Journal of Applied Pharmaceutical Science Vol. 7 (12), pp. 008-013, December, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.71202
ISSN 2231-3354

Optimization of Ionic Liquid 1-Butyl-3-Methylimidazolium Hexafluorophosphate ([Bmim] PF_6)-Based Microwave Assisted Extraction Method for Gamma Oryzanol from Rice Bran (*Oryza Sativa* L.)

Elsa Trinovita[1], Efendy Sigalingging[2], Fadlina Chany Saputri[1], Abdul Mun'im[2*]

[1]Graduate Program of Herbal Medicine, Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia.
[2]Laboratory of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia.

ARTICLE INFO

Article history:
Received on: 13/07/2017
Accepted on: 26/08/2017
Available online: 30/12/2017

Key words:
[Bmim] PF_6, ionic liquid, gamma oryzanol, IL-MAE, response surface methodology (RSM).

ABSTRACT

The effect of IL solvent and the microwave-assisted extraction (MAE) including concentration IL, the ratio of liquid/solid, time extraction and microwave power in the result of gamma oryzanol extract in rice bran (*Oryza Sativa* L.) were investigated. The application of IL [Bmim] PF_6 as a selected solvent in the MAE method was developed for the extraction of gamma oryzanol from rice bran. The Box–Behnken design on four factors with response surface methodology version 10 was used to optimize experimental conditions. The optimum process for the MAE using ionic liquids ([Bmim]PF_6) concentration 0.7 M [Bmim]PF_6 solution, the ratio of liquid/solid 15mL/g, extraction time 10 min, and 30% microwave power with the gamma oryzanol value of 0.27 mg/g.The IL-MAE method is more efficient for the extraction of gamma oryzanol compounds from rice bran.

Abbreviation: [Bmim] PF_6 : 1-butyl-3-methylimidazolium hexafluorophosphate; IL : ionic liquid; MAE : microwave-assisted extraction; RSM : Response Surface Methodology; HPLC : High-performance liquid chromatography

INTRODUCTION

Currently, Indonesia produces a lot amount of paddy which results an increasing by-product rice milling and rice bran is one of such by product. Some researchers have used rice bran as raw materials in the extraction process because rice bran has high nutrition and contains antioxidants such as tocopherol, oryzanol, tocotrienol, squalene, and polyphenol. Gamma oryzanol is a mixture compound of at least 10 components of ferulic acid esters and alcohols triterpene (Liazid *et al*., 2007). The various pharmacological activities of gamma oryzanol including anti-inflammatory, antineoplastic, hypoallergic, antidiabetic, antiulcerogenic, hypolipidemic (Islam *et al.*, 2011) and anti-cancer (Forster *et al.*, 2013). Suitable of solvents is needed in increasing the levels of gamma oryzanol in the extract. Some previous studies have been done in the extraction of rice bran oil by using conventional solvents such as isopropanol and hexane. Some disadvantages of conventional solvents such as the amount of solvent used, long extraction time and environmentally unfriendly (Ballard *et al.*, 2010). Therefore, the use of anionic liquid solvent as the application of green chemistry principles can increase the active compounds from natural materials (Espino *et al.*, 2016). Ionic liquids are known as safe, environmentally friendly, non-toxic solvents, vapor ignored pressure, good thermal stability, and non-flammable. IL-MAE approach is a green, simple, rapid, efficient extraction method for extracting bioactive compounds from plant materials (Xu *et al.*, 2012). But in our knowledge, until now, IL-MAE has not yet been reported for extraction of gamma oryzanol from rice bran (*Oryza Sativa* L.)

* *Corresponding Author*
Dr Abdul Mun'im, Laboratory of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia. Email: munim_@ farmasi.ui.ac.id, Phone: 0852-1610-4550

In this experiment, IL 1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim]PF_6) was used as a hydrophobic solvent in microwave-assisted extraction (MAE) method in gamma oryzanol extraction from rice bran. The microwave-assisted extraction (MAE) method is a faster extraction method than conventional extraction methods such as Soxhlet (Du *et al*., 2007). The experimental results were analyzed by response surface methodology (RSM). Response surface methodology is a technique that can be used in the process of studying parameters or variables used in the study. Therefore, the purpose of this experiment was to investigate the effect of ionic liquid concentration, the ratio of liquid/solid, time extraction and microwave power in the level of gamma oryzanol extract in rice bran. The optimum conditions for extraction are determined by response surface methodology (RSM) version 10 so that the highest gamma oryzanol levels can be obtained.

MATERIALS AND METHODS

Plant materials and reagents

Sample fresh rice bran varieties IR 64 *(Oryza Sativa* L.) was obtained from Bogor, West Java. Standards gamma oryzanol was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). [Bmim]PF_6 (> 99%) was obtained from Chengjie Chemical Co., Ltd., (Shanghai, China). An analytical grade of isopropanol was purchased from Merck. HPLC Grade (methanol, acetonitrile, and isopropanol) were obtained from Merck.

Instrumentation

Microwave (Modena MV-3002 with slight modification), volume pipette (Pyrex), micropipette 100-1000 µl (Thermo Scientific), rotary vacuum evaporator (Buchi, German), centrifuge (Heraeus-Christ GmbH, Osterode Germany), and HPLC system (Shimadzu Corp., Japan) were used in this study. The column used is a type Zorbax Eclipse Plus C-18 Analytical 4.6 x 150 mm, 5 µm (Agilent Technologies-USA).

Stabilization of rice bran

Sample rice bran fresh with IR-64 varieties was stabilized using an oven at 110° C for 15 minutes. After that rice bran sample was cooled in a container for 30 minutes to reach room temperature. The stabilized rice bran was put in clear plastic and stored at room temperature (Thanonkaew *et al*., 2012;Nasir *et al*., 2009).

Extraction method conventional solvent-MAE

The stabilized samples (1 g) were placed into a flat-bottom flask, then was added isopropanol with the appropriate experimental design (w/v).The boiling flask was placed into a microwave by the treatment of variations of the ratio of liquid/solid (10-20 mL/g), extraction time (5, 10, 15 minutes) and the microwave power (10%, 30%, 50%). The results of the extract were cooled at room temperature for 10 min and diluted to 10 mL with isopropanol. Then 20 µL of the extract solution was injected using a syringe into the HPLC system. Optimization condition on extraction was employed by response surface methodology (RSM) version 10, so the extraction conditions were varied to some factors as described in Table 1.

Table 1: The experimental conditions of isopropanol-MAE method with RSM.

Run	A:The ratio of liquid/solid	B:Time	C:Power	Levels of gamma oryzanol
	(mL/g)	min	%	(mg/g)
1	10	5	30	0.06
2	15	10	30	0.25
3	10	15	30	0.07
4	15	5	10	0.21
5	20	5	30	0.07
6	15	15	10	0.11
7	20	10	50	0.22
8	20	10	10	0.10
9	15	5	50	0.15
10	10	10	50	0.11
11	15	15	50	0.13
12	20	15	30	0.08
13	10	10	10	0.23

Optimization IL-MAE by response surface methodology (RSM)

The Box-Behnken design on four factors with response surface methodology with Design-Expert® Software Version 10 (Stat-Ease, Inc., Minneapolis, USA) and it was used to optimize experimental conditions (Table 2) and analysis the interaction between some factors and optimize experimental conditions to get 25 sample of treatment. The factors for microwaved-assisted extraction of gamma oryzanol included IL [Bmim]PF_6 concentration (0.4-0.7 M), the ratio of liquid/solid (10-20 mL/g) extraction time was varied on several conditions (5-15 minute), microwave power of 10, 30 and 50% of 900 W for 100% microwave power supply. The response value of this study gamma oryzanol was a yield (mg/g) with ANOVA analysis for the significance of the model.

Table 2: The experimental conditions of IL-MAE method with RSM.

IL Concentration	The ratio of liquid/solid	Extraction time	Power microwave	Levels of gamma oryzanol
(M)	(mL/g)	(Minutes)	(%)	(mg/g)
0.4	10	10	30	0.19
1	10	10	30	0.25
0.7	15	15	50	0.17
1	15	15	30	0.22
0.7	15	10	30	0.27
0.4	20	10	30	0.17
1	15	5	30	0.18
0.4	15	10	50	0.17
1	15	10	10	0.19
0.7	10	10	50	0.21
0.7	10	10	10	0.2
0.4	15	5	30	0.23
0.7	15	5	50	0.17
0.7	10	5	30	0.15
0.7	20	15	30	0.19
0.7	20	5	30	0.19
0.4	15	15	30	0.13
0.7	20	10	10	0.21
1	20	10	30	0.14

0.7	10	15	30	0.2
0.7	15	15	10	0.24
0.7	15	5	10	0.15
1	15	10	50	0.19
0.4	15	10	10	0.17
0.7	20	10	50	0.22

Extraction method IL-MAE

The stabilized rice bran (1 g) was put in a flat-bottom flask. The ionic liquid solution with different concentration and volume was added into the flask. The boiling flask was placed into a microwave. The extraction was performed under different power level and time. After the extraction process was completed, the sample was cooled to room temperature ± 10 minutes, then was filtered using a filter paper to obtain the desired filtrate as a result of extraction MAE. Furthermore, the filtrate was added to 1 ml of solvent N-hexane and 1 ml KH_2PO_4 0.01mol. Then was vortexed for 10 seconds and followed centrifuged at 3000 rpm for 15 minutes to separate the residue with the supernatant.

Determination of gamma oryzanol levels with HPLC

Calibration standard gamma oryzanol curve was constructed with correlating concentration in the range of concentration of 1 ppm-45 ppm and area under curve value (Figure 1).

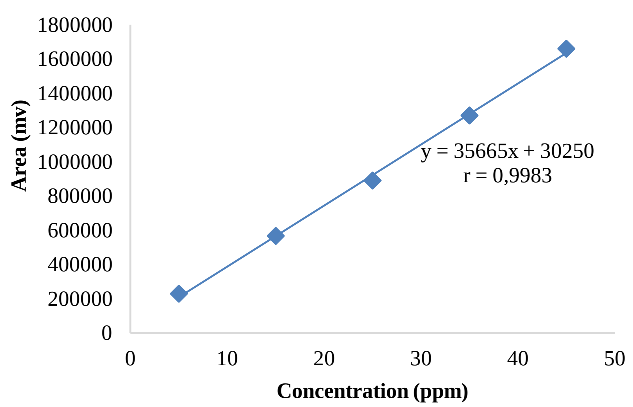

Fig. 1: Calibration Curve of Standard Gamma Oryzanol.

The supernatant (0.5 ml) was added isopropanol and put in a flask up to 10 ml. Then 20 µL was injected into the HPLC system (Shimadzu Corp., Japan). A mixture of methanol (50%), acetonitrile (40%), and isopropanol (10%) was used as mobile phase under isocratic conditions. UV detector wavelength set at 327 nm and the flow rate was set 1 ml/ min. Each sample was measured with 3 repetitions.

RESULTS AND DISCUSSION

Optimum conditions of conventional solvent-MAE

Based on the results of the statistical analysis using RSM version 10 retrieved the value F of 5.07 and value P of 0.1042 thus the model was not significant in influencing levels of gamma oryzanol. Several factors indicated a significant relationship with the levels of gamma oryzanol included model AC (the ratio of liquid/solid and power) showed of P-value (0.0392), A^2 (the ratio of liquid/solid) of 0.0359 and B^2 (extraction time) of 0.0226. based on the analysis of the optimum conditions acquired RSM in increasing levels of gamma oryzanol on sample RUN 2 (the ratio of liquid/solid 15 mL/g, 10 min, and 30% power) and acquired the levels of gamma oryzanol of 0.25 mg/g.

MAE method was using microwave radiation to rapid selective extraction through solvent warming quickly and efficiently (Jain *et al*., 2009). The selection of a suitable solvent on extraction process by MAE method will affect the solubility of targeted analytes, the penetration of solvents and interactions with the matrix of the sample. Furthermore the ratio between solvents and samples that will be concerned in the extraction process. The MAE methods were used in extraction rice bran oil can increase the amount of oil and antioxidant content with isopropanol solvent (Duvernay *et al*., 2005).

Optimum conditions of solvent and sample ratio affected the process of warming on vessels to be effective and were distributed evenly. Over of solvents caused microwave warming became worse so that microwave radiation was absorbed by solvent and energy required when warming became too much (Chan *et al*., 2011). On the research of conventional solvent was used in the method of MAE is isopropanol caused gamma oryzanol was due to a combination of alcohol triterpene and ferulate acid slightly polar, thus easy to dissolve in the polar solvent like isopropanol (Xu and Godber, 1999). Isopropanol solvent used in the extraction process because of the polar solvent can absorb microwave energy better than nonpolar solvents (Duvernay *et al*., 2005).

According to previous research explains that a polarity compound was affected by the solubility of a solvent, it is shown in the ability of the extraction of oryzanol better-using isopropanol and methanol than hexane solvent. This was caused due to an interaction of the hydroxyl group on the benzene ring of ferulate esters part of gamma oryzanol compound with alcohol functional group of isopropanol solvent (Chen and Bergman, 2005).

Time is an important parameter in the extraction. Extraction time is affected by the value of dielectric solvent. The exposure the solvents such as water, ethanol, and methanol were too long at risk on the thermolabile of the target compound (Mandal *et al*., 2007). Microwave power was chosen precisely to avoid the degradation temperature in target compounds and over pressure in the extraction process. The breakdown of cells at low power happened gradually. Microwave power mutually influenced by temperature and time in extraction. The combination of low-power medium and long extraction time was the best extraction conditions approach. High temperature and power caused breakdown cell walls (Mandal *et al*., 2007)

Optimum Conditions of IL-MAE

Research on the optimum extraction condition of the specified IL-MAE with the application of RSM, that are included in the application Design Expert 10.03. Quadratic models vs-2 had a value of Prob > F and all samples normally distributed, it can be seen in Figure 2 below. Based on the analysis of ANOVA for the optimization of the IL-MAE concluded that the value of F 1.46 and value of P 0.27. This means that this model showed the relationship was not significant in the levels of gamma oryzanol.

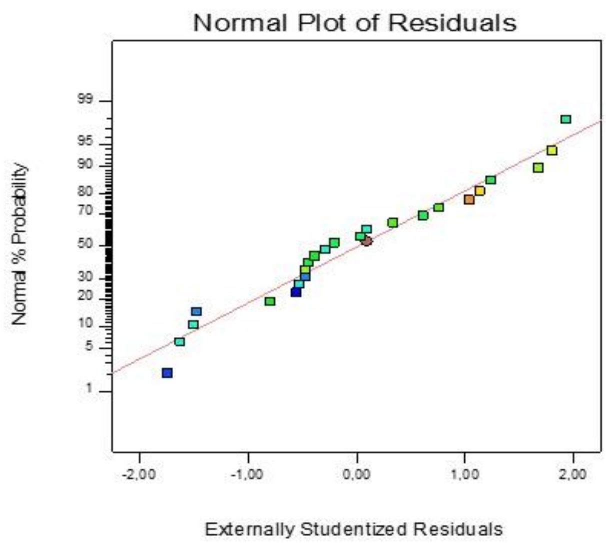

Fig. 2: Normal Distribution Chart.

In this case, there were three models that show a significant relationship with the levels of gamma oryzanol (value of Prob> F under 0.05) include AC (concentration of IL and extraction time) of 0.0444, A^2 (concentration) of 0.0324 and C^2 (extraction time) of 0.0288.

Mathematical equation from a model that was used to show the relationship between the independent variables and the variable response was: Y = 0.11 + 9.167 E-003 A – E-003 6.667 B + E 6.667-003 C + 2,500 E-003 D – 0.023 0.023 AB + AC + BC AD-0.013 0000 + 0000 BD – 0.022 CD-0034-0045 A2 B2 – C2 D2 0037-0046, Y represented the level of gamma oryzanol (mg/g), A was IL concentration (M), B was the ratio of liquid/solid (mL/g), C was a microwave power (%), and D was extraction time (minute). Figure 3 showed the image coordinates of the hot areas under one condition on extraction by the concentration of 0.7 M, the ratio of liquid/solid 15 (mL/g) in the extraction of 10 minutes, microwave power of 30% and obtained the levels of gamma oryzanol of 0.27 mg/g.It can be concluded that this condition had the highest response. Information of hot coordinate area was very useful for optimization.

It can also be due to the concentration of 0.7 M has closed to the level of polarity γ-oryzanol compound compared with the concentration of 0.4 M and 1 M. The condition of the extraction time of 15 minutes and microwave power of 30% was optimum condition due to exposure to the temperatures was not too hot so that the potential damage to the compound gamma oryzanol was decreased.Determination of optimum conditions can be done by showing a 3D surface with response peaks in the central point is the location instructions of optimum conditions (Figure 4). Levels of gamma oryzanol with conventional Soxhlet method obtained 0.047 mg/g (Marriod *et al*., 2014). This is compared with the results of the isopropanol-MAE 0.25 mg/g and IL-MAE of 0.27 mg/g, therefore be concluded that the MAE method was more effective in increased levels of gamma oryzanol as compared to the conventional method. IL-MAE is very suitable to extract the unstable compounds on heating. The IL-MAE method showed high extraction results with using less of solvents and energy (Bogdanov, 2014).

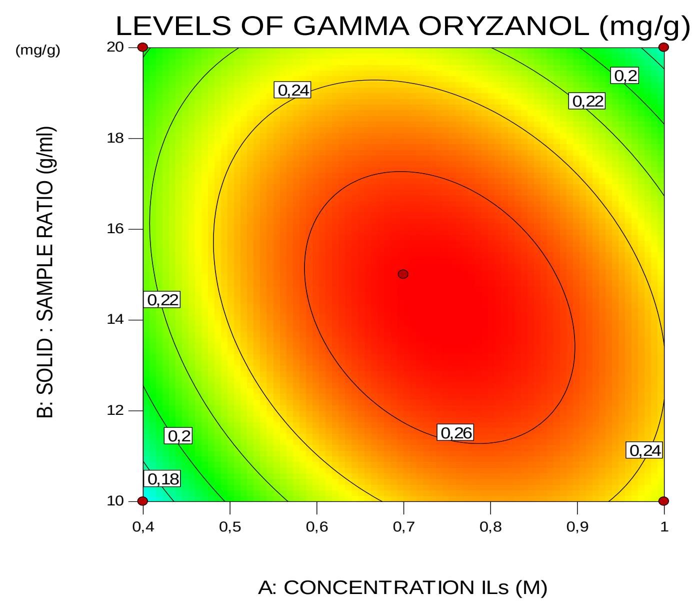

Fig. 3: Model Contour Plot.

In this research, the utilizing of eco-friendly solvents such as ionic liquids are more effective as solvents in the extraction of gamma oryzanol than organic solvents because of the flexibility of combinations ions in the customize properties of physicochemical in the target compounds. In addition of ionic liquid had the potential to replace organic solvents which characteristic are flammable, toxic and volatile (Jessop *et al*., 2012).

The efficiency of extraction with the ionic liquid can be increased with decreasing alkyl groups, where the alkyl residues can be reduced by lengthening alkyl group (Ying etal., 2007). Ionic liquid $[Bmim]PF_6$ was used as an absorption medium and applied to the extraction of essential oil from dried fruits of *Illicium verum* hook. F. and *Cuminum cyminum* L. by microwave-assisted extraction (Zhai *et al*., 2009). Ionic liquids as solvents can be used to extract the compounds from *Peperomia pellucida* (L.) Kunth plants and also the extraction process becomes faster, easier, and efficient compared conventional methods in general (Ahmad *et al*., 2017).

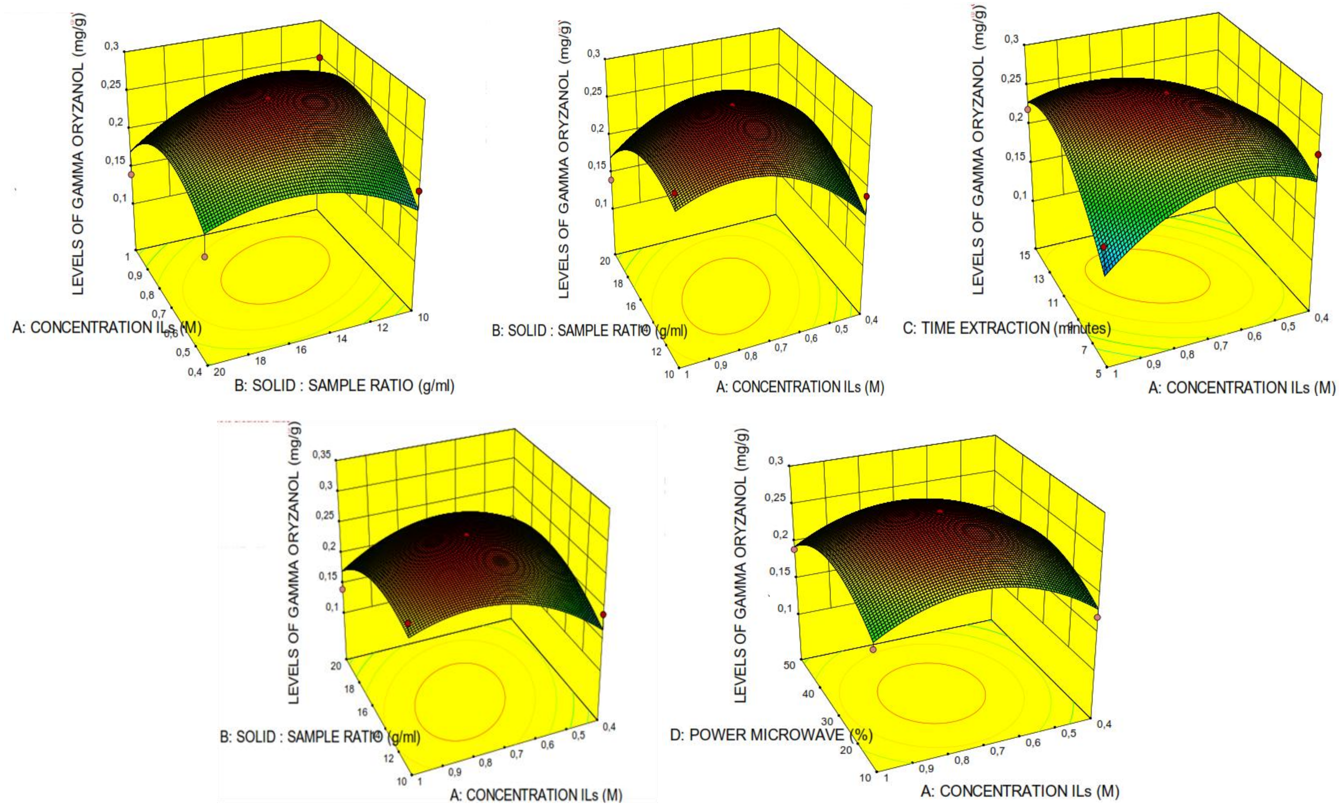

Fig. 4: The 3D response surface methodology (RSM) models.

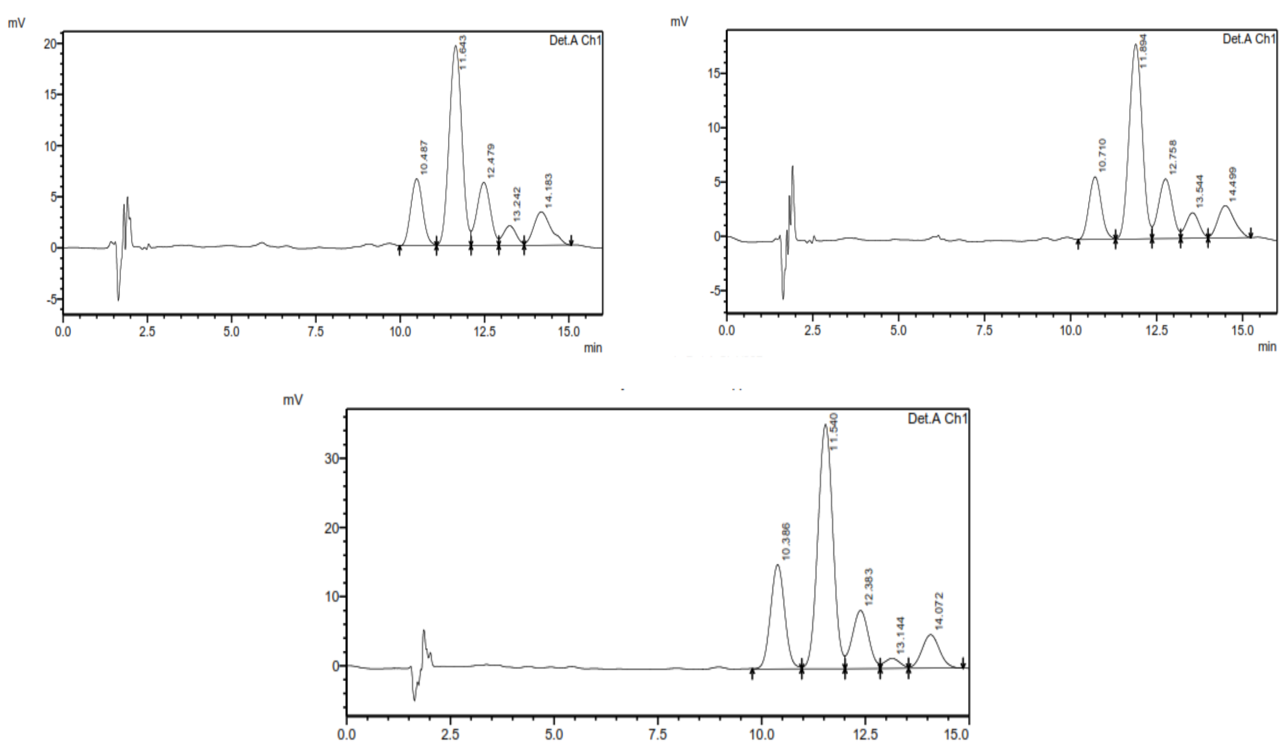

Fig. 5: The Results of HPLC Chromatogram: (A) Standard gamma oryzanol; (B) Extract of rice bran (gamma oryzanol) by isopropanol-MAE; (C) Extract of rice bran (gamma oryzanol) by IL [BMIM]PF_6-MAE

CONCLUSION

IL-MAE is an alternative method extraction in the separation process of a bioactive compound from the plant. It showed an increasing level of gamma oryzanol utilizing IL-MAE compared to the conventional method extraction.

ACKNOWLEDGMENT

This research was supported by the Ministry of Research and Technology, The Republic of Indonesia and the Directorate of Research and Community Service, Universitas Indonesia through PITTA 2017.

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

Ahmad I, Ahmad I, Yanuar A, Mulia K, Mun'im A. Application of ionic liquid based microwave-assisted extraction of the secondary metabolite from *Peperomia pellucida* (L) Kunth. Pharmacogn J, 2017; 9(2):227-234.

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

Bogdanov MG. Ionic Liquids as Alternative Solvents for Extraction of Natural Products. Green Chemistry and Sustainable Technology, 2014; pp 127-166.

Chan C, Yusoff R, Ngoh G, Wai-Lee KF. Microwave-assisted extractions of active ingredients from plants. Journal of Chromatography A, 2011;1218:6213-6225.

Chen MH, Bergman CJ. A rapid Procedure for Analysing Rice Bran, Tocopherol, Tocotrienol and γ-Oryzanol Contents. Journal of Food Analysis, 2005; 18: 139–151.

Du FY, Xiao HX, Gong KL. Application of ionic liquids in the microwave-assisted extraction of trans-resveratrol from Rhizoma Polygoni Cuspidati. Journal of Chromatography A, 2007;1140:56–62.

Duvernay, WH, Assad, JM, Sabliov, CM, Lima, M., Xu, Z. Microwave extraction of antioxidant components from rice bran. Pharmaceutical Engineering, 2005; 25: 126-130.

Espino M, Fernandez M, Gomez FJV, Silva MF. Natural designer solvents for greening analytical chemistry. Trends in Analytical Chemistry, 2016; 76:126–136.

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

Islam S, Nagasaka R, Ohara K, Hosoya T, Ozaki H, Ushio H, Hori M. Biological Abilities of Rice Bran-Derived Antioxidant Phytochemicals for Medical Therapy. Current Topics in Medicinal Chemistry, 2011; 1:1847–1853.

Jain T, Jain V, Pandey R, Vyas A, Shukla SS. Microwave Assisted Extraction for Phyto Constituents - An Overview. Asian Research Journal of Chemistry, 2009;1(2): 19-25.

Jessop PG, Jessop DA, Fu D, Phan L. Solvatochromic parameters for solvents ofinterest in green chemistry. Green

Chem, 2012 ;14: 1245.

Liazid A, Palma M, Brigui J, Barroso CG. Investigation on phenolic compounds stability during microwave-assisted extraction. J Chromatogr A, 2007; 1140(1-2):29-34.

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

Mariod A, Ismail M, Abd Rahman NF, Matthaus B. Stability of rice bran oil extracted by SFE and soxhlet methods during accelerated shelf life storage. Grasas Aceites, 2014; 65 (1): 1-10.

Nasir, S, Fitriyanti, Kamila H. Extraction of rice bran to crude oil (Crude rice bran oil) with solvent N-hexane and ethanol. Journal of Chemical Engineering, 2009; 2(16): 1-10.

Thanonkaew A, Wongyai S, McClements, DJ, Decker, EA. 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.). Food Science and Technology, 2012;48: 231-236.

Xu, Z., Godber, JS. Purification and Identification of Components of gamma oryzanol in rice bran oil. Journal of Agriculture and Food Chemistry, 1999; 47: 2724-2728.

Xu W, Chu K, Li H, Zhang Y, Zheng H, Chen R, Chen L. Ionic Ionic Liquid- Based Microwave-Assisted Extraction of Flavonoids from *Bauhinia championii* (Benth.) Benth. Molecules, 2012;17: 14323–14335.

Ying YY, Zhang W, Cao SW. Extraction of Ferulic Acid and Caffeic Acid with Ionic Liquids. Chin J Anal Chem, 2007;35(12): 1726–1730.

Zhai Y, Shuo S, Ziming W, Jianhua C, Yantao S, Lu W, Yupu Z, Hanqi Z, Aimin Y. Microwave extraction of essential oil from dried fruits of *Illicium verum* Hook. f. and *Cuminum cyminum* L. using ionic liquid as the microwave absorption medium. J. Sep. Sci., 2009; 32: 3544 – 3549.

How to cite this article:

Trinovita E, Sigalingging E, Saputri FC, Mun'im A. Optimization of Ionic Liquid 1-Butyl-3-Methylimidazolium Hexafluoro-phosphate ([Bmim] Pf_6)-Based Microwave Assisted Extraction Method Forγ-Oryzanol From Rice Bran (*Oryza Sativa* L.). J App Pharm Sci, 2017; 7 (12): 008-013.