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Pulsed electric field (PEF)-assisted Extraction of Phenolic Compounds from Mangosteen Pericarp

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Abstract

Mangosteen (*Garcinia mangostana* Linn.) pericarp is a rich source of phytochemicals. Therefore, It is used as a traditional medicine. This study aims to investigate effects of pulsed electric field (PEF)-assisted extraction of mangosteen pericarp on total phenolic content (TPC) and antioxidant activity using ferric reducing ability power (FRAP) assay. The study conditions were varied by PEF intensity (4, 5 and 6 kV/cm-1) with 1000, 3000 and 5000 pulses. Mangosteen pericarp macerated in water was used as a control. The study revealed that TPC value increased with higher intensity and pulses. The highest TPC was observed, in the pericarp extracted with 6 kV/cm^{-1} and 5000 pulses, at 0.17 mgGAE/gFW. The PEF-assisted extraction showed 425% increment compared to its untreated counterpart. Similar trend was found with FRAP assay where the pericarp extracted with PEF (6 kV/cm^{-1}, 5000 pulses) showed the highest FRAP value at 0.38 mgGAE/gFW. Moreover, TPC value and FRAP value exhibited positive correlation with r = 0.9869.

Keywords: *Pulsed electric field-assisted extraction, mangosteen pericarp, phenolic content, antioxidant activity*

1. Introduction

Mangosteen (*Garcinia mangostana* Linn.) fruit is also known as the "Queen of fruits" because of its excellent flavor. The edible pulp of mangosteen fruit is white, soft and juicy with a slightly acidic, sweet flavor and a pleasant aroma. The fruit is round with a smooth, thick and tough pericarp. The color of the pericarp is dark purple when fully ripe (Satong-aun, Assawarachan, & Noomhorm, 2011). Mangosteen pericarp was used as an alternative treatment, such as heart medications, lowering the blood sugar, malaria medicines, anti-inflammatory agent, antimicrobial, and antioxidants (Muchtaridi, Prasetio, Saptarini, & Saputri, 2018).

There are important components in mangosteen pericarp which possess antioxidant properties and other medicinal properties which called xanthones alpha (α)-, beta (β)- and gamma (γ)-mangostins (Satong-aun et al., 2011). Xanthones are polyphenol compounds that are found abundantly in mangosteen pericarp which contain α-mangostin as the major xanthones compound (Figure 1) followed by γ-mangostin (Mohammad et al., 2018). There are many extraction techniques to recover bioactive xanthones from mangosteen pericarp.

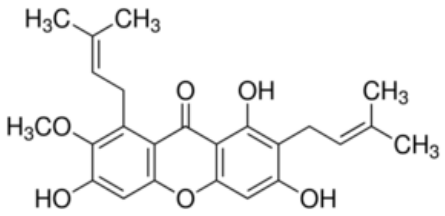

Figure 1. Chemical structure of α-mangostin

Various solvents extraction techniques such as Soxhlet, maceration and ultrasonication, with different solvents (methanol, ethanol, 70% acetone, ethyl acetate etc.) have been commonly used for extracting α-mangostin from mangosteen pericarps (Boonrat & Indranupakorn, 2015). However, it is a toxic solvent with high energy consumption. In addition, the disposal of organic solvents is also a big environmental issue. Meanwhile, temperature played an important role in increasing the solubility of bioactive compounds. However, thermal degradation of bioactive compounds occurred at the same time.

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At present pulsed electric field treatment (PEF) or high-voltage, high-current electrical signal supply for a short time under conductive liquid between high-voltage electrodes, is an emerging technique for environmentally friendly extraction method. It has a variety of applications such as disinfection of ready-to-eat foods such as fruit, meat, beverages, or dairy products. PEF treatment has also been applied to the destruction of plant cell walls to enhance phytochemical compounds released from the cells. A basic principle of this technique is by using a switching type power supply that can generate high voltage up to 20 kV electricity. The high voltage is stored in the capacitor and then the pulse generator supplies a short pulse of high voltage to the plant extraction chamber with the solvent. A short pulse electric field travels through a high voltage electrode and penetrates plant cells causing them to break and the important substances are released in the solvent.

This project aimed to study the effect of pulse number and pulse intensity on phenolic compounds and antioxidant activity extracted from mangosteen pericarp.

2. Methodology

2.1 Raw material

Mangosteens were purchased from a local market in Chiang Mai. Mangosteen pericarp was cleaned with 10% sodium bicarbonate solution then dried at room temperature. The sample was cut into small pieces which were approximately 5 x 5 x 5 mm^3. The sample was treated with an electric pulsed field immediately after processing.

2.2 Chemicals

Folin-Ciocaltue reagent gallic acid, gallic acid, sodium acetate hydrate and ferrous sulphate were purchased from Sigma-Aldrich (Singapore). Potassium chloride, sodium carbonate and hydrochloric acid was purchased from Merck (Darmstadt, Germany). Deionized water was purchased from LABSCAN.

2.3 Sample extraction using pulsed electric field

15 g of chopped mangosteen pericarp and 40 ml of distilled water was added to an extraction chamber. PEF intensity at 4, 5 and 6 kV/cm-1 were studied with 1000, 3000 and 5000 pulses. The pulse frequency used in this study was 2 Hz. After the PEF treatment, the mixture was centrifuged at 5000 rpm for 10 minutes at room temperature and the supernatant was collected. The supernatant was immediately analyzed for total phenolic content (TPC) and antioxidant activity using ferric reducing ability power (FRAP) assay.

2.4 Determination of total phenolic content (TPC)

Total phenolic content (TPC) was measured by Folin- Ciocalteau method as described previously (Folin & Ciocalteu, 1927) with some modifications. A reaction mixture contained 0.2 ml of extract, 0.2 ml of 10% Folin ciocalteu phenol, and 2 ml of 7.5% sodium carbonate solution. The mixture was set aside for 30 minutes in the dark. The absorbance was measured at 765 nm using a spectrophotometer. Gallic acid was used as the standard for the calibration curve.

2.5 Determination of antioxidant activity

The antioxidant activity of the samples was determined by ferric reducing ability power (FRAP) assay according to Benzie and Strain (ref). Briefly, 2 ml of working FRAP reagent (0.1 M acetate buffer: 0.02 M $FeCl_3$: 0.01 M TPTZ = 10: 1: 1) prepared freshly were mixed with 0.2 ml of the mangosteen pericarp supernatant and mixed well. The mixture was set aside for 30 minutes in the dark. The absorbance was measured at 593 nm using a spectrophotometer. Gallic acid was used as the standard for the calibration curve.

3. Results and Discussion

According to the study, PEF-assisted extraction resulted in significant temperature increment ($p < 0.05$) when the pulse intensity and pulse number increased.

Table 1 Temperature (℃) during PEF-assisted extraction at different conditions

PEF intensity (kV/cm-1)	Pulse number		
	1000	3000	5000
4	32	41	47
5	32	44	53.6
6	31	44	62.3

3.1 Effect of pulsed electric total phenolic content

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An analysis of total phenolic contents (TPC) in untreated and PEF treated mangosteen pericarp extract revealed that PEF-assisted extraction showed higher TPC than control samples.

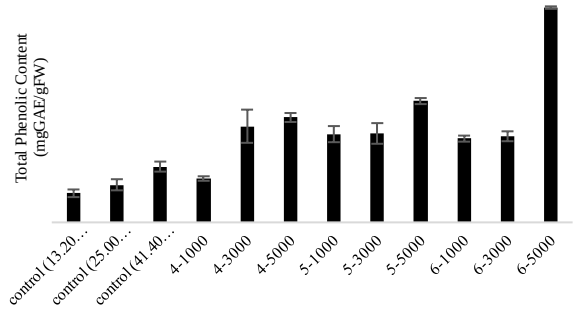


Figure 2 Total phenolic content of mangosteen pericarp extracted with PEF at various condition

As presented in figure 2, the higher PEF intensity resulted in the higher TPC. TPC values were 0.04, 0.08, 0.1 and 0.17 mg GAE/g FW when the extracting conditions were maceration, 4, 5, 6 kV/cm^{-1} with 5000 pulses, respectively. The highest TPC was found with the sample extracted with pulse intensity of 6 kV/cm^{-1} and 5000 pulses. The highest TPC was 425% increment compared to its untreated counterpart.

There are very few studies regarding PEF-assisted mangosteen pericarp extraction. However, TPC values of mangosteen pericarp varied depending on extraction methods. PEF-assisted extraction in plant material has shown to enhance TPC values. A study of Alide and colleagues (2020) showed that TPC values of garlic extracted using PEF was higher than non-PEF extraction. This may also result from temperature increment during PEF treatment. Moreover, PEF also affected cell wall of the plant material which led to more phenolic compounds released in extraction medium (Shaimaa, Mahmoud, Mohamed, & Emam, 2016).

3.2 Effect of pulsed electric field on radical scavenging activities using FRAP assay.

The analysis of FRAP values exhibited a similar trend with TPC values.

As presented in figure 3, the higher PEF intensity resulted in the higher FRAP. FRAP values were 0.02, 0.05, 0.06 and 0.13 mg GAE/g FW when the extracting conditions were maceration, 4, 5, 6 kV/cm^{-1} with 5000 pulses, respectively. The highest FRAP value was found with the sample extracted with pulse intensity of 6 kV/cm^{-1} and 5000 pulses. The highest FRAP value was 650 % increment compared to its untreated counterpart.

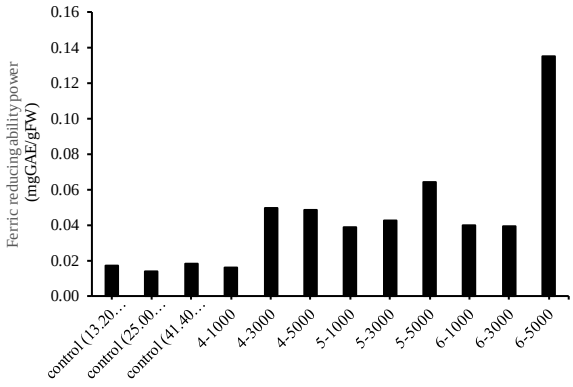
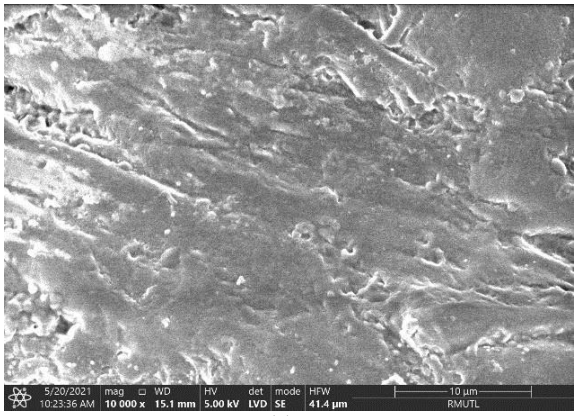


Figure 3 Ferric reducing ability power of mangosteen pericarp extracted with PEF at various condition

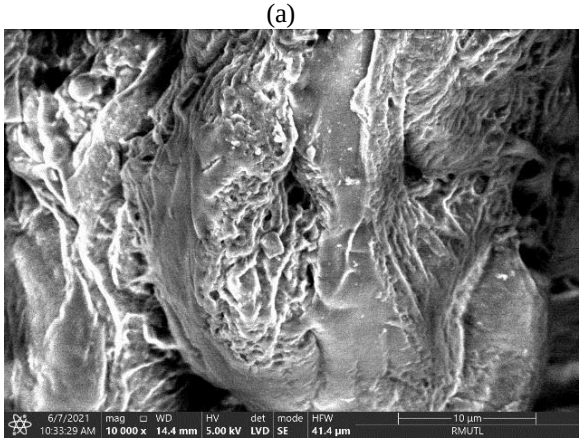
According to the findings, increasing pulse intensity and pulse numbers led to temperature increment which subsequently resulted in higher TPC and FRAP values. This may attribute to cell wall damage by PEF. The scanning electron microscope (SEM) exhibited that when the PEF intensity increased, more damages occurred (Figure 4). Similar study on PEF-assisted herb extraction using steam distillation also found that cell wall deconstruction from PEF resulted in higher essential oil extraction (Dobreva, Tintchev, Dzhurmanski, & Toepfl, 2013).

Moreover, a study revealed that the yield of α-mangostin, xanthones polyphenol, extracted from mangosteen pericarp may enhance when exposed to high temperature (65 ℃) which may cause by the shifting of functional group in other mangostin compound to the α-mangostin structure (Mulia, Hasanah, & Krisanti, 2018). The author also stated that polyphenol oxidase enzyme degradation during high temperature treatment may give a rise to the α-mangostin content. Similar study also showed that cytotoxicity of mangosteen pericarp extract positively correlated with temperature used in the experiment.

According to the information on cytotoxicity of α-mangostin therefore extraction method as well as extraction condition must be carefully selected to minimize cytotoxic effect that may occur.



(a)



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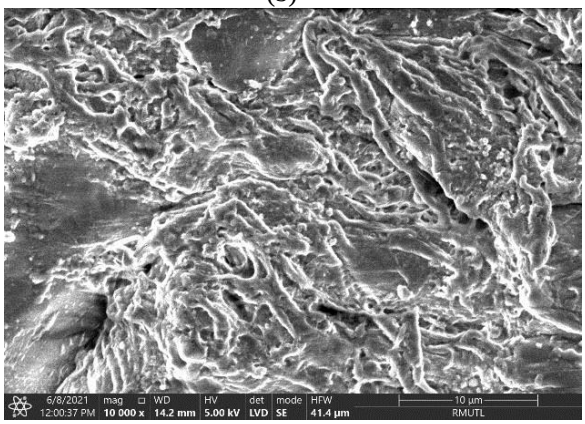

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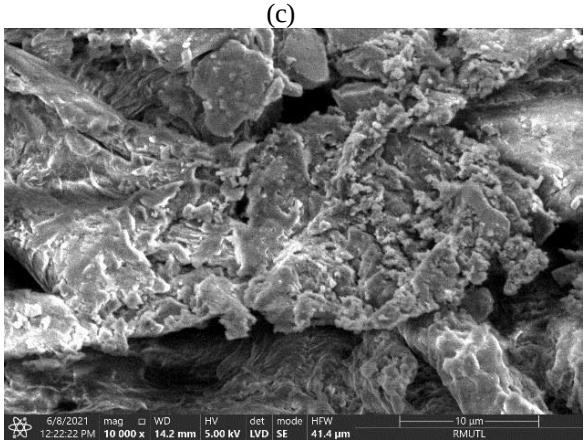

(d)

Figure 4 SEM images of mangosteen pericarp extracted with PEF at various conditions were (a) control (b), (c) and (d) 4, 5 and 6 kV/cm with 5000 pulses, respectively.

Significant positive correlations were found between total phenolic content and FRAP values ($p \leq 0.01$) ($r = 0.9869$). It could be assumed that phenolic compounds in plant extracts contribute significantly to their antioxidant potential. Similar finding was reported that total phenolic content determined by the Folin–Ciocalteu method presented strong correlation with DPPH, ABTS, and FRAP assays (Dudonne, Vitrac, Coutiere, Woillez, & Merillon, 2009).

4. Conclusion

The total phenolic content extracted from mangosteen pericarp was enhanced using PEF-assisted extraction. When the PEF intensity was raised, the extraction capability was elevated. Furthermore, total phenolic content and FRAP value were significantly positively correlated.

5. Acknowledgement

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