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Effect of pulse electric field assisted extraction on anthocyanin content and antioxidant activity of purple rice

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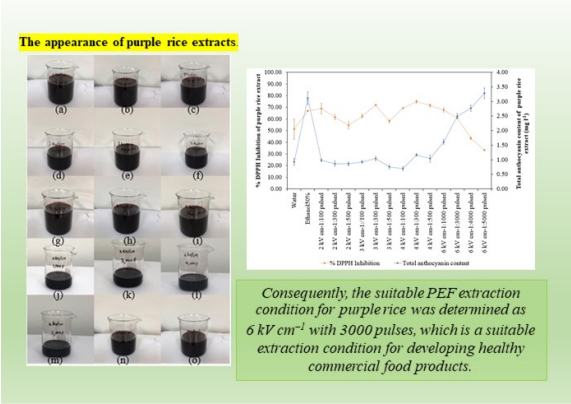
Abstract

Purple rice (*Oryza sativa* L.) is popular for planting and consuming in North and Northeast Thailand. The important pigment on the purple rice grain is anthocyanin which has high antioxidant activity. This research studied the effect of applying a pulse electric field (PEF) technique on the extraction of the total anthocyanin content, and the antioxidant activity of purple rice. The extraction treatment was divided into two levels of PEF [low and high PEF]. The low PEF treatment condition used low pulse numbers (0, 100, 300, and 500) at three levels of electric field intensity (2, 3, and 4 kilovolts / centimetres (kV cm^{-1}) in 1 Hz). The high treatment condition used an electric field intensity of 6 kV cm^{-1} in 1 Hz with the pulse numbers of 1,000, 3,000, 4,000, and 5,000. The results indicated that a sample extracted with low PEF showed low anthocyanin content but high % of DPPH inhibition. In contrast, a high level of anthocyanin content and low % of DPPH inhibition were found in the high PEF extraction treatment. The result demonstrated that PEF technology did not cause a chemical change to anthocyanin but affected antioxidant activity. This effect was due to high energy and temperature generated in the high PEF treatment. The PEF technique has a benefit in decreasing the time required for the extraction process and does not negatively affect the anthocyanin extracted. The appropriate PEF extraction condition for purple rice was obtained at 6 kV cm^{-1} with 3,000 pulses, which provided an acceptable total anthocyanin content (24.99 ± 0.92 mg l^{-1}) and was consistent with a percent inhibition of DPPH (60.97 ± 0.64). The results of this study have implications for the ongoing development and use of food-based products.



Keywords: Purple rice; Anthocyanin; Antioxidant; Pulse electric field

1. Introduction

Purple rice (*Oryza sativa* L.) or black rice is a popular local variety for planting and consuming in North and Northeast Thailand. Jaranjit and Suwat found that important organic compounds in purple rice include Gamma-oryzanol and anthocyanin which have benefits for the body by helping to improve body's immune system [1]. Anthocyanin has antioxidant properties, with claims of curing chronic

diseases such as cancer, cardiovascular ailments, and diabetes [2]. A critical feature of purple rice is the color on its pericarp which is composed of anthocyanin particularly; cyanidin hexoside (1%), cyanidin dihexoside (3%), peonidin 3-glucoside (6%), and cyanidin 3-glucoside (90%). Cyanidin 3-glucoside is the major anthocyanin in purple rice [3]. Moreover, purple rice also has other nutrients such as protein, phosphorus, potassium, calcium, iron, and zinc at higher levels than white rice [4]. The components in purple rice seed have beneficial nutrients available in their rice hulls, rice barn and rice barn oils, all of which can be used as functional foods [5]. Thus, purple rice has the potential to be made into many food and cosmetic products because of its benefits. However, anthocyanin in purple rice may have a stability problem that could reduce total anthocyanin and phenolic contents, also antioxidant activity, may be impaired as a result of pH, light, and heat factors [6].

Pulse electric field (PEF) technology is a new challenge for the food industry [7]. PEF technology is a non-thermal technology which could help to prevent the degradation of nutrients and beneficial characteristics during extraction compared to traditional thermal processing [8]. PEF is a key factor for inhibiting growth of micro-organisms responsible for pathogens and spoilage in beverages or lipid foods, thereby assisting the product to maintain a fresh taste [9]. The use of PEF also improves polyphenol, anthocyanin and tannins found in Cabernet franc grapes. Use of PEF in moderate and high intensity treatments resulted in high total polyphenols yield and the highest pigmentation of the extract compared to the control [10].

The objective of this study is to investigate suitable conditions for purple rice extraction using a PEF treatment. The affect on important compounds, including total anthocyanin content and antioxidant activity is the determining measure. The outcome will have application for the further study and development of nutrient benefits from purple rice.

2. Materials and Methods

Materials

Purple rice (*Oryza sativa* L.) was provided by the local farmers at Doi Saket Chiangmai, Thailand. The rice was harvested during November to December 2020, vacuum-packed and stored at room temperature in a humidity-controlled room.

Potassium chloride (KCl) and sodium acetate 3-hydrate ($CH_3COONa \cdot 3H_2O$) were purchased from KemAus (Australia). Hydrochloric acid 37% (HCl) and Methanol were purchased from Qrec (NewZeland). Ethanol was purchased from Duksan (Korea). 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) were purchased from Sigma-Aldrich (Singapore). All chemicals are analytical grade.

Extraction

Samples for testing were prepared in the ratio, purple rice (1 kg) per two litres of extraction solution. The control samples solutions were (a) water, (b) ethanol 50%. The PEF treatment sample was purple rice (1 kg) to two litres of water only. PEF extraction was carried out using a pulse electric system developed by the College of Integrated Science and Technology, Rajamangala University of Technology, Thailand. PEF extraction conditions were achieved by varying the electric field intensity of 2, 3, and 4 kV cm^{-1} at a fixed frequency of 1 Hz and the number of pulses at 100, 300, and 500 pulses (low PEF treatment) and 6 kV cm^{-1} with 1,000, 3,000, 4,000, and 5,000 pulses (high PEF treatment) shown in Fig. 1.

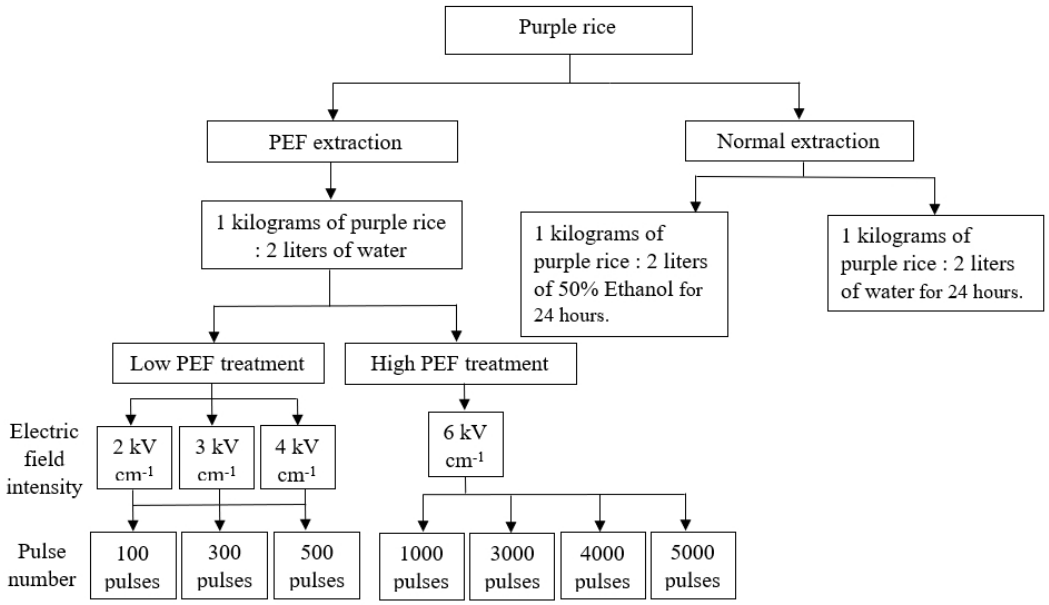


Fig. 1 Extraction process.

Determination of anthocyanin content

Total anthocyanin content was determined by pH differential method. A buffer solution pH1 was prepared by dissolving 1.49 grams of KCl in 100 ml of distilled water and pH adjusted using HCl (1 M) to achieve pH (1 ± 0.05). A buffer solution pH 4.5 was prepared by dissolving 1.64 grams of $CH_3COONa{\cdot}3H_2O$ in 100 ml of distilled water and pH adjusted using HCl (1 M) to achieve a pH (4.5 ± 0.05). Extract samples were prepared by diluting 1 ml of sample with 9 ml of distilled water and centrifuged at 6,000 rpm for 8 minutes (Hettich zentrifugen, EBA 20). A sample of 3 ml was taken from resulting supernatant solution. To this was added buffer solution pH 1 and pH 4.5 up to a total volume of 30 ml. The sample was then incubated for 30 minutes in the absence of light, and at room temperature. The light absorption (A) of each mixture sample was measured using a UV–Vis spectrophotometer at 510 and 700 nm (Spectrum Instruments, SP-UV 200 spectrophotometer) [11]. The total anthocyanin content was calculated using the following Eq. (1) [12].

Where MW is the molecular weight of cyanidin-3-glucoside (449.20 g mol^{-1}), DF is the dilution factor of the sample (DF = 10), and ε is the molar absorptivity coefficient values (ε = 26900 M cm^{-1})

$$\text{Total anthocyanin content (mg } l^{-1}) = \frac{(A510 - A700)pH1 - (A510 - A700)pH4.5 \times MW \times DF \times 1000}{\varepsilon} \quad (1)$$

Determination of antioxidant activity with 2,2-Diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging method.

The antioxidant activity of samples was performed by DPPH free radical scavenging method. The method was modified from Sunan and Sirithon [13] using 1 ml of sample diluted by mixing with 10 ml of methanol. From this mixture, a 1.50 ml sample was mixed with 0.50 ml of DPPH methanolic solution (3.56 mM). The resulting mixture was incubated in the dark for 30 minutes at room temperature. The control solution consisting of 1.50 ml of methanol, 0.50 ml of DPPH solution and 2 ml of methanol served as a blank. The absorbance of each mixture was measured using a UV–Vis spectrophotometer at 517 nm wavelength. The percent inhibition of DPPH was calculated using the following Eq. (2);

Statistical analysis

The experiment was performed in triplicate for each sample. The results were statistically analyzed using one-way ANOVA and Duncan test. Differences were considered statistically significant if p value < 0.05.

3. Results and Discussion

Extraction

Extracted solutions from the purple rice using normal and PEF technology are shown in Fig. 2. The color of the final extract of purple is a dark red-purple solution due to the water-soluble vacuolar of anthocyanin pigment in which coloration is dependent on pH of the extraction medium [14]. From Table 1, the pH of the purple rice extracts is between 5.54 and 6.67 which is similar to the pH of water and ethanol 50% extraction solutions. The yield percentage of purple rice extracts shows a lower level of extract when using low numbers of pulse treatments compared to a high number of pulse treatments.

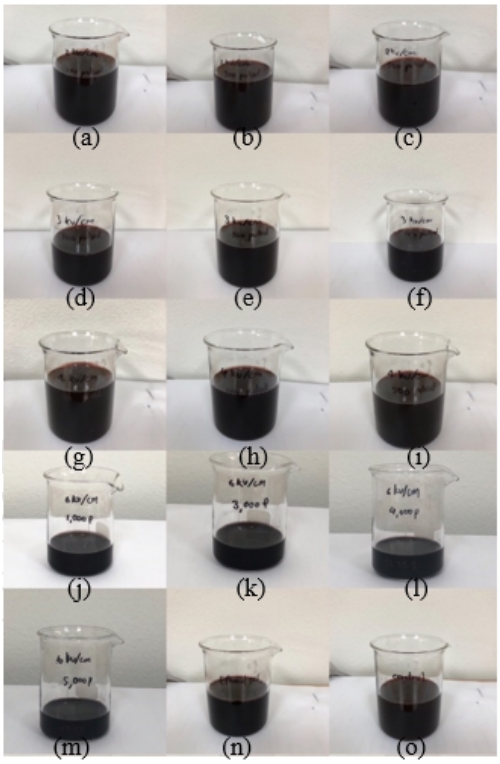


Fig. 2 The appearance of purple rice extracts**.** (a – c) 2 kV cm^{-1} with 100, 300, and 500 pulses, (d – f) 3 kV cm^{-1} with 100, 300, and 500 pulses, (g – i) 4 kV cm^{-1} with 100, 300, and 500 pulses, (j – l) 6 kV cm^{-1} with 1,000, 3,000, 4,000, and 5,000 pulses, (n) ethanol 50%, and (o) water, respectively.

$$\text{Percentage of DPPH inhibition} = \left[\frac{Acontrol - Asample}{Acontrol}\right] \times 100 \quad (2)$$

Table 1 pH and yield percentage of purple rice extracts.

Treatment of electric field intensity	Number of Pulses	pH ± SD	% yield
Water	0	6.23 ± 0.04	49.32
Ethanol 50%	0	6.26 ± 0.03	48.96
2 kV cm^{-1}	100	6.22 ± 0.05	46.76
	300	6.05 ± 0.06	50.55
	500	6.05 ± 0.09	46.00
3 kV cm^{-1}	100	6.22 ± 0.05	47.83
	300	6.25 ± 0.01	48.75
	500	6.05 ± 0.05	48.59
4 kV cm^{-1}	100	5.54 ± 0.31	48.69
	300	6.01 ± 0.12	46.67
	500	6.05 ± 0.04	47.15
6 kV cm^{-1}	1,000	6.50 ± 0.01	73.60
	3,000	6.32 ± 0.01	85.93
	4,000	6.67 ± 0.02	86.28
	5,000	6.56 ± 0.01	77.43

Determination of anthocyanin content

The total anthocyanin content of the samples extracted with PEF technique compared with control water and water/ethanol shown in Table 2. The PEF extraction was achieved by applying 2 levels of electric field intensity and a varying number of pulses. These were low PEF treatment (2, 3, and 4 kV cm^{-1} at 100, 300, and 500 pulses) and high PEF treatment (6 kV cm^{-1} at 1,000, 3,000, 4,000, and 5,000 pulses). It was found that there is no trend in total anthocyanin content of the purple rice extracts extracted across low PEF treatments, however the highest total anthocyanin content of the purple rice extracts for low PEF treatment was obtained at 4 kV cm^{-1} with 300 pulses (11.69 ± 0.44 mg l^{-1}) which was significantly different to the level of extract using ethanol 50% ($p < 0.05$). For high PEF treatment, the highest total anthocyanin content (32.84 ± 1.84 mg l^{-1}) of the purple rice extracts was found at 6 kV cm^{-1} with 5,000 pulses which was not significantly different from the amount extracted by ethanol medium ($p > 0.05$). The extraction method using ethanol 50% presented the highest total anthocyanin content (31.12 ± 2.22 mg l^{-1}) (Table 2). Total anthocyanin content of the purple rice extracts using low PEF treatment were all low but increased when using the high PEF treatment. This reflects a similar result to a study using PEF extraction with red grapes. The PEF extraction condition used a fixed electric field at 1.50 kV cm^{-1} and a varying the number of pulses (0, 1, 5, and 10 msec) corresponding to a total specific energy of 0, 2, 10, and 20 kilojoules / kilogram (kJ kg^{-1}), respectively. The study found that total anthocyanin of the PEF-extracted from samples tended to increase as an increasing of the number of pulses [15]. A similar result was observed in a study of the effect of PEF treatment on Tempranillo grapes. In this study a fixed number of pulses (50) were applied at varied electric fields with total specific energy at 5 kV cm^{-1} with 1.80 kJ kg^{-1} and 10 kV cm^{-1} with 6.70 kJ kg^{-1}. The result found that the application of PEF treatment at 5 kV cm^{-1} with 1.80 kJ kg^{-1} and 10 kV cm^{-1} with 6.70 kJ kg^{-1} increased total anthocyanin content of the extract samples by 21.50% and 28.60% respectively. These results were higher than that of the control sample after maceration for 96 hours [16]. The PEF method was also applied in red cabbage extraction. An electric field of 2.50 kV cm^{-1} and 50 pulses was used as the extraction condition. The result showed that PEF treatment enhanced total anthocyanin content by 2.12 times when compared with the conventional extraction [17]. The finding of

these studies indicate that the number of pulses and electric field intensity is able to encourage the release of anthocyanin pigment by transfer from the moisture mass inside the cellular structure of the sample [18, 19].

Table 2 Total anthocyanin content (mg l^{-1}) of the purple rice extracts using PEF technique.

Treatment of electric field intensity	Number of Pulses	Total anthocyanin pigment (mg l^{-1})
Water	0	9.30 ± 1.11^{def}
Ethanol 50%	0	31.12 ± 2.22^{a}
2 kV cm^{-1}	100	9.85 ± 0.44^{def}
	300	8.68 ± 0.93^{def}
	500	8.63 ± 0.67^{def}
3 kV cm^{-1}	100	9.30 ± 0.48^{def}
	300	10.41 ± 0.75^{de}
	500	7.57 ± 0.67^{ef}
4 kV cm^{-1}	100	6.96 ± 0.70^{f}
	300	11.69 ± 0.44^{d}
	500	10.41 ± 1.28^{de}
6 kV cm^{-1}	1,000	16.09 ± 0.77^{c}
	3,000	24.99 ± 0.92^{b}
	4,000	27.66 ± 1.09^{b}
	5,000	32.84 ± 1.84^{a}

$^{a-h}$*Means in the same column with different superscript differ ($p < 0.05$).*

Determination of antioxidant activity

The antioxidant activity was evaluated by the radical scavenging activity (% of DPPH inhibition). The results (Table 3) show that the percent inhibition of DPPH of all samples extracted with low PEF treatment was higher than that of the water. There was some fluctuating trend in each set of electric field intensity treatment, namely; the percentage of DPPH inhibition of the PEF-extracted samples increased with an increasing number of pulses and then decreased at the highest pulse number. A possible explanation is that the percentage of DPPH inhibition decreased according to the effect of the high temperature of higher pulses number treatment. The highest percentage of DPPH inhibition of the purple rice extracts using low PEF treatment was found at 4 kV cm^{-1} with 300 pulses (74.79 ± 1.45) which was significantly different from the inhibition measure of the sample extracted using ethanol 50% ($p > 0.05$**).** Research by Eleni *et al.* investigated the application of the pulsed electric field (PEF) technique to the production of extracts from Moringa oleifera plant material (freeze-dried leaves). The results found that the percent inhibition of DPPH increased as the number of pulses increased (pulse duration of 10 μs) until it reached the maximum value of the percentage of DPPH inhibition at a pulse duration of 20 msec and then decreased at pulse durations of 40 and 50 msec [20]. A similar result was also found in a study using PEF technology in longan [21]. The experimental result concluded that the PEF technology was less time consuming than a maceration method and reflux extraction method but the antioxidant activity (IC50) decreased by increasing of the number of pulses over time (15 mins = 51.60 ± 1.10 μg ml^{-1}, 30 mins = 42.30 ± 0.60 μg ml^{-1}, and 45 mins = 39.50 ± 0.80 μg ml^{-1}, respectively). The results of this current study are consistent with these other observations that PEF treatment affected the percentage of DPPH inhibition, especially the effect of the number of pulses delivered during the extraction.

For high PEF treatment, there was no fluctuation in results of the percentage of DPPH

inhibition of the purple rice extract samples. The value continuously decreased with an increasing number of pulses [See Table 3]. High levels of pulses generate high energy and temperature which results in the degradation of the percentage of DPPH inhibition. The highest level of the percentage of DPPH inhibition was found at PEF treatment of 6 kV cm^{-1} with 1,000 pulses (67.74 ± 1.88) which was significantly different when compared to results obtained from ethanol 50% extraction ($p > 0.05$).

Table 3 % of DPPH inhibition of the purple rice extracts using PEF technique.

Treatment of electric field intensity	Number of Pulses	% of DPPH inhibition
Water	0	51.27 ± 8.60^{f}
Ethanol 50%	0	66.89 ± 0.43^{bc}
2 kV cm^{-1}	100	68.89 ± 4.48^{b}
	300	61.57 ± 2.22^{d}
	500	54.55 ± 2.94^{ef}
3 kV cm^{-1}	100	62.45 ± 1.61^{cd}
	300	72.05 ± 0.38^{ab}
	500	58.11 ± 1.09^{de}
4 kV cm^{-1}	100	69.37 ± 0.27^{b}
	300	74.79 ± 1.45^{a}
	500	71.61 ± 1.34^{ab}
6 kV cm^{-1}	1,000	67.74 ± 1.88^{b}
	3,000	60.97 ± 0.64^{d}
	4,000	43.50 ± 1.26^{g}
	5,000	33.24 ± 0.43^{h}

$^{a-h}$*Means in the same column with different superscript differ ($p<0.05$).*

Determination of the optimum PEF extraction condition for purple rice

In order to investigate the optimum extraction condition for PEF method, the relationship between total anthocyanin content and % of DPPH inhibition was plotted and shown in Fig. 3. From the graph, it can be seen that the % of DPPH inhibition of all PEF-extracted samples using low PEF treatment were high but total anthocyanin content of all samples were low. However, both these values trend in the opposite direction when using high PEF treatment: the percentage of DPPH inhibition decreased and total anthocyanin increased. This was because high temperature and energy was produced at high electric field intensity and number of pulses, which influence the total anthocyanin content and percent inhibition of DPPH. High PEF promoted the release of anthocyanin pigment from the cell but resulting high temperature also affected antioxidant activity.

The same evidence can be observed in each set of electric field intensity treatment (2, 3, 4, and 6 kV cm^{-1}) that % of DPPH inhibition and total anthocyanin content increased by increasing of the number of pulse but decreased at the highest pulse number. Moreover, an inverse correlation between total anthocyanin content and percent inhibition of DPPH was also explained in a previous study using rice berry bran which reported that there are others antioxidative compound in purple rice anthocyanin was not the main antioxidative compound in purple rice [22]. The optimum PEF extraction condition was determined by considering the optimal level of total anthocyanin content in conjunction with a suitable level of DPPH inhibition. At the PEF condition of 6 kV cm^{-1} at 3,000 pulses, acceptable levels of total anthocyanin content

and percentage of DPPH inhibition, 24.99 ± 0.92 mg l^{-1} and 60.97 ± 0.64, respectively were achieved.

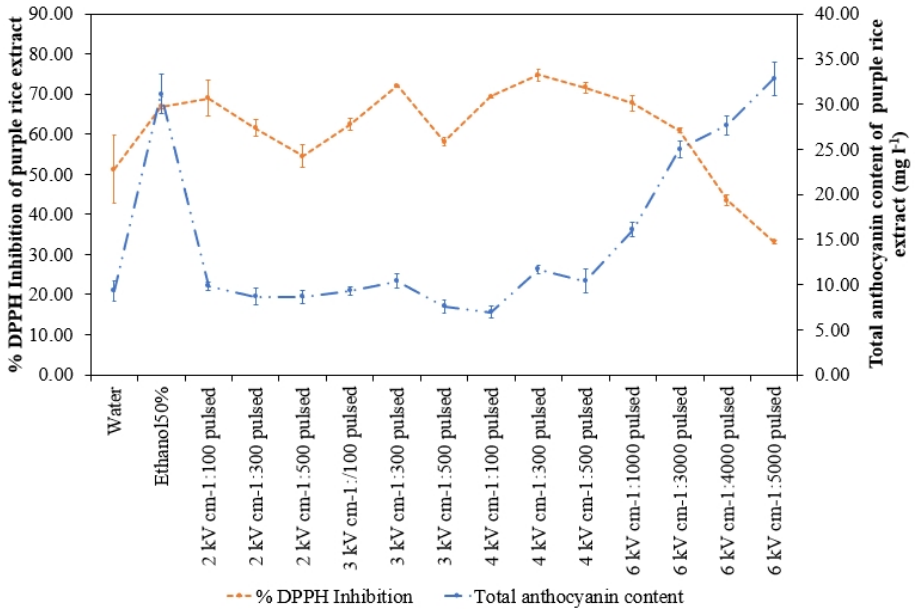


Fig. 3 The relationship between total anthocyanin content (mg l^{-1}) and % of DPPH inhibition of purple rice extracts.

4. Conclusion

The results of this study highlighted that pulse electric field (PEF) technique was suitable for use in extraction of anthocyanin from purple rice. The PEF process improves total anthocyanin content and percent inhibition of DPPH of purple rice extracts compared to the conventional extraction methods. The experimental result showed that PEF treatment did not cause any change of pH or chemical content. However, applying of high electric field intensity and the number of pulses can increase total anthocyanin content but decrease the percent inhibition of DPPH because of an influence of temperature. Consequently, the suitable PEF extraction condition for purple rice was determined as 6 kV cm^{-1} with 3,000 pulses, which is a suitable extraction condition for developing healthy commercial food products.

5. Suggestions

Despite the fact that the findings revealed that the electric intensity and number of pulses have a significant effect on total anthocyanin content and antioxidant activity. The total anthocyanin content and antioxidant activity, on the other hand, were unrelated. As a result, the extraction conditions will depend on whether anthocyanin content or antioxidant activity is required.

6. Acknowledgement

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