Utilization of [Bmim]BF$_4$-MAE on enhancement of γ-oryzanol extraction from rice bran and its tyrosinase inhibitory activity

Arif Arrahman[ID][1], Efendy Sigalingging[3], Elsa Trinovita[2], Fadlina Chany Saputri[4], Abdul Mun'im[3]*

[1]Department of Pharmaceutical-Medicinal Chemistry and Bioanalysis, Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia, [2]Graduate Program of Herbal Medicine, Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia, [3]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia, [4]Department of Pharmacology and Toxycology, Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia

This study investigated the effect of ionic liquid (IL)-microwave assisted extraction (MAE) on γ-oryzanol extraction from rice bran (*Oryza sativa* L.). The conditions included the concentration of IL, liquid (IL)/solid (sample) ratio, extraction time, and microwave power. The use of 1-butyl-3-methylimidazolium ([Bmim]BF$_4$) as a selected solvent in IL-MAE method was developed for extraction of γ-oryzanol from rice bran. The Box-Behnken design on four factors with response surface methodology (RSM) was used to optimize the experimental conditions. The optimum conditions for the MAE were a [Bmim]BF$_4$ concentration of 0.7 M, liquid/solid ratio of 15 mL/g, extraction time of 10 minutes, and 30% microwave power with a γ-oryzanol value of 0.41 mg/g. The experimental values agreed with those predicted by the RSM models. A microplate reader method was used to determine the IC$_{50}$ value in tyrosinase inhibitory activity of the extract. An IC$_{50}$ value of the extract obtained from IL-MAE was 1240 μg/mL. This study suggests that IL-MAE is suitable for the extraction of γ-oryzanol from rice bran and that under the optimum conditions the extract has low activity as a tyrosinase inhibitor.

Keywords [Bmim]BF$_4$. Ionic liquid. IL-MAE. γ-oryzanol. Tyrosinase.

INTRODUCTION

Indonesia is known as agricultural country, and paddy is one of its most significant agricultural commodities. As paddy production has increased, the process of milling paddy into rice has expanded, and many by-products have been created. One of the by-products of rice milling is rice bran. In previous research, rice bran was used as a raw material in the extraction process because it has many bioactive compounds and antioxidants, such as tocopherol, tocotrienol, oryzanol, squalene, and polyphenol (Goffman, 2003). γ-oryzanol is a mixture of ferulate (4-hydroxo-3-methoxy cinnamic acid) esters of sterols (campesterol, stigmasterol, and β-stigmasterol) and triterpene alcohols (cycloartenol, and 24-methylene cycloartenol) that are responsible for the biological properties of rice bran (Miller *et al.*,

2003). These include antioxidant, antihypertensive, antimicrobial, cholesterol reducing agents, antidiabetic, and anticancer properties (Bhatnagar *et al.*, 2014; Gul *et al.*, 2015; Kaur *et al.*, 2015; Wang *et al.*, 2015; Foster *et al.*, 2013).

The extraction of metabolite from plants is necessary for finding a suitable solvent to increase the levels of targeted compounds such as γ-oryzanol. Previous studies have focused on the extraction of rice bran oil using conventional solvents such as ethanol, methanol, isopropanol, and hexane (Xu, Godber, 2000; Chen, Bergman, 2005). A disadvantage of using conventional solvents was that too many solvents had to be used. This practice was time-consuming, inefficient, and environmentally unfriendly (Ballard *et al.*, 2010). Therefore, ionic solvents (IL) have been used because of their maximum yield value and the potential for obtaining active compounds from natural products (Espino *et al.*, 2016). Ionic liquid has a low melting point (<100 °C). They are safe, non-toxic solvents, with excellent thermal stability. In addition, they are environmentally

*Correspondence: A. Mun'im. Laboratory of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Depok 16424, West Java, Indonesia. Phone: +6221 788 49001-3. Email: munim@farmasi.ui.ac.id

friendly, have a negligible vapor pressure, and are non-combustible. The ionic liquid microwave-assisted extraction (IL-MAE) approach is a green, simple, rapid, efficient method for extracting bioactive compounds from plant materials (Xu *et al.*, 2012). So far, the extraction of γ-oryzanol from rice bran (*Oryza sativa* L.) using IL-MAE methods has been reported (Trinovita *et al.*, 2017), but the γ-oryzanol level was still low. In this study, IL, 1-butyl-3-methylimidazolium tetrafluoroborate [Bmim]BF$_4$ was used as a hydrophilic solvent in the microwave-assisted extraction (MAE) method in γ-oryzanol extraction from rice bran. The MAE method is more efficient than conventional extraction methods such as Soxhlet and reflux. Response surface methodology (RSM) is an application that can be used to determine the process for studying the parameters and variables in the study. The experimental results were analyzed by RSM after the optimization by IL-MAE, along with high-performance liquid chromatography (HPLC), was performed to analyze the chemical composition of the rice bran. Thus, the purpose of this study was to determine the optimum conditions for the extraction of γ-oryzanol. Four parameters were considered in obtaining the γ-oryzanol extracted from rice bran: ionic liquid concentration, liquid (IL) to solid (sample) ratio, extraction time, and microwave power. This experiment sought to determine the strength of the tyrosinase inhibitory activity of γ-oryzanol from rice bran, given that the γ-oryzanol antioxidant function is considered to affect the process of melanogenesis in human skin (Jun *et al.,* 2012). The tyrosinase inhibitory activity was measured under the optimum conditions of extraction from optimization using IL-MAE, and a larger sample than have been previously used was to obtain a more significant amount of extract.

MATERIAL AND METHODS

Material

Samples of varieties of rice bran (*Oryza sativa* L.) were obtained from Bogor (West Java). Standards of γ-oryzanol were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). [Bmim]BF$_4$ (>99%) was purchased from Chengjie Chemical Co., Ltd., (Shanghai, China). Isopropanol pro analysis was obtained from Merck (Darmstadt, Germany). Solvents based HPLC grade (methanol, isopropanol, and acetonitrile) and dimethyl sulfoxide were obtained from Merck (Darmstadt, Germany). Tyrosinase and L-DOPA were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

METHODS

Preparation of rice bran

Sample rice bran was stabilized for 15 minutes using an oven heated to 110 °C. After that, the rice bran sample was cooled in a container to room temperature for ±30 minutes. Last, the stabilized rice bran was put in clear plastic and stored at room temperature.

Pre-optimization of various salts in extraction after IL-MAE

1 gram of rice bran sample was extracted with 10 mL of solvent, 1 M [Bmim]BF$_4$ in ethanol solution, using the microwave power at 30%. It was then filtered using filter paper to obtain the desired filtrate resulting from extraction MAE. Also, the filtrate was added to 1 mL n-hexane solvent and 1 mL of 1 M salts (NaNO$_3$, KH$_2$PO$_4$, and NaCl). It was then vortexed for 10 seconds. The process was continued with centrifugation at 3,000 rpm for 15 minutes to separate the residue from the supernatant.

Extraction method: Microwave-assisted extraction (MAE) with IL ([Bmim]BF$_4$)

The stabilized rice bran was put in a flat-bottomed flask (1 gram rice bran), and ionic liquid-ethanol solutions (10, 15, and 20 mL) with different concentrations (0.4, 0.7, and 1.0 M) were added. The flask was placed in a microwave oven and set to extraction with microwave energy. The extraction was performed at various power levels and times (based on running). After the extraction was completed, the sample was cooled to room temperature for ±10 minutes and then filtered to obtain the desired filtrate resulting from IL-MAE. Then, the filtrate was added to 1 mL of *n*-hexane and 1 mL of 1 M NaNO$_3$; then it was vortexed for 10 seconds, followed by centrifugation at 3,000 rpm for 15 minutes to separate the residue with the supernatant.

Determination of levels of γ-oryzanol with HPLC

Before the yield from the sample was determined, the supernatant (0.5 mL) was added to isopropanol; then it was put into a flask of up to 10.0 mL. Next, 20 µL of the mixture was injected into the HPLC system (Shimadzu Corp., Japan). The column used was a Zorbax Eclipse Plus, C-18 analytical column, 4.6 x 150 mm, 5 µm (Agilent Technology, USA). A mixture of methanol, acetonitrile, and isopropanol (in a 50:40:10 ratio) was used in the mobile phase under isocratic conditions. The wavelength of UV detector was set at 327 nm, and the flow rate was set at 1 mL/minutes. Each sample was measured with three replicates.

Tyrosinase inhibitory activity of standard kojic acid

Kojic acid was dissolved in 50 mM phosphate buffer (pH 6.8) to obtain solutions with concentrations of 5, 10, 20, 40, and 80 µg/mL. A total mixture of 80 µL phosphate buffer solution (50 mM, pH 6.8), 40 µL L-DOPA solution (20 mM), 40 µL kojic acid solution, and 40 µL tyrosinase enzyme solution (620 IU/mL) was put into 96 well-plates. It was incubated at 37 °C for 10 minutes; then the absorbance was measured using a microplate reader at 490 nm (VersaMax Elisa Microplate Reader, USA). The tests were replicated three times (Masuda *et al.*, 2005; Kamkaen, Mulsri, Treesak, 2007). The composition of each substance can be seen in Table I.

Tyrosinase inhibitory activity on the extract from the optimum conditions of IL-MAE

The extract (100 mg) was dissolved in 1 mL of dimethyl sulfoxide (DMSO) and adjusted to 10.0 mL in the flask with a phosphate buffer solution (50 mM, pH 6.8). The extract solution (1.25 mL) was transferred to a 10.0 mL flask, and the volume was adjusted with the phosphate buffer solution. The mixture was diluted to a concentration of 50, 100, 200, 400, and 800 µg/mL. In control, the sample solution was replaced with 1.25% DMSO solution with the blank treated the same but not given a tyrosinase enzyme solution. The composition of each substance can be seen in Table II. The sample blanks were treated the same as the test sample, but without the addition of the tyrosinase enzyme solution. The mixed solution was incubated at 37 °C for 15 minutes. After incubation, the absorbance of the mixed solution was measured using a microplate reader at 490 nm.

Based on the method developed by Kamkaen *et al.* (2007), the percentage of tyrosinase inhibitory activity was calculated as:

$$\% Inhibition = \frac{(A-B)-(C-D)}{(A-B)} \times 100\%$$

A represents the absorbance of controls with an enzyme, B represents the absorbance of control-blanks without enzyme, C represents the absorbance of the sample with an enzyme, and D represents the absorbance of sample-blank without enzyme.

Samples with a percentage of inhibition of more than 50% or extracts with the highest percentage inhibition were calculated. The concentrations of the sample for the IC$_{50}$ values were 50, 100, 200, 400, and 800 µg/mL. The IC$_{50}$ was calculated by making curve-% inhibition vs. sample concentration. A linear equation was sought from the curve, and the IC$_{50}$ was calculated.

RESULTS

Calibration curve of standard γ-oryzanol

A calibration standard curve of γ-oryzanol in the

TABLE I - Substance composition of tyrosinase inhibitory acitivity of Kojic acid as positive control

Substances	Volume (µL)			
	C	CB	S	SB
Phosphate buffer solution	120	160	80	120
L-DOPA (20 mM)	40	40	40	40
Kojic acid solution	-	-	40	40
Tyrosinase enzyme (620 unit/mL)	40	-	40	-

C = Control, CB = Control-Blank, S = Sample, SB = Sample-Blank

TABLE II - Substance composition ot tyrosinase inhibitory activity of IL-MAE extract

Substances	Volume (µL)			
	C	CB	S	SB
Phosphate buffer solution	80	120	80	120
1.25% DMSO solution	40	40	-	-
L-DOPA (20 mM)	40	40	40	40
Samples solution	-	-	40	40
Tyrosinase enzyme (620 unit/mL)	40	-	40	-

C = Control, CB = Control-Blank, S = Sample, SB = Sample-Blank

range concentration of 5 – 45 ppm was constructed by HPLC analysis. This process is represented in the linear regression curve at Figure 1.

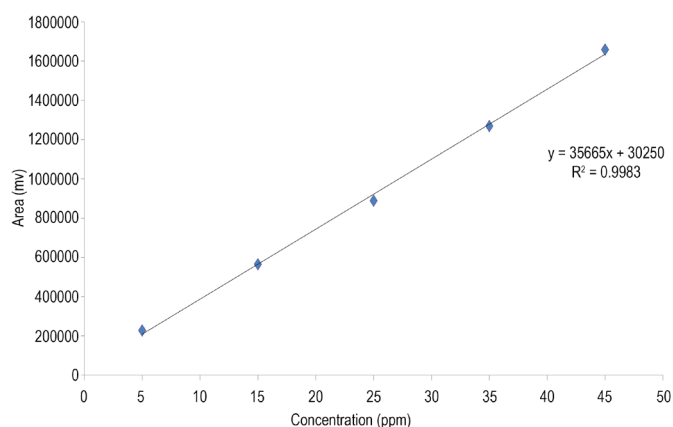$$y = 35665x + 30250 \qquad \text{(Equation 1.)}$$

and r = 0.9991.

FIGURE 1 - Calibration curve of standard γ-oryzanol.

Pre-optimization of various salts in IL-MAE

Figure 2 shows the results of the pre-optimization of the salt sample with a concentration of 1 M. The figure illustrates the conclusions. The highest yield of γ-oryzanol was 0.0208 mg/g rice bran using a $NaNO_3$ solution, and the lowest yield of γ-oryzanol was 0.0104 mg/g rice bran using a NaH_2PO_4 solution.
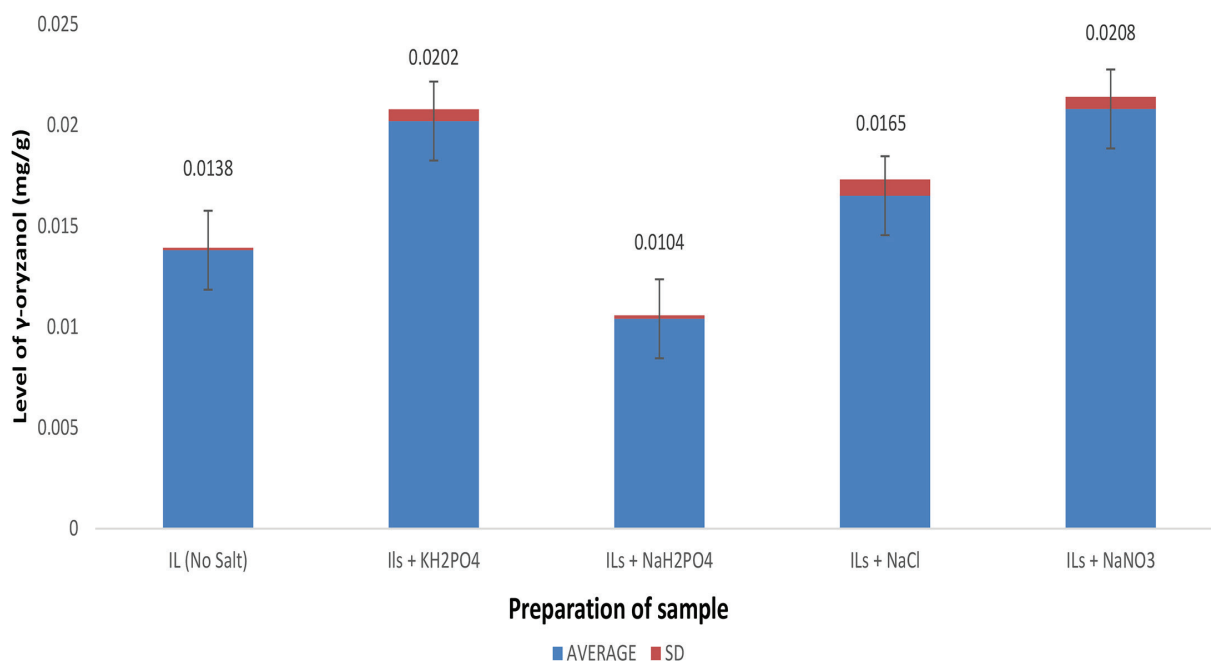
IL-MAE for γ-oryzanol extractions

A Box-Behnken design of four factors with RSM version 10 was used to optimize the experimental conditions. Table III shows the obtained levels of γ-oryzanol from 25 running samples that were calculated using Microsoft Excel.
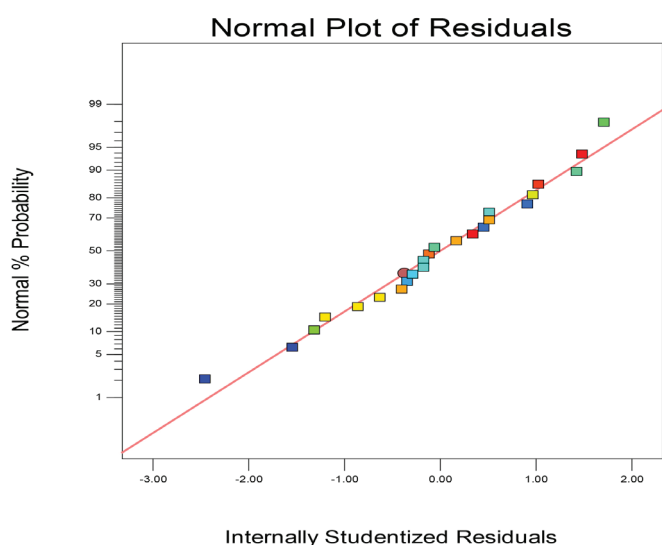
Statistical analysis by RSM

Figure 3 showed that the data for all sample (Table 3) had a normal distribution. This made it easier to analyze the optimum conditions for IL-MAE method. The result of ANOVA analysis showed that two models had a significant relationship with γ-oryzanol level (Prob value > F) under 0.05 including an AD (concentration of IL and microwave power) of 0.0490 and a B (sample/liquid ratio) of 0.016. Therefore, it can be concluded that there was a significant correlation between the concentration of IL and the sample/liquid ratio regarding the increases in the γ-oryzanol levels.

The experimental data were analyzed using the quadratic polynomial equations. The final equation to investigate the relationship between the independent variables and the response variable is shown below:

$$y = -4.167 \times 10^{-3}A + 8.333 \times 10^{-4}B + 4.617 \times 10^{-3}C + 5.833 \times 10^{-3}D - 0.015AB + 2.5 \times 10^{-3}AD + 0.013BD + 2.50 \times 10^{-3}CD \qquad \text{(Equation 2.)}$$

FIGURE 2 - Levels of γ-oryzanol in various slats.

TABLE III - The yields of γ-oryzanol in IL-MAE

Factor 1	Factor 2	Factor 3	Factor 4	Response 1
IL concentration (M)	IL/Rice bran (mL/g)	Extraction time (Minutes)	Power microwave (%)	Levels of gamma oryzanol (mg / g rice bran)
0.4	10	10	30	0.264
0.4	15	15	30	0.301
0.4	15	5	30	0.291
0.4	20	10	30	0.310
0.4	15	10	10	0.243
0.4	15	10	50	0.291
0.7	15	15	10	0.421
0.7	15	10	30	0.410
0.7	10	15	30	0.391
0.7	20	15	30	0.380
0.7	10	10	10	0.371
0.7	10	10	50	0.382
0.7	15	5	50	0.400
0.7	20	10	10	0.390
0.7	15	15	50	0.420
0.7	20	5	10	0.382
0.7	15	5	30	0.411
0.7	10	5	30	0.391
0.7	20	10	50	0.350
1	15	5	30	0.281
1	15	10	10	0.240
1	10	10	30	0.272
1	15	10	50	0.302
1	20	10	30	0.261
1	15	15	30	0.294

FIGURE 3 - Normal distribution chart.

In equation 2, y represents the γ-oryzanol yield (mg/g), A represents the IL concentration, B represents the sample/liquid ratio, C represents the extraction time, and D represents the microwave power.

Figure 4 shows that run 8 in Table 3 occupied hot area coordinates in extraction conditions of 0.7 M concentration, with a ratio of 15 mL/g solvent-sample in 10 min. of extraction time and 30% microwave power. This condition had the highest response. Based on RSM result, it can be concluded that the solvent with a concentration of 0.7 M had a closer polarity level to he γ-oryzanol than the solvent with concentrations of 0.4 and 1 M. the extraction results by using a relatively short extraction time so that potential damage of the γ-oryzanol compound is reduced. Figure 5 illustrates that a 3D image of surface response shows a hyperbolic curve with a peak area at the midpoint, indicating the location
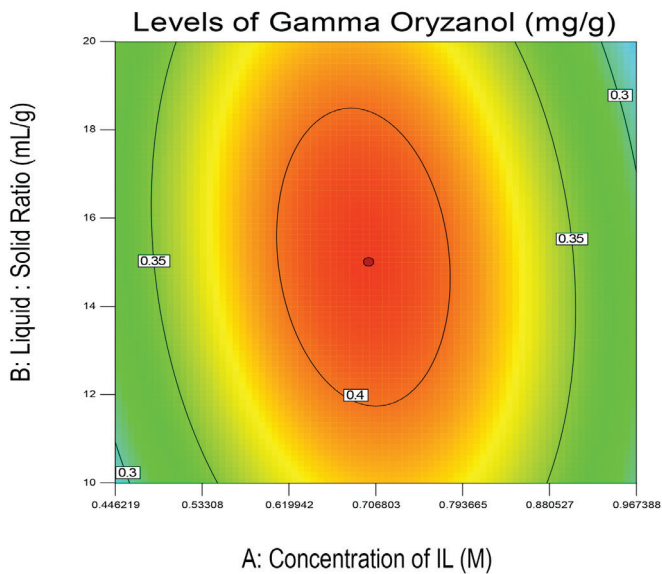
FIGURE 4 - Heat area coordinates.

of the optimum conditions and the presence of a response relationship between the variables.

Tyrosinase inhibitory activity of the kojic acid

Figure 7 shows that the highest inhibition obtained

in a concentration of 16 µg/mL was 59.05%, and the IC_{50} value of kojic acid as a positive control was 11.64 µg/mL.

Tyrosinase inhibitory activity of IL-MAE extract under the optimum condition

The absorbance was obtained using a microplate reader. As seen in Table IV below, it was concluded that the highest inhibitory of 800 µg/mL was 43.96%. It was calculated the IC_{50} value of the extracted sample was 1240 µg/mL

DISCUSSION

Previous studies have discussed many methods for the stabilization of rice bran. These include chemical stabilization, the hydrothermal process, ohmic heating, and infrared radiation. However, in this study, the stabilization of the rice bran was done by drying for 15 minutes using an oven heated to 110 °C. Previous research has identified a few stabilization (heating) methods for increasing extraction. These are hot-water heating, roasting, steaming, oven heating and microwave heating (Thanonkaew *et al.*, 2012). However, the stabilization method using a microwave oven was not suitable as
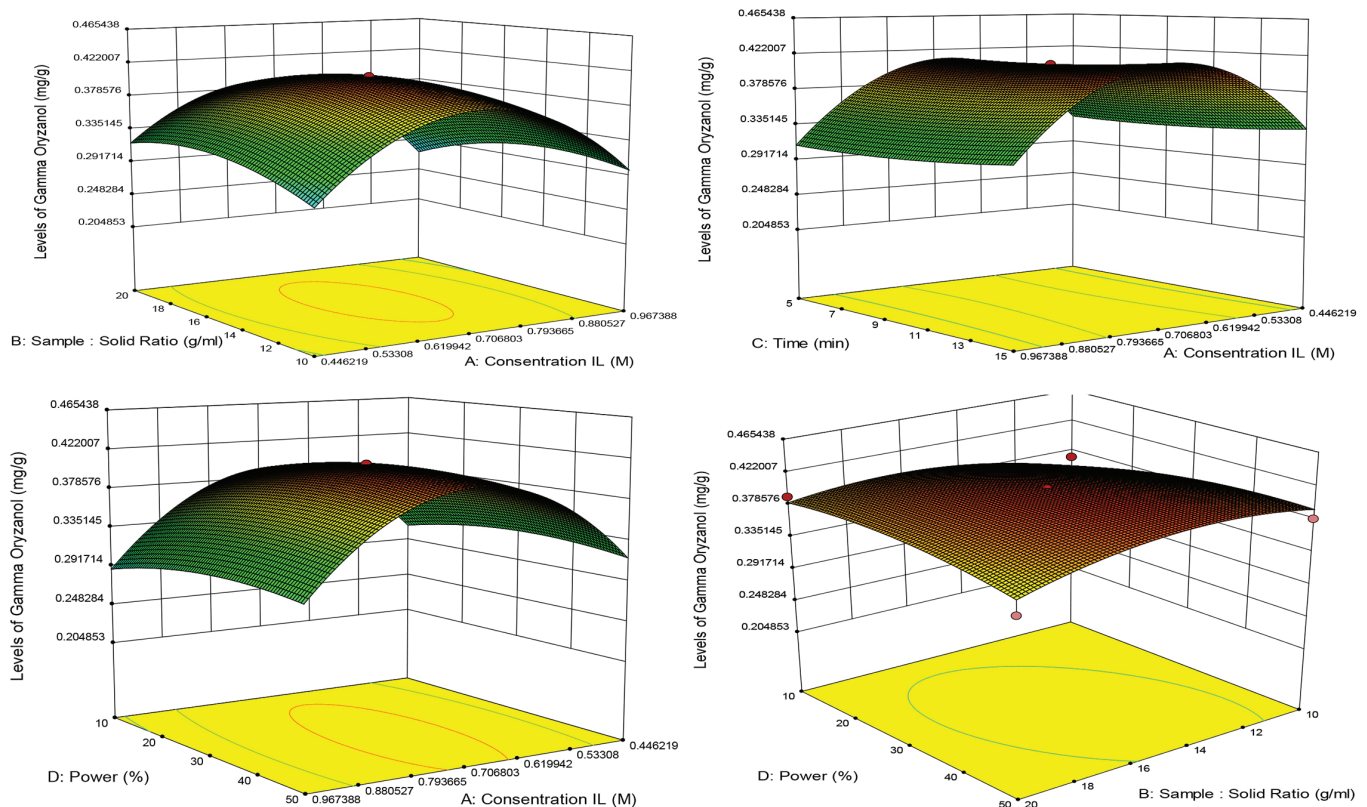

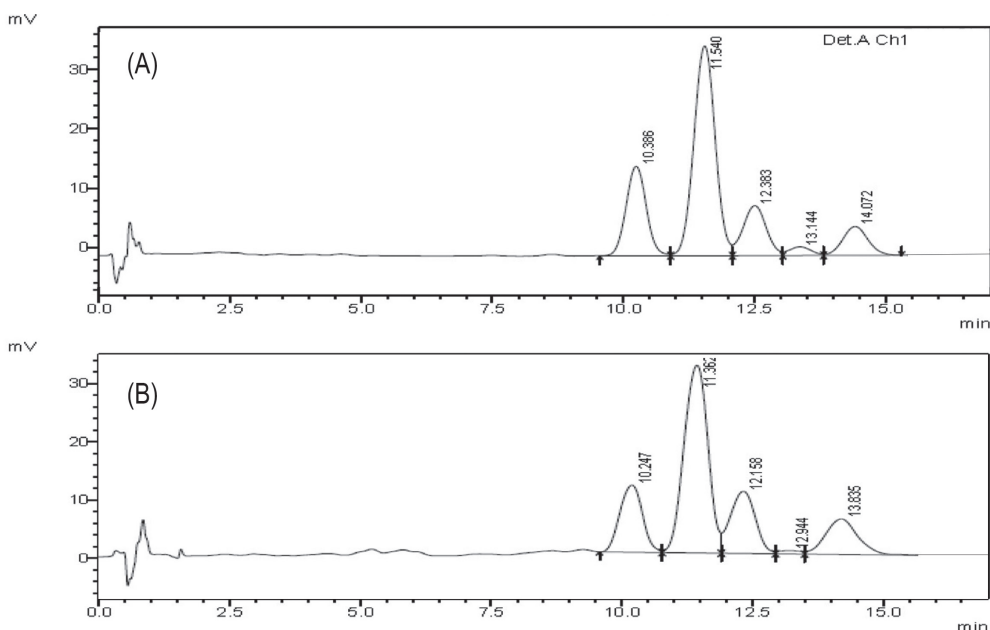**FIGURE 5 -** The 3D response surface methodology (RSM) models.

FIGURE 6 - The results of HPLC. (A) standard γ-oryzanol; (B) the sample (optimum extraction conditions, 0.7 M [Bmim]BF$_4$ solution; ratio of sample/liquid 15 mL/g; extraction time, 10 min; and 30% microwave power).
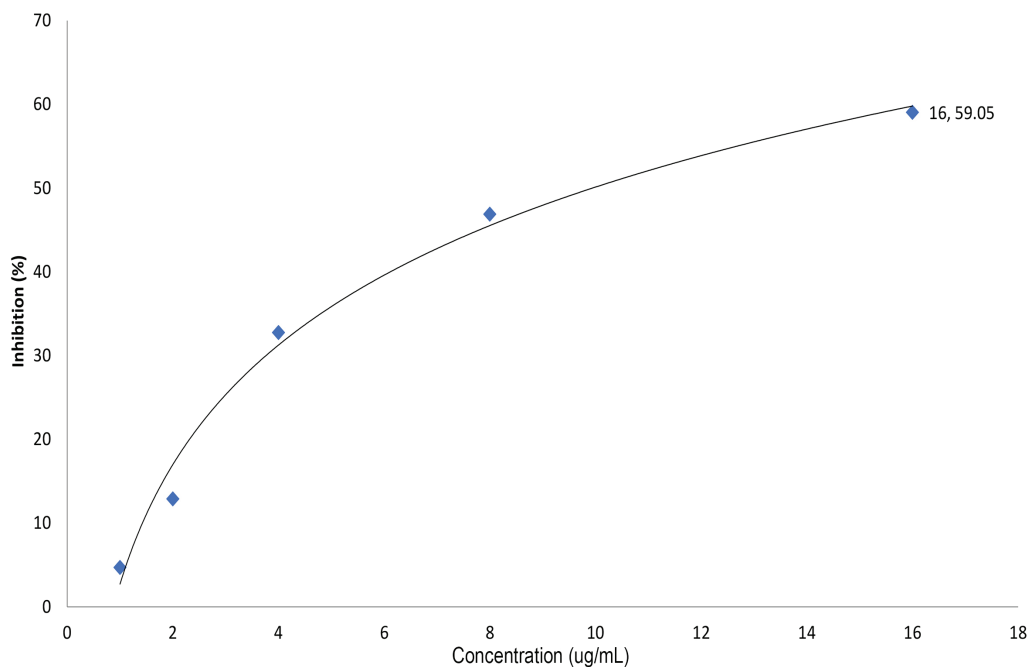

FIGURE 7 - Curve of tyrosinase inhibitory activity of the kojic acid.

TABLE IV - Tyrosinase inhibitory activity of IL-MAE extract

Concentration of samples (µg/mL)	% Inhibition
50	33.68
100	36.59
200	37.54
400	40.54
800	43.96

a stabilization method for a small- or a medium-scale project. The oven was more efficient for a small-scale project. This occurred because a temperature higher than 120 °C can cause the volume of the components contained in the rice bran to decrease and thus, the oil yield. In the extraction process, it was necessary for selecting a suitable solvent using the MAE method to have an effect on the solubility of the targetted compound and solvent penetration.

Based on the results in Table III, the highest levels of γ-oryzanol, 0.421 mg/g, were obtained in run 7. The result was higher than that of a previous study (Trinovita *et al.*, 2017). The optimum levels of γ-oryzanol concentration were based on the response to the surface analysis conducted in run 8 (Optimization), where a yield of 0.41 mg/g. The optimum conditions was shown to be 0.7 M [Bmim]BF$_4$ solution, liquid/solid ratio of 15 mL/g, extraction time of 10 min, and microwave power of 30%. Nevertheless, in this experiment, the optimum conditions for extraction were not the same as the conditions with the highest yield because the optimum condition for extraction has a relationship with all the parameters for extraction. One reason is that an extraction time is too long can decrease the levels of γ-oryzanol or damage the targeted compounds in the rice bran. In such a case, the dissolution of [Bmim]BF$_4$ in ethanol is recommended because, according to Xiaohong *et al.* (2015), the solubility of [Bmim]BF$_4$ in ethanol is high. The study showed that many solvents are not suitable for microwave extraction because microwave radiation is absorbed by [Bmim]BF$_4$. Besides, increased energy for heating is required. The IL [Bmim]BF$_4$ can e quickly heated to a high temperature under microwave power (irradiation), although the polarity of IL [Bmim]BF$_4$ referred by the dielectric constant is far lower than that of water. Also, the existence of [Bmim]BF$_4$ enables [Bmim]BF$_4$-ethanol solution to be heated much faster than water by microwave irradiation. In the study conducted by Mandal, Mohan and Hemalatha (2007), this result may have stemmed from the breakdown of the cell walls because of the intensity of the power and the heat.

Microwave irradiation as an energy source has become a favorite method in modern chemistry (Lyopy, 2012). Microwave irradiation transfers energy through microwaves to the targeted compound by two mechanisms: dipole rotation and ionic conduction. Microwave power is very much influenced by extraction time and extraction temperature. There is an interaction between the solvent (ethanol) and matrix material (rice bran) so that it can absorb microwave energy. Therefore, eventually reduced reaction time from hours to minutes with significant increase targeted compound yield (Kappe, Dallinger, Murphree, 2009). Heat from microwave power reduces the enzymatic activity that damages γ-oryzanol or targeted compounds (Salas *et al.*, 2010). In this experiment, the microwaves decreased the lipase enzyme activity in the rice bran.

This study used solvent ethanol to dilute the ionic liquid [Bmim]BF$_4$. The next parameter in the extraction was the length of the extraction time. Generally, the extraction time was positively correlated with the amount of the target compound, although there was a risk of degradation of the target compound itself. An extraction time depending on the extracted material. Research on the optimization of the extraction time is needed because the extraction time may vary with different kinds of materials. The extraction time was affected by the value of the dielectric solvent. Selection of the appropriate power can avoid the degradation of the target compound and the excess pressure in an extraction process.

The pre-optimization of various salts informed the selection of NaNO$_3$ as the process for salting out in the rice ran extraction, because the results showed that NaNO$_3$ salt had higher levels of γ-oryzanol than those of other spices that were used. This result is different with our previous study (Trinovita *et al.*, 2017). The primary purpose for using salt in this study was to increase the partition coefficient to increase the yield of γ-oryzanol. Salt weakens the chemical bonding between IL and targeted compounds (Han *et al.*, 2010). The ionization process occurred in the process of salting out where nitrate ion was bonded with ethanol so that the combination of both liquids formed two phases: the aqueous two-phase system (ATPS) increased hydrophilic intensity and showed an increase in the yield of the target compounds (Wang *et al.*, 2011).

Response surface methodology (RSM) is a design and model for the evaluation of variable effects and the interactions of variable responses. Thus, RSM is a collection of statistical and mathematical techniques that have been successfully used for developing, improving, and optimizing processes (Liyana, Shahidi, 2005). Figure 6 shows that a rice bran sample and a standard of γ-oryzanol had the same retention times and the same peaks in derivatives of γ-oryzanol.

Based on research by Lu *et al.* (2008), [Bmim] BF$_4$ was used as a solvent in the extraction of *Nelumbo nucifera* Gaertn. to find phenolic and alkaloid compounds where they were extracted by MAE. This was the main reason for the use of liquid ionic solvent, 1-butyl-3-methylimidazolium tetrafluoroborate to increase the levels of γ-oryzanol. In other extraction methods [Bmim]BF$_4$ was coupled with ultrasonic-assisted extraction was used to extract *Stephanie tetrandrine* which targeted compounds of fangchinoline and tetrandrine (Zhang *et al.*, 2009). Also, at the extraction of white pepper which targeted compound of piperine (Ma *et al.*, 2010). Ionic liquid (IL) can be applied to microwave-assisted extraction (MAE) methods and can be used as a solvent in extraction and extracted other natural products.

The test of kojic acid was performed as a positive

control to ensure that the method was correct by comparing the IC$_{50}$ values obtained in this study with the IC$_{50}$ values obtained in studies reviewed in the literature. If the IC$_{50}$ values obtained in this study were within the range of IC$_{50}$ values obtained in previous studies, then it could be concluded that the method can be used. The test was done by the protocols for achieving optimal results. The results of the experiment on the tyrosinase inhibitory activity of kojic acid as a positive control can be seen in Figure 7. An IC$_{50}$ value that is equal to 11.64 µg/mL was obtained. This IC$_{50}$ value was not much different from the amount reported by Lee *et al.* (2006), who got an IC$_{50}$ kojic acid value of 30.61 µM or 4.35 µg/mL. It can, therefore, be concluded that this method can be used.

The optimum conditions for extraction with the IL-MAE method were obtained by run 8 in Table III with a γ-oryzanol content of 0.41 mg/g. A regression equation was used to calculate the IC$_{50}$ value for the optimum results of rice bran extraction. The IC$_{50}$ values obtained from the optimum extract at a various initial concentration of samples were 50, 100, 200, 400, dan 800 µg/mL. Based on this experiment, the IC$_{50}$ value of the optimum condition extract is 1,240 µg/mL, as illustrated in Table 4.

Research conducted by Moon *et al.* (2010) showed that tyrosinase inhibitory activity was not active if the percentage of inhibition at 500 µg/mL concentration was less than 30%. Activity was considered as weak if this inhibition was 30-60%, and it was strong if this inhibition was more than 60%. The IC$_{50}$ value of arbutin was 180 µg/mL, where arbutin is commonly used as the whitening agent. Table 4 shows the percentage of inhibition from the extract of the optimum IL-MAE at 800 µg/mL concentration to be 43.96%. It can, therefore, be concluded from the results that the extraction from optimum IL-MAE has low activity as a tyrosinase inhibitor.

CONCLUSIONS

According to the experimental design and response surface analysis, quadratic polynomial models can be used to predict the yield of γ-oryzanol. This suggests that IL-MAE be more efficient for the extraction of γ-oryzanol compounds from rice bran. The optimum condition for the MAE using ionic liquids was 0.7 M [Bmim]BF$_4$ solution, a 15 mL/g liquid/solid ratio, 10 minutes extraction time, and 30% microwave power, with a γ-oryzanol value of 0.41 mg/g. Ionic liquid-based MAE was used as an alternative method for the extraction of γ-oryzanol from rice bran. In a future experiment, other ionic liquids can be used to obtain a higher yield of γ-oryzanol. Based on the observed tyrosinase inhibitory activity, it can be concluded

that the IC$_{50}$ value of sample under an optimum condition in IL-MAE is 1240 µg/mL. This means γ-oryzanol has weak/low activity in tyrosinase inhibition, so further research is needed to discover the best activity from rice bran, specifically from γ-oryzanol, the main component in rice bran.

ACKNOWLEDGMENT

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

REFERENCES

Ballard TS, Mallikarjunan P, Zhou K, O'Keefe S. Microwave-assisted extraction of phenolic antioxidant compounds from peanut skins. Food Chem. 2010;120(4):1185-1192.

Bhatnagar AS, Prabhakar DS, Kumar PKP, Rajan RGR, Krishna AGG. Processing of commercial rice bran for the production of fat and nutraceutical rich rice brokers, rice germ and pure bran. J Food Sci Technol. 2014;58(1):306-311.

Chen M-H, Bergman CJ. A rapid procedure for analysing rice bran, tocopherol, tocotrienol and γ-oryzanol contents. J Food Composition Anal. 2005;18(2-3):139-151.

Espino M, Fernández MA, Federico JV, Gomez MFS. Natural designer solvents for greening analytical chemistry. Trends Anal Chem. 2016;76:126-136.

Forster GM, Raina K, Kumar A, Kumar S, Agarwal R, Chen M, Ryan EP. Rice varietal differences in bioactive bran components for inhibition of colorectal cancer cell growth. Food Chem. 2013;141(2):1545-1552.

Goffman FD. Genetic diversity for lipid content and fatty acid profile in rice bran. J Am Oil Chem Soc. 2003;80(5):485-490.

Gul K, Yousuf B, Singh AK, Singh P, Wani AA. Rice bran nutritional value and its emerging potential for development of functional food. A review. Bioactiv Carbohydr Diet Fibre. 2015;6(1):24-30.

Han J, Wang Y, Kang W, Li C, Yan Y, Pan J, Xie X. Phase equilibrium and macrolide antibiotics partitioning in real water samples using a two-phase system composed of the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate and an aqueous solution of an inorganic salt. Microchim Acta. 2010;169(1-2):15-22.

Jun H, Lee JH, Cho B, Seo W, Kang H, Kim D, Cho K, Lee S. Dual Inhibition of γ-Oryzanol on cellular melanogenesis: Inhibition of tyrosinase activity and reduction of melanogenic gene expression by a protein kinase A-dependent mechanism. J Nat Prod. 2012;75(10):1706-1711.

Kamkaen N, Mulsri N, Treesak C. Screening of some tropical vegetables for anti-tyrosinase activity. Thai Pharm Health Sci. J. 2007;2(1):15-19.

Kappe CO, Dallinger D, Murphree SS. Practical Microwave Synthesis for Organic Chemists: Strategies, Instruments, and Protocols. Weinheim: Wiley-VCH; 2009.

Kaur H, Kaur N, Bedi O, Kaur M. Conventional steaming retains tools and γ-oryzanol better than boiling and frying in the jasmine rice variety. Khao Dok Mali 105. Indian J Pharmacol. 2015;2(5):229-233.

Lee YS, Park JH, Kim MH, Seo SH, Kim HJ. Sinthesis of tyrosinase inhibitory kojic acid derivative. Arch Pharm. 2006;339(3):111-114.

Liyana-Pathirana C, Shahidi F. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chem. 2005;93(1):47-56.

Lu Y, Ma W, Hu R, Dai X, Pan Y. Ionic liquid-based microwave-assisted extraction of phenolic alkaloids from the medicinal plant Nelumbo nucifera Gaertn. J Chromatogr A. 2008;1208(1-2):42-46.

Lyopy A. Microwaves in Organic Synthesis. 3rd edn. Weinheim: Wiley-VCH; 2012.

Ma W, Lu Y, Hu R, Chen J, Zhang Z, Pan Y. Application of ionic liquids based microwave-assisted extraction of three alkaloids N-nor-nuciferine, O-nor-nuciferine, and nuciferine-from lotus leaf. Talanta. 2010;80(3):1292-1297.

Mandal V, Mohan Y, Hemalatha S. Microwave assisted extraction-an innovative and promising tool for medicinal plant research. Pharmacol Rev. 2007;1(1):7-18.

Masuda T, Yamashita D, Takeda Y, Yonemori S. Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from Garcinia subelliptica. Biosci Biotech Biochem. 2005;69(1):197-201.

Miller A, Frenzel T, Schmarr HG, Engel KH. Coupled liquid chromatography-gas chromatography for the rapid analysis of Gamma oryzanol in rice lipids. J Chormatogr A. 2003;985(1-2):403-410.

Moon J-Y, Yim E-Y, Song G, Lee NM, Hyun C-G. Screening of elastase and tyrosinase inhibitory activity from Jeju Island plants. Eur Asian J BioSci. 2010;4(6):41-53.

Salas PG, Aranzazu M-S, Antonio S-C, Alberto F-G. Phenolic-compound extraction systems for fruit and vegetable samples. Molecules. 2010;15(12):8813-8826.

Thanonkaew A, Wongyai S, McClements DJ, Decker A. Effect of stabilization of rice bran by domestic heating on mechanical extraction yield, quality, and antioxidant properties of cold-pressed rice bran oil (Oryza sativa L.). J Food Sci Technol. 2012;48:231-236.

Trinovita E, Sutriyo, Saputri FC, Munim, A. Enrichment of the gamma oryzanol level from rice bran by addition of inorganic salts on ion liquid 1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim]PF_6) extraction. J Young Pharm. 2017;9(4):555-558.

Wang L, Zhu H, Sun Y-T, Xu Y-J, Wang Q-W, Yan Y-S. Determination of trace chlorophenols endocrine disrupting chemicals in water sample using [Bmim]BF_4-nah2po4 aqueous two-phase extraction system coupled with high-performance liquid chromatography. Chin J Anal Chem. 2011;39(5):709-712.

Wang W, Guo J, Zhang J, Peng J, Liu T, Xin Z. Isolation identification and antioxidant activity of bound phenolic compounds present in rice bran. Food Chem. 2015;171:40-49.

Xiaohong MA, Wang Q, Li X, Tang J, Zhang Z. Determination of the solubility parameter of ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate by inverse gas chromatography. Chin J Chromatogr. 2015;33(11):1192-1198.

Xu W, Chu K, Li H, Zhang Y, Zheng H, Chen R, Chen L. Ionic liquid-based microwave-assisted extraction of flavonoids from Bauhinia championii (Benth.) Benth. Molecules. 2012;17(12):14323-14335.

Xu Z, Godber JS. Comparison of supercritical fluid and solvent extraction methods in extracting gamma-oryzanol from rice bran. J Am Oil Chem Soc. 2000;77(5):1127-1131.

Zhang L, Geng Y, Duan W, Wang D, Fu M, Wang X. Ionic liquid-based ultrasound assisted extraction of fangchinoline and tetrandrine from Stephaniae tetrandrine. J Sep Sci. 2009;32(20):3550-3554.