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Comparison of pulse electric field, microwave and ultrasonic pretreatment prior to black rice extraction on antioxidant and sirtuin1 enzyme stimulating activities

Nuttinee SALEE[1*], Srisuwan NARUENARTWONGSAKUL[2], Wantida CHAIYANA[3], Artit YAWOOTTI[4], Kanrawee HUNSAKUL[1], Bow TINPOVONG[5], Niramon UTAMA-ANG[1*]

Abstract

The objective of this research was to investigate the effects of pulse electric field, microwave, and ultrasonic assisted water extractions on the bioactive compounds in black rice, and their antioxidant and sirtuin1 enzyme stimulating activities. The number of pulses were changed from 1,000 to 2,000 and 3,000 at a constant electric field intensity of 5 kV/cm. Microwave and ultrasonic conditions were followed by 800 W for 20 min and 60 °C for 20 min, respectively. The results found that using pulse electric field with 3,000 pulses and microwave achieved high concentrations of total phenolic compound and cyanidin-3-glucoside with non-significantly difference. Extracts from the pulse electric field with 3,000 pulses showed the significantly highest DPPH scavenging activity (30.83 ± 2.06%) and the sirtuin1 enzyme stimulating activity (27.260 ± 0.418 of fluorescence intensity) when compared with microwave and ultrasonic extractions. Scanning electron microscopy and fourier transform infrared spectroscopy results showed distinct differences between the effects of these methods on the outer surface. The overall results revealed that pulse electric field and microwave were similarly effective at increasing the content of bioactive compounds in extract and their antioxidant activities. However, the pulse electric field was considerably more effective for activating sirtuin1 enzyme activity.

Keywords: pulse electric field; microwave; ultrasonic; black rice grain; antioxidant activity; surtuin1 enzyme.

Practical Application: In the food and pharmaceutical industries, pulse electric field and microwave can be applied as novel technologies to improve extraction yield and health-promoting benefits properties of extract prior to black rice extraction.

1 Introduction

In Southeast Asian countries, pigmented rice has been produced and consumed for centuries (Wongsa, 2020). The colored rice is coated with pericarp layers of diverse colors, such as black and dark purple. Amrinola et al. (2022) reviewed that the rice bran layer was abundant in bioactive compounds, including anthocyanins, proanthocyanins flavonoids and phenolic compounds, and also minerals, essential fatty acids, and essential amino acids. Black rice (*Oryza sativa* L.) is a type of pigmented rice that contains significant amounts of anthocyanins in the bran layer, and is regarded as an effective health-promoting functional food. Anthocyanin from black rice was identified by cyanidin-3-glucoside (C3G) and peonidin-3-glucoside (P3G) (Salee et al., 2022) which demonstrated scientifically proven health benefits, as well as promoted immune responses in leukemia (Fan et al., 2017), anti-diabetes and hyperlipidemia (Yang et al., 2011; Zhang et al., 2013) and cholesterol and lipid metabolism. Colored rice could affect the expansion of life-span by reducing the reactive oxygen species (ROS) via Sir2-dependent signaling pathways, with human homologs of NAD-dependent deacetylase sirtuin-1 (surtuin1) (Sunthonkun et al., 2019). The enzymatic activity of sirtuin1 could influence gene expression, DNA repair, metabolism, the response to oxidative stress, mitochondrial function, and even biogenesis. Several human diseases, including cancer, diabetes, and cardiovascular disease, may have their origins within the degeneration of specific tissues due to their unusual activity (Iside et al., 2020). The effectiveness of the surtuin1 modulator could be demonstrated through sirtuin1 enzyme-stimulating activities. Literature suggested that a surtuin1 modulator could be a natural polyphenol extract from roots of codonopsis pilosula, kaempferia parviflora, drosophila melanogaster and fruits of lycium ruthenicun (Kayashima et al., 2017; Nakata et al., 2014; Qi et al., 2018; Zheng et al., 2018).

As a result of their lower toxicity compared to that of methanol and hydrochloric acid, ethanol and water acidified with acetic acid and organic acids were extensively used as solvents for anthocyanins and other bioactive chemicals extraction from black rice (Escribano-Bailón et al., 2004). In addition, water was considered as an optional new and environmentally friendly solvent for the extraction of bioactive compounds, as well as anthocyanins from black rice (He et al., 2017; Prommachart et al., 2020; Salee et al., 2022). Furthermore, extraction procedures that use water as a solvent were linked to an increase in environmental and health concerns (Carpentieri et al., 2021). Non-convention processing has been interesting for extraction methods that use less solvent, short time and increase extraction yield (Roobab et al.,

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[1] *Division of Product Development Technology, Faculty of Agro Industry, Chiang Mai University, Chiang Mai, Thailand*
[2] *Division of Food Engineering Development Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand*
[3] *Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand*
[4] *Department of Electrical Engineering, Faculty of Engineering, Rajamangala University of Technology Lanna, Chiang Mai, Thailand*
[5] *Program of Food Production and Innovation, College of Integrated Science and Technology, Rajamangala University of Technology Lanna, Chiang Mai, Thailand*
*Corresponding author: nattinee@rmutl.ac.th; niramon.u@cmu.ac.th

2018; Wijngaard et al., 2012). Despite this, there are gaps in the data of the comparisons of non-conventional water extraction methods used for the anthocyanin extraction from black rice.

In this research, we examined three non-conventional water extraction methods; Pulse electric field (PEF), Microwave (MAE) and Ultrasonic (UAE). PEF technology involves applying pulses of high voltage electricity, typically with the strong electric fields (Hernández-Hernández et al., 2019). Applying an external electric field induces electroporation, which generates pores in the cell membrane and enables bioactive compounds diffuse from plant tissue (Lakka et al., 2021; Zderic & Zondervan, 2016). MAE is nonionizing electromagnetic radiation between 300 MHz to 300 GHz (Chuyen et al., 2018). These radiations are delivered as waves that are capable of penetrating the matrix and acting directly on polar molecules like water to generate heat via dipole rotation and ionic conduction (Zia et al., 2022). UAE are high frequency (>20 kHz) waves. These waves passing through a medium induces compression and expansion, causing a cavitation effect (Garavand et al., 2019). All techniques were designed to improve the quantity of C3G and P3G which could be extracted from black rice. The antioxidant properties of the extract, which included diphenyl-picryhydrazyl (DPPH) radical scavenging, 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation decolorization, and ferric ion reducing antioxidant power (FRAP) were measured and compared. Black rice extract was also investigated by in vitro assay for its potential to activate sirtuin1 enzyme stimulation.

2 Materials and methods

2.1 Materials

Black rice (*Oryza sativa* L.) was harvested in 2018, Northern Thailand. It was also de-husked by rice milling machines and freezed at -20 °C prior, for experimental purposes.

2.2 Reagents, solvents and standards

The standards of C3G and P3G were acquired from S.M. Chemical Supplies Co.,Ltd. (Bangkok, Thailand). The sirtuin1 Activity Assay Kit (Fluorometric) ab156065 was obtained from Abcam, USA and was used according to the instructions of the manufacturer. All additional reagents and chemicals were commercially accessible and to an analytical standard.

2.3 Assisted water extraction methods

The extraction ratio of black rice and water was fixed at 0.5 g/mL. Prior to the water extraction, the effects of treatments were determined on black rice.

Pulse electric field-assisted water extraction (PEF)

The electric field strength (I) was generated at 5 kV/cm with 1 μs of constant pulse width, and 5 Hz of pulse repetition frequency. The PEF apparatus is shown in Figure 1. The electric field generator was powered by 500 W with 220 VAC of voltage and 50 Hz of frequency. The energy storage capacitor had a capacitance of 0.1 μF, and a voltage of 20 kV. A high voltage electric field with specification of 0–10 kV/cm and pulse length

Figure 1. The pulse electric field apparatus, located at the Faculty of Engineering, Rajamangala University of Technology Lanna, Chiang Mai, Thailand.

of 1s was then supplied to the extraction chamber by switching the spinning gap. The electric field strength was controlled as 5 kV/cm which chosen via the condition of anthocyanin extraction from purple potato (Puértolas et al., 2013). The number of pulses (Np) was one of the effective pretreatment factors since it affected the extraction time and temperature. Np was varied at 1,000 (PEF1) 2,000 (PEF2) and 3,000 (PEF3) pulses. The mixture of black rice and water was accurately weighed and placed into a PEF chamber.

Microwave-assisted water extraction (MAE)

Microwave-assisted extraction was performed with 220 V and 50 Hz (ME711K, Samsung, Bangkok, Thailand). The mixture of black rice and water was placed into an extraction bowl. Microwave conditions were at 800 W for 20 minutes, with slight modification conditions of Setyaningsih et al. (2015) for phenolic compounds extraction from black rice.

Ultrasonic-assisted water extraction (UAE)

Ultrasonic device (Bath type, D-78224 Singen/Htw, Elma, Germany) 550 W and 50/60 Hz was conducted prior to extraction. A mixture of black rice and water was placed into a beaker and settled in an ultrasonic bath. The extraction temperature and time were set to 60 °C for 20 minutes, with a few minor modifications of Surin et al. (2020) which was extraction condition of purple glutinous rice bran (*Oryza sativa* L.).

2.4 Water extraction methods

After the assissted-extraction water process, the water extraction was followed. The mixture was immediately poured into an Erlenmeyer flask for continuous extraction. The duration time of water extraction was 6 h and it was shaken on an electrical shaker (Unimax2010, Heidolph) at 150 rpm. The mixture solution was filtered by Whatman® No.4 filter paper (Merck, Germany). The filtrate solution was freeze-dried and then kept at -20 °C until analysis.

2.5 Overall experimental design

The overall experimental design was illustrated in Figure 2. Initially, black rice grain was subjected to a pulse electric field under conditions of PEF1, PEF2 and PEF3. Additionally, MAE and UAE were also studied. A sample was collected to evaluate the effect on the outer surface via SEM and FTIR. After water extraction, the extract solution was filtered and respectively freeze-dried. The obtained extract was analyzed.

2.6 Analytical analysis of black rice extract

Determination of total phenolic content (TPC)

TPC of black rice extracts were measured by the Folin–Ciocalteu colorimetric followed method of Chaiyana et al. (2017). Summarily, the extract solution with a concentration of 0.02 mg/mL (20 µL) was combined completely with 100 µL of Folin–Ciocalteu reagent (1:10 dilution) in a 96-well plate for 4 min, followed by added 80 µL of 7.5% (w/v) Na_2CO_3. The mixture was allowed in the dark for 2 h. The absorbance of samples was determined at 760 nm using a microplate reader (DTX880, Beckman Coulter, Austria). The result of TPC was calculated from a callibration curve, and represented as mg of gallic acid equivalent per gram of extract (GAE/ g).

Determination of antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging activity of the extracts was examined using a method with slight modifications from Chaiyana et al. (2017). In brief, 20 µL of 0.001 mg/mL extract solution was blended with 180 µL of the DPPH solution and standed for 30 minutes in the dark during incubation. The absorbance of the mixture solutions was measured at 520 nm. The capability of the scavenge DPPH radical of sample was calculated using the following Equation 1:

$$\%\ DPPH\ scavenging\ activity\ =\ \left(\left(C\ -\ S\right)\ /\ C\right)\ x\ 100\% \quad (1)$$

where S represents UV absorbance of the sample, and C represents UV absorbance of control solution.

ABTS radical scavenging activity

Scavenging activity against ABTS radicals of sample was examined using an ABTS assay following a procedure used by Chaiyana et al. (2017). The ABTS solution was produced by a mixture of of 2.45 mM of $K_2S_2O_8$ and 7 mM of ABTS solution with a ratio of 2:3 (v/v), and then incubated for 24 h in the dark. On the analysis, 20 µL of each sample was combined with 180 µL of ABTS solution in a 96-well plate and allowed at room temperature for 5 minutes. The absorbance of the sample was measured at 750 nm using a microplate reader. Trolox was used to setup the standard curve. The ABTS scavenging activity of the sample was represented as the amount of Trolox equivalent per gram of extract (mol Trolox/g).

Ferric reducing antioxidant power (FRAP)

Ferric reducing power of sample was evaluated in accordance with Chaiyana et al. (2017). The solution of 10 mM 2,4,6-TPTZ in 40 mM HCl (1 mL) was combined with 20 mM ferric chloride solution (1 mL) and 0.3 M acetate buffer (10 mL) to produce a fresh FRAP solution (pH3.6). FRAP assay method was performed

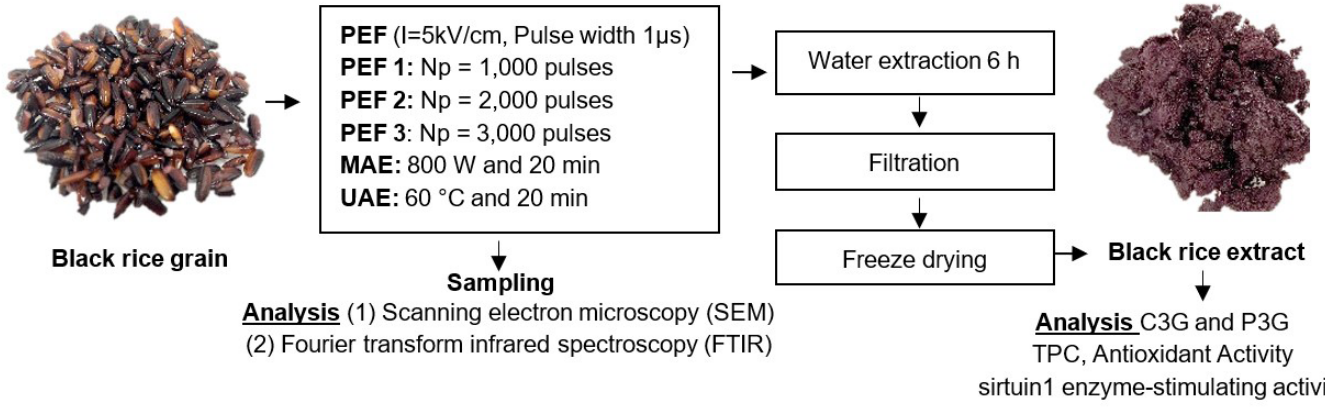

Figure 2. The overall experimental design.

by added extract solution (20 µL) in FRAP solution (180 µL). The mixture solution was blended homogeneously, and then incubated in a dark area for 5 minutes. The standard curve was created using $FeSO_4$. The ferric reducing capacity was measured as the amount of $FeSO_4$ equivalent per gram of extract (mmol $FeSO_4$/g).

Determination of C3G and P3G content

High-performance liquid chromatography was applied to separate and also to measure the amount of C3G and P3G in the extract (HPLC, Agilent Technologies, Santa Clara, CA, USA), and using a C18 rapid resolution column. The mobile phase consisted of water, methanol, and formic acid in the proportions of 75:18:7 v/v, with isocratic elution at a flow rate of 0.5 mL/min. C3G and P3G were separated and detected at 510 nm (Settapramote et al., 2018).

Determination of the surtuin1 enzyme-stimulating activity

The surtuin1 Activity Assa Kit (Fluorometric) of Abcam (ab156065) was used to evaluate the surtuin1 enzyme-stimulating activity of extract in accordance with the instructions from manufacturer. The in vitro assay of surtuin1 enzyme-stimulating activity of extract was examined according to Salee et al. (2022). The mixture solution of water HPLC-grade (25 µL), surtuin1 assay buffer (5 µL), fluoro-substrate peptide (5 µL) and NAD (+)-dependent histone deacetylase (5 µL) was added and mixed gently to the wells of a microtiter plate. After that, 5 µL of an extract solution was added to each well and mixed homogenized. The initial reaction was started by adding the developer solution (5 µL), and thoroughly mixing it together at room temperature. Microplate fluorimeters (SpectraMax M3, Molecular Devices, USA) with excitation wavelengths of 360 nm and emission wavelengths of 485 nm, were used to measure the intensity of fluorescence for 60 minutes at 2-minute intervals. The fluorescent intensity was measured while the reaction velocity remains constant. The surtuin1 enzyme-stimulating activity of the extract was calculated by dividing the fluorescence intensity of the extract solution by the fluorescent intensity of the control solution, and stated in terms of the ratio of fluorescent intensities (FIR).

2.7 Experimental design and statistical analysis

The experimental design consisted of three replications conducted by completely random design. The mean standard deviation of three duplicate results was provided. Analysis of variance (ANOVA) with Duncan's multiple range test (DMRT) were conducted using SPSS (Version 17, SPSS Inc., Chicago, IL, USA) at a significance level of 95%.

2.8 Scanning electron microscopy (SEM)

The scanning electron microscope (Prisma E, thermo scientific, USA) was used to visually show the impact of each assisted-extraction technique on the surface microstructure. Samples were affixed to the aluminum studs with double-sided adhesive tape, and observed by SEM at a magnification of 3,000x under an acceleration voltage of 10 kV.

2.9 Fourier transform infrared (FT-IR) spectroscopy analysis

FT-IR Spectrometer (FT/IR-4700, Jasco International Co. Ltd., Tokyo, Japan) with a wavenumber range of 4000-500 cm^{-1} and a resolution of 4 cm^{-1} were used to investigate the functional groups of the bioactive compounds present on the outer surface. The transmission on outer surfaces of untreated and treated black rice by assisted water extraction were measured.

3 Results and discussion

3.1 Effect of assisted-water extraction methods on TPC and antioxidant activities

Table 1 displays the TPC and antioxidant activities of extracts which were produced using different of assisted-water extraction techniques.

There was no statistically significant difference between the TPC of extracts extracted using PEF3 and MAE. The TPC correlated with anthocyanin content in Table 2, as C3G and P3G were the large subgroup of phenolic compounds identified in rice bran (Chen et al., 2017; Settapramote et al., 2018). For the PEF, increasing the number of pulses with a constant electric field strength could increase the content of extractable polyphenols from black rice similarly to previously study (Ciulu et al., 2018; Quagliariello et al., 2016). The results illustrated that enhancing the number of pulses from 1,000 to 3,000 significantly increased the TPC. The number of pulses was related to the extraction time; therefore, a high number of pulses indicated an extended extraction time. This could increase the contact time between the solvent and the rice bran. Essentially, the extraction efficiency of the phenolic compounds found in rice bran could also increase (Kim, 2017). Increasing the number of pulses could also improve membrane disruption and electroporation on the outer surface

Table 1. TPC and antioxidant activities of extracts with different assisted extractions.

Methods	Conditions	TPC (mg GAE/g)	DPPH (% inhibition)	ABTS (mol Trolox/g)	FRAP (mmol $FeSO_4$/g)
PEF 1	I=5 kV, Np=1,000 pulses	417.173 ± 3.235^{b}	26.36 ± 0.06^{cd}	5.820 ± 0.034^{bc}	7.404 ± 0.081^{a}
PEF 2	I=5 kV, Np=2,000 pulses	424.235 ± 1.842^{b}	27.17 ± 0.76^{bc}	5.751 ± 0.204^{cd}	7.400 ± 0.051^{a}
PEF 3	I=5 kV, Np=3,000 pulses	486.866 ± 8.442^{a}	30.83 ± 0.45^{a}	6.253 ± 0.025^{a}	7.674 ± 0.177^{a}
MAE	800 W, 20 min	486.252 ± 8.306^{a}	28.36 ± 0.94^{b}	6.010 ± 0.060^{ab}	7.408 ± 0.108^{a}
UAE	60 °C, 20 min	327.832 ± 1.064^{c}	25.36 ± 1.04^{d}	5.516 ± 0.232^{d}	6.625 ± 0.271^{b}
Control	No pretreatment	287.306 ± 4.636^{d}	21.28 ± 0.79^{e}	4.156 ± 0.666^{e}	6.062 ± 0.044^{c}

Means of three replicates experimental (mean ± SD). $^{a-e}$Different superscript letters indicate statistically significant differences at P≤ 0.05 for values within the same column.

Table 2. C3G and P3G of black rice extract with different assisted-extraction methods.

Methods	Conditions	C3G (mg/g extract)	P3G (mg/g extract)
PEF 1	I=5 kV, Np=1,000 pulse	78.602 ± 2.162^{b}	4.741 ± 0.107^{ab}
PEF 2	I=5 kV, Np=2,000 pulse	83.403 ± 1.324^{ab}	4.794 ± 0.020^{ab}
PEF 3	I=5 kV, Np=3,000 pulse	89.566 ± 3.272^{a}	4.914 ± 0.144^{a}
MAE	800 W, 20 min	88.543 ± 0.945^{a}	4.613 ± 0.208^{bc}
UAE	60 °C, 20 min	59.738 ± 7.206^{c}	4.420 ± 0.012^{c}
Control	No pretreatment	43.267 ± 5.353^{d}	3.537 ± 0.268^{d}

Means of three replicated experiments (mean ± SD). $^{a-d}$Different superscript letters indicate statistically significant differences at P≤ 0.05 for values within the same column.

of rice grains. This resulted in the transfer of intracellular components or phenolic compounds from the ruptured cell to the solvent, which was associated with the increasing of TPC in the extract (Parniakov et al., 2015; Toumi et al., 2022). Nonetheless, Zhou et al. (2015) found that increasing the number of pulses by more than 10 pulses under high electric field strength (>20 kV/cm) reduced TPC extraction. Energy was rapidly transferred to the black rice matrix and absorbed by a particular polyphenol inside rice bran, which could explain for the increased TPC values observed with MAE. According to its absorption of microwave radiation and subsequent transformation into heat, the moisture started to evaporation. The pressure building within the cell wall caused by evaporation of water finally results in cell rupture, allowing the leaching out of active components into the contacted solvent and enhancing extraction yield (Ballard et al., 2010; Zhang et al., 2011). UAE assisted in isolating intracellular components by dissolving the cell wall structure of black rice via cavitation and mechanical interactions (Iscimen & Hayta, 2018). Moreover, there was a considerable correlation between TPC values and antioxidant activity, indicating that phenolic chemicals greatly contributed. The PEF3 extract demonstrated the greatest antioxidant activity in all three assays. MAE yielded similar findings to PEF, with the exception of DPPH assay, which yielded significantly lower results. The obtained result correlated with the C3G and P3G concentration of extract in Table 2. C3G and P3G have a unique antioxidant activity, possibly because the quantity and location of hydroxyl groups in the B ring affects the antioxidant activity (Aqil et al., 2014; Buraidah et al., 2011). The higher of C3G and P3G content of extract from PEF3 demonstrated a greater ability to scavenge DPPH free radicals than MAE extract which, similarly to the result of Siquet et al. (2006), explained the ability to scavenge free radicals was proportional to the amount and position of hydroxyl groups in phenolic compounds. Comparing the ABTS assay, the extract obtained by PEF3 and MAE exhibited greater antioxidant capabilities than the extract with UAE and no assisted-extraction. The finding regarding TPC in the extract was based on an electron transfer. Nonetheless, the lower concentration of P3G in the MAE-obtained extract had no difference on the antioxidant capacity of ABTS when compared with PEF3. Attributed to the reason that high content of pigmented antioxidant compounds is found in a variety of plant foods, the ABTS assay performed better than the DPPH assay (Floegel et al., 2011). The strongest of ferric reducing antioxidant power of extract obtained by PEF and MAE. The results indicated a strong positive of FRAP correlated between TPC and polyphenolic compounds, similar to the findings of Yu et al. (2022) and Chávez Santiago et al. (2022).

3.2 Effect of different assisted-extraction methods on C3G and P3G contents

The concentration of C3G and P3G obtained by different procedures were determined based on the results of an HPLC analysis, as shown in Table 2.

From the results, the use of PEF2, PEF3 and MAE extraction led to the significantly highest yields of C3G content which offered 83.403 ± 1.324, 89.566 ± 3.272 and 88.543 ± 0.945 mg of C3G/g of extract, respectively. While black rice extract obtained from PEF3 contained more P3G than extract from MAE and UAE. The highest yield of P3G (4.914 ± 0.144 mg of P3G/g extract) was achieved from PEF under an electric field strength of 5 kV/cm, and a number of pulses of 3,000 pulses. Compared to the effect of PEF, MAE and UAE indicated that the anthocyanin content depended on extraction mechanism and condition. For PEF, moderate electric intensity (<10 kV/cm) (Aşık-Canbaz et al., 2022) and repetitive short duration pulses (1 μs) were carried out to extract anthocyanin from black rice. By the reason of the high voltage of electric field induced permeabilization of cell membranes by pores (Raso et al., 2016), and also micro-pores on the surface of rice grain (Qiu et al., 2021). Anthocyanins were located in the pericarp layer. Yoshimura et al. (2012) validated the distribution of C3G and P3G in the pericarp and seed coat layers. The mass transport between anthocyanin and water could be enhanced through micro-pores at the surface. Therefore, this mechanism led to enhance the yield of C3G and P3G in the extract. Moreover, the moisture content of raw materials was related to the efficiency of PEF because of their electrical conductivity. Electrical conductivity is the ability of electrical charges to move through a material when subjected to PEF. Electrical conductivity of food material has been found to be increasing with moisture content and temperature (Banti, 2020; Nowacka et al., 2019). The moisture content of black rice under this study was relatively low (14-15%). Therefore, black rice grains should be filled with moisture to enhance the electrical conductivity before being treated by PEF for future study. Moreover, PEF was also relevant to the enhancement of anthocyanin extraction from variety of food resources such as blueberry, grape, and plum peel (Barba et al., 2015; Medina-Meza et al., 2016; Zhou et al., 2015). Microwave irradiation's penetrating can breakdown cell structure and lead to water diffusion to anthocyanin in rice pericarp for solid-liquid extraction. Moreover, anthocyanin extraction can be enhanced because of the water temperature

increase during microwave heating. Duan et al. (2015) analyzed Chinese bayberry extract by HPLC-DAD-ESI-MS analysis and found that C3G was hydrolyzed to cyanidin during the MAE extraction procedure. However, the extraction process did not significantly affect the antioxidant activity because cyanidin was released. The results exhibited that the amount of anthocyains extracted from UAE was less than that obtained by PEF and MAE under similar extraction time. Ultrasound waves during UAE are attributed to mechanical effects of cell disruption on black rice pericarp, and increased solvent diffusion leading to enhance ultrasonic capillary effect (Thakur et al., 2022). These effects are similar to the effects UAE on other plant materials (Yin et al., 2022; Zhong & Wang, 2010). Pham et al. (2022) demonstrated that the UAE requires additional time when compared to the effectiveness of MAE. Therefore, UAE by ultrasonic bath at 60 °C can enhance the yield of anthocyanin extract from black rice if the time of extraction was extended for more than 20 minutes. In addition, temperature was the most important factor during anthocyanin extraction. The temperature during MAE in this study was 75.5 ± 0.6 °C. It led to some thermal degradation of the anthocyanin although there was a high amount of compound leaching out of the outer surface from irradiation's penetrating. The temperature during PEF with moderate electric intensity and short duration pulses was 48.0 ± 0.6 °C, so the thermal degradation of the anthocyanin may have been less affected than with MAE. For the important reason of thermal degradation of the anthocyanin, the results of He et al. (2017) and Figueiredo et al. (1996) found that anthocyanins were evenly oxidized at temperatures higher than 50 °C, therefore it was appropriate to select 50 °C as the optimum process temperature. For the purpose of enhancing the anthocyanin extraction from black rice, it was necessary to consider both the mechanism of each technique and the extraction temperature. The results of the comparison of extraction with no treatment showed that PEF, MAE, and UAE made it highly effective to get C3G and P3G out of the pericarp. The overall effect of PEF3 and MAE on increasing of C3G and P3G contents were approximately 2.05 and 1.34 times respectively. In addition, the UAE could increase the amount of C3G and P3G by approximately 1.38 and 1.25 time.

3.3 Effect of assisted-extraction methods on surtuin1 enzyme-stimulating activity

Figure 3 illustrates the effect of assisted-extraction techniques on the enzyme-stimulating activity of surtuin1. As depicted in Figure 3, the maximum surtuin1 enzyme-stimulating activity (27.26 ± 0.41 of fluorescence intensity) was obtained when PEF 3 was utilized. The fluorescence intensity of the surtuin1 enzyme-stimulating activity of the MAE and UAE techniques were 23.99 ± 0.48 and 19.40 ±0.11 of fluorescence intensity, respectively. The surtuin1 enzyme-stimulating activity was found to be related to the C3G content of black rice, as shown in Table 2. As evidenced by the data, a quantifiable change in the concentration of C3G stimulated surtuin1 enzyme activity.

This was attributed to the induction of peroxisome proliferator-activated receptor gamma coactivator 1-alpha by C3G (PGC-1a activity). This increase in PGC-1a gene expression was accompanied by an increase in surtuin1 gene expression. (Mogalli et al., 2018). In addition, Quagliariello et al. (2016) revealed that PEF treatment efficiently extracted various components, including polyphenol and phenolic acid, from brown rice. This may be evidence that PEF3 extract can extract polyphenol and phenolic acid derivatives that are effective as surtuin 1 activators due to the role of phenolic compounds in inducing surtuin 1 activity (Corbi et al., 2018). Therefore, the other phenolic compounds in black rice, excluding C3G and P3G, should be identified for future research to support the extract as a surtuin 1 activator.

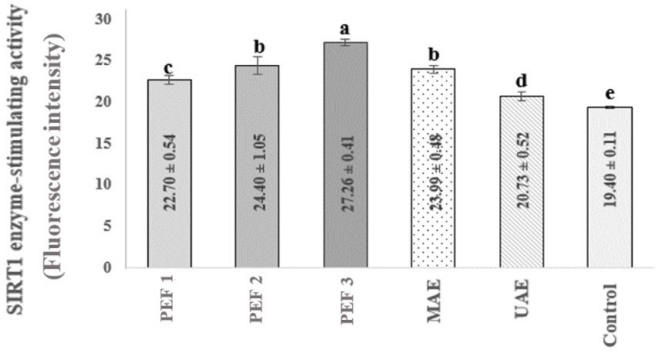

Figure 3. Effect of assisted-extraction on surtuin1 enzyme-stimulating activity. [a-e]Different letters indicate statistically significant differences at P≤ 0.05.

3.4 Effect of assisted-extraction techniques on the grain surface of black rice

Figure 4 shows that the SEM results show that the different methods had different effects on the surface of the black rice. As seen in Figure 4a, the outside surface of untreated black rice appeared to be smooth and matte. Figure 4b shows the UAE approach, some areas of the surface were damaged and resembled the polygonal ridges particle leached from rice kernels, whereas some areas were slightly damaged. As a result of sound waves passing through the rice bran, the outer coating cavitation phenomena occurs which evenly heats the entire sample during the extraction method (Wen et al., 2018). The outer surface after MAE method shows in Figure 4c that the surface was extensively destroyed, and that there were enormous holes and what appears to be a starch particle leaking out, and also that some areas have an inflated appearance. The issue was caused by microwave heating, which resulted in the opening of the cell walls of a portion of the kernel, which may had increased the rate of water penetration (Le et al., 2014). Furthermore, the heat was produced by the interaction between the radiation and the molecules or extracted substances in rice bran, as the molecules sought to align with the waves of the electromagnetic field (Chan et al., 2011; Setyaningsih et al., 2015). Figures 4d illustrate pericarp attached by PEF. The rough skin and both deep and shallow holes were observed. Due to water diffusing into the rice kernel, the permeability index and electrical conductivity between electrodes and rice grain increased (Quagliariello et al., 2016). Therefore, anthocyanin species and bioactive compounds localized throughout the pericarp could be easily leached out (Yoshimura et al., 2012).

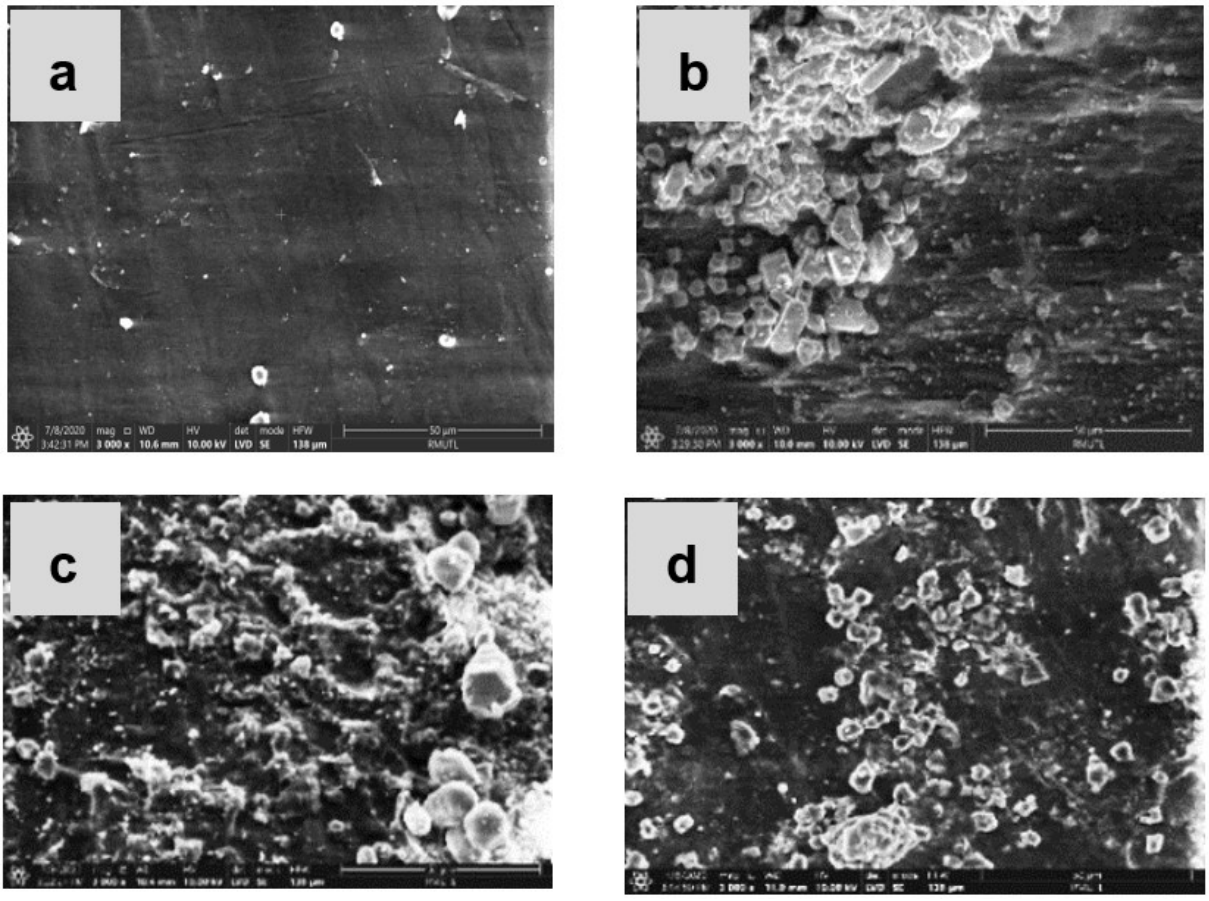

Figure 4. SEM of black rice surface. (a) control sample or non-pretreatment and through different pretreatment (b) UAE, (c) MAE and (d) PEF.

The results of the FT-IR analysis depicted in Figure 5 approved the use of a different extraction method to improve extraction efficiency. Before and after extraction, the hydroxy groups exhibited peak vibrations at ~ 3292 cm^{-1} (O–H stretching) that were representative of the phenolic ring (Jia et al., 2017; Maylinda et al., 2019). The peak intensities of PEF and MAE were lower than those of UAE and non-treated samples, which indicated a lower concentration of residual hydroxy groups of phenols on the outer surface. This result is related to the TPC, C3G and P3G content of the extract, which can be found in Tables 1 and 2. In addition, similar trends of vibration band reduction were observed at ~ 994 cm^{-1} (C=C bending), ~ 1744 cm^{-1} (C-H bending), and ~ 2922 cm^{-1} (C-H stretching) (Fatchiyah et al., 2020).

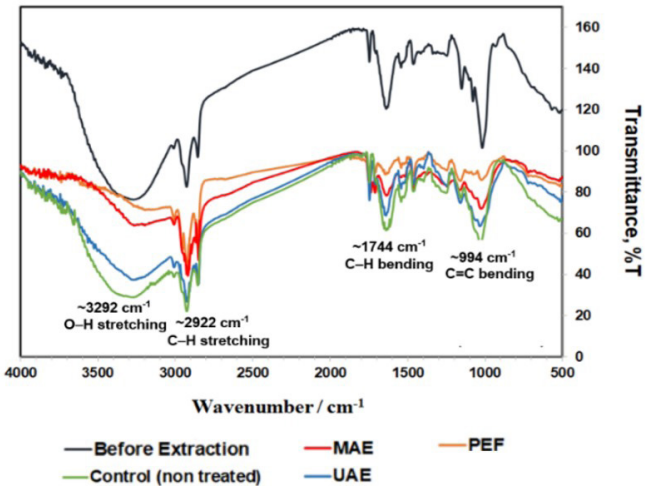

Figure 5. FTIR spectra of outer surface of black rice before extraction, non-pretreated and through different pretreatments.

4 Conclusions

The result can be summarized by the extract from PEF3 and MAE treatment, which contributes to significantly higher TPC and antioxidant activities than UAE treatment. PEF3 produced extract with the highest yield of C3G, P3G when compared with MAE and UAE. Surprisingly, the extract obtained from PEF3 stimulated sirtuin1 enzyme activity significantly more effectively than MAE and UAE, which corresponds to the amount of surtuin 1 activator as C3G in the extract. We propose that PEF can effectively produce black rice extract with powerful and effective antioxidant and sirtuin1 enzyme-stimulating properties. In addition, PEF was applicable to non-conventional extraction procedures in plant materials that can be used in the food industry to improve the biological and nutritional qualities, as well as the recovery of anthocyanins or bioactive chemicals from food by-products.

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