted to remove dirt or rotten fruit. Areca fruits were then peeled and the nuts were removed. The areca nuts were crushed and dried in the oven for 24 hours at 50^{o}C. Dry areca nuts were mashed and sifted on 60 mesh. Areca nut powder was weighed 50 g each, was added with distilled water (1: 4), was covered with aluminum foil, and was macerated kinetically for 120 minutes at 60^{o}C. It was filtered through filter paper to obtain the areca nut filtrate. It was roasted for 24 hours at 60ºC. Yield calculation, water content, antioxidant activity, and tannin content analysis were performed.

Data Analysis and Selection of Best Treatment. Data analysis was processed using ANOVA with SPSS software and continuous test with Duncan Multiple Range Test 5%. Determination of the best treatment of this research was based on the lowest water content and IC_{50} values and the highest yield and tannin content of all treatments. A low IC_{50} value means a better antioxidant activity, while a high yield means a higher economic value, resulting in more effective production. Fourier Transform Infra-Red (FTIR) analysis was then performed to the best treatment in order to identify the functional group.

Yield. The yield of areca nut extract is the percentage of extract produced from the extraction process of areca nut powder. The final form of the areca nut extract in this research is solid. Table 2 presents the results of the yield of areca nut extract in this study.

The results of the analysis of variance (ANOVA) with 95% confidence interval (α = 0.05) indicated that the interaction and each PEF frequency and duration factor did not give significant effect to yield of areca nut extract. This is indicated by the sig value greater than 0.05. The absence of influence of both factors on the yield value showed that the treatment variation needed to be modified again. It was assumed that the variation in exposure time of electric pulses had not fully opened the cells pores so that the resulting tannin has not shown any difference at various frequencies. However, when compared with the yield control without PEF (2.71%), the value was less than the yield value using the PEF preliminary treatment. By using PEF, there was an increase of 44.73%.

Table 2 – Extract Yield

Frequency (kHz)	Time (seconds)	Average Yield (%)
1.2	30	4.63
	35	4.73
	40	5.27
1.4	30	5.11
	35	5.32
	40	4.39
1.6	30	5.11
	35	4.70
	40	4.92
Control without PEF		2.71

In accordance with Table 2, it can be seen that the pre-treatment resulted in an increase in the areca nuts extract yield. It was suspected that the electric shock could open the pores of the areca nuts' cell wall so that it can facilitate the transfer of tannin active ingredients into the distilled water solvent. Gould (1995) confirmed this assumption by stating that the presence of electric shock could create holes in the cell wall, whereas in the absence of electric shocks to the plant cell, there was no hole or pore. The presence of electric shock caused modification on the surface of plant cells, resulting in an increase of the extraction ability.

Water Content. Table 3 presents the results of the water content of the extracts in this study. The results of the analysis of variance (ANOVA) with 95% confidence level (α = 0.05) indicated that the interaction and each PEF frequency and duration factor did not give a significant effect on the water content of the extract. This is indicated by the sig value greater than 0.05.

Table 3 – Water Content

Frequency (kHz)	Time (seconds)	Average Water Content (%)
1.2	30	9.18
	35	7.64
	40	8.03
1.4	30	8.13
	35	8.55
	40	7.50
1.6	30	8.40
	35	8.70
	40	8.63
Control without PEF		11.75

The absence of the influence of both factors on the water content value of areca nut extract indicated that the treatment variation needed to be modified again. It was assumed that the variation of exposure time of electric pulses had not fully opened the pores of the cells so that the areca nut extract had not shown any different water content at various frequencies. However, when compared to the water content control without PEF (11.75%), the value was greater than the water content value of the extract using the PEF pre-treatment. By using PEF, there was a decrease in water content of 29.30%. In accordance

with Table 3, it can be seen that the pre-treatment caused a decrease in the water content of areca nut extract. Water content is the amount of water contained in the extract. It was assumed that electric shocks could open the pores of the areca nuts' cell wall to facilitate the transfer of the active ingredient from the material into the distilled water solvent.

Antioxidant Activity (IC_{50}). The antioxidant activity (IC_{50}) is the residual DPPH concentration (ppm) which does not react with antioxidant compounds of areca nut extract from the extraction process of areca nut powder. Table 4 presents the results of IC_{50} of areca nut extract in this study. The result of analysis of variance (ANOVA) with 95% confidence interval ($\alpha = 0.05$) found that the interaction and each PEF frequency and duration factor did not give significant effect to the antioxidant activity of areca nut extract. This is indicated by the sig value greater than 0.05. It was assumed that the variation of exposure time of electric pulses had not fully opened the pores of the cells so that the areca nut extract had not shown any difference in antioxidant activity at various frequencies. However, when compared with antioxidant activity control without PEF (179.86%), the value showed the similar antioxidant activity with PEF pre-treatment.

Table 4 – Antioxidant Activity

Frequency (kHz)	Time (seconds)	Average IC_{50} (ppm)
1.2	30	162.23
	35	170.31
	40	162.96
1.4	30	169.61
	35	174.63
	40	180.37
1.6	30	177.48
	35	177.74
	40	171.06
Control without PEF		179.86

According to Table 4, it can be seen that the value of IC_{50} areca nut extract with and without PEF resulted in the similar antioxidant activity. Both have antioxidant activity in the medium category that was between 100-250 ppm (Molyneux, 2004). It was assumed that the electric shock exposure time was too short, causing not all tannin compounds could diffuse into the distilled water solvent. The increase in time resulted in an increase in the extract yield value, however, long periods of time may decrease the yield due to reduced cell membrane resistance (Sukardi, 2016; Janositz, 2010).

The frequency of this study was believed to be too small so that cell membrane polarization had not occurred. Good PEF frequency applications were in the range of 10 kHz-1MHz. The polarization of cell membranes produced dispersions between 1 kHz and 10 MHz depending on the size of the cell and the surrounding electrolyte conductivity. Dispersion is defined as the movement of ions in cells that affect cell electroporation (Pliquet, 2010; Donsi et al., 2010; Shen et al., 1997).

Tannin Content. The tannin content is the amount of mg of tannin equivalent to the gallic acid of each gram (mg GAE/g) resulting from the extraction process of areca nut powder. Table 5 presents the results of tannin content in this study. The results of the analysis of variance (ANOVA) with 95% confidence interval ($\alpha = 0.05$) found that the interaction and each PEF frequency and duration factor did not give significant effect to tannins. This is indicated by the sig value greater than 0.05.

The absence of the influence of both factors on the value of tannin content indicated that the treatment variation needed to be modified again. It was assumed that the variation in exposure time of electric pulses had not fully opened the pores of the cells so that the tannin content had not shown any difference at various frequencies. However, when compared to the tannin content control without PEF (451.75 mg GAE/g), the tannin content using PEF pre-treatment was higher.

PEF pre-treatment was able to produce the areca nut extract with higher tannin content. Tannin is a polar compound that is soluble in polar compounds. The presence of

PEF was able to support the diffusion process of tannin compounds from the inside out of the cell. With the pre-treatment, the pores of cell membranes had been damaged so that the bioactive components can be easily extracted with the solvent, resulting in the faster time required in the maceration process.

Table 5 – Tannin Content

Frequency (kHz)	Time (seconds)	Average Tannin Content (mg GAE / g)
1.2	30	507.42
	35	594.02
	40	495.05
1.4	30	539.73
	35	523.23
	40	553.47
1.6	30	582.34
	35	607.08
	40	483.37
Control without PEF		451.75

Selection of the best treatment. The selection of best treatment was based on the economic aspects of ANOVA treatment and analysis because each parameter and each treatment were not significantly different. The best PEF frequency and duration on yield and antioxidant quality of areca nut extract were at 1.2 kHz and 30 seconds with yield value of 4.63%; water content of 9.18%; the IC_{50} value of 162.23 ppm; and tannin levels of 507.42 mg GAE / g.

FTIR Spectrophotometric Analysis Results. FTIR analysis to identify the functional group of the extract was conducted on the best treatment resulted from PEF pre-treatment with the frequency of 1.2 kHz and duration of 30 seconds. To ensure the functional group of extract had the same profile, FTIR analysis was done at the highest frequency of PEF that was at frequency of 1.6 kHz and time of 40 seconds. Table 6, Figures 1 and 2 present the results of FTIR analysis. Based on Table 6, the values of wave numbers (cm-1) in the bands show the same remark value as the standard (William and Ian 1995) and tannins (Rosyda and Taslim, 2009).

Table 6 – FTIR Spectra

No.	Sample A (Frequency 1,2 kHz; 30 seconds)	Sample B (Frequency 1,6 kHz; 40 seconds)	Merbau Wood Tannin Extract (Rosyda and Taslim, 2009)	Remarks (William and Ian, 1995)	
1.	3381.74; 3260.24	3377.89	3356.25	3650-3590; 3200-3600	-OH (alcohol, phenol)
2.	2922.72	2930.43	2966.62; 2924.18	2960-2850	C-H and C-C aliphatic
3.	1613.14; 1522.49	1613.14; 1524.42	1616.4; 1512.24;	1600-1500	C=C aromatic
4.	1443.42	1447.27	1450.52	1430-1470	-CH2; -CH3 deformation
5.	1377.84; 1206.19	1379.77; 1208.12	*1303.92*	1385-1355	C-O-C hetero-tannin ring
6.	1061.54	1061.54	*1033.88*	1150-1040	-C-O stretching
7.	822.38	822.38	840.99	840-790	*Para substitution* as characteristic of benzene(conjugated aromatics)

The best treatment (lowest frequency - Sample A) and the highest frequency (Sample B) also showed the same band. The -OH group (alcohol, phenol) was indicated on the wave number 3381.74; 3260.24 for the frequency of 1.2 kHz and 3377.89 cm-1 for the frequency of 1.6 kHz. The C-H and C-C aliphatic clusters were shown at wave numbers 2922.72 cm-1 for frequencies of 1.2 kHz and 2930.43 cm-1 for the frequency of 1.6 kHz. C = C aromatic cluster; -CH2; -CH3 deformation; C-O-C hetero-tannin ring; -C-O stretching and Para substitution as characteristic of benzene (conjugated aromatics) were also found at a frequency of 1.2 kHz as the best treatment and 1.6 kHz as the highest frequency treatment. The bands showed that the extract was believed to contain strong tannins.

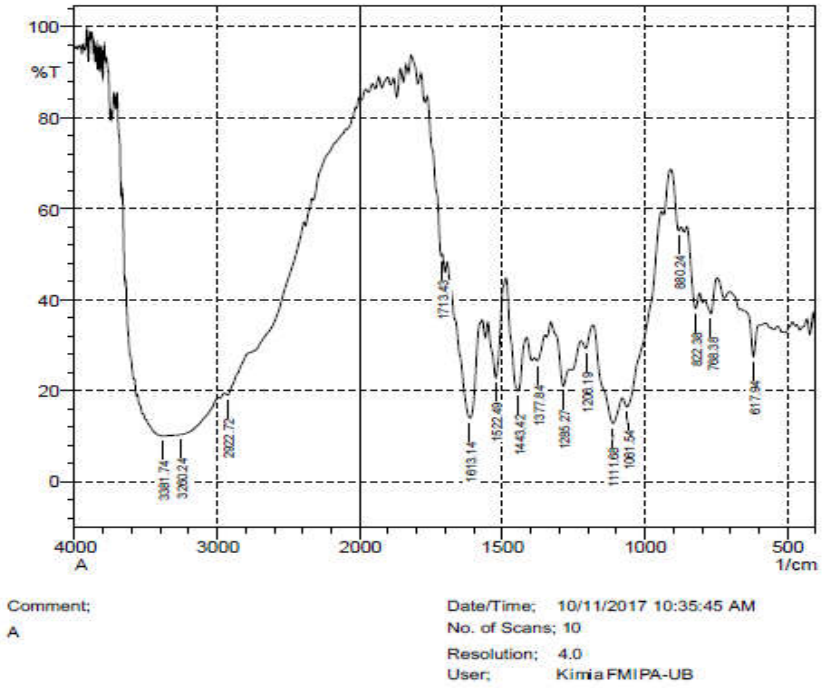

Figure 1 – The best IR spectra treatment results (PEF frequency of 1.2 kHz)

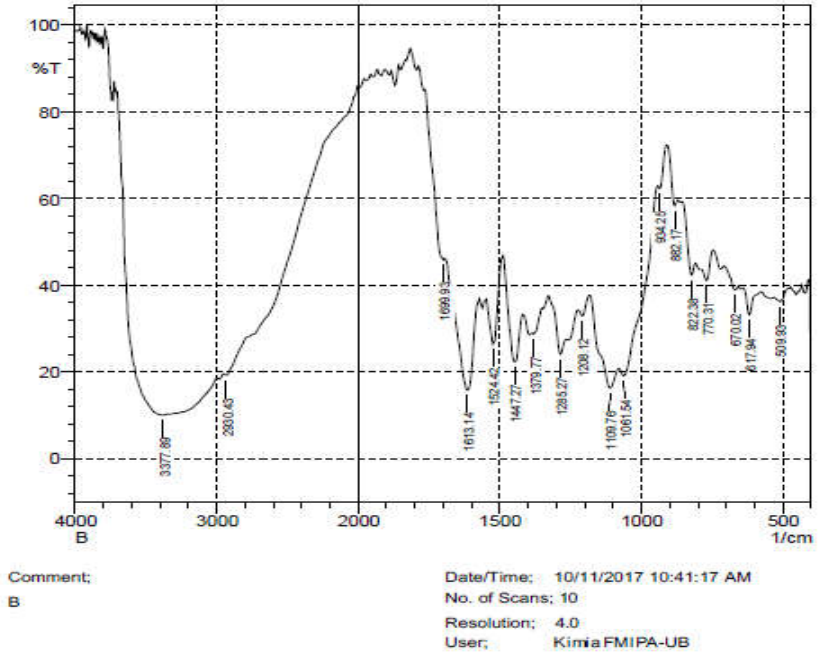

Figure 2 – IR spectra results (PEF frequency of 1.6 kHz)

CONCLUSION AND SUGGESTIONS

The extract of areca nut from the extraction with PEF (Pulsed Electric Field) pre-treatment with a combination of PEF frequency and duration did not produce significantly different yield and quality. The best PEF frequency and duration on yield and antioxidant quality of areca nut extract were 1.2 kHz and 30 s.

Modifications were needed to increase the frequency and duration value of the PEF pre-treatment of dry areca nut powder.

ACKNOWLEDGEMENTS

The researchers would like to thank to the Institute for Research and Community Services of University of Brawijaya for the fund support that has been given for this study.

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