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Original Article

Enrichment of the Gamma Oryzanol Level from Rice Bran by Addition of Inorganic Salts on Ionic Liquid 1-Butyl-3-Methylimidazolium Hexafluorophosphate ([BMIM] PF_6) Extraction

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ABSTRACT

Objective: Rice bran oil has many helath benefit. The biologically active compounds of rice bran oil are fatty acids, squalene, tocopherols, phytosterols, tocotrienols, oryzanol, and polyphenols. Gamma oryzanol is a combination of at least 10 components of ferulic acid esters and alcohols triterpene. Gamma oryzanol has pharmacological activity includes cardiovascular disease, antioxidant, anticancer, antidiabetic, antiulcerogenic, neuroprotective and action immunomodulator. Ionic liquids are an environmentally friendly solvent used in the extraction and separation of bioactive compounds from plants. This study was conducted to evaluate the effect of inorganic salts addition on ionic liquid [Bmim]PF_6 in increasing levels of gamma oryzanol from rice bran. **Method**: In the experiments, IL [Bmim]PF_6 - MAE method was used to separated gamma oryzanol compound, and then was partitioned with hexane after the addition of inorganic salts: KH_2PO_4, NaCl, $NaNO_3$ and Na_2CO_3, respectively. Gamma oryzanol content was determined by HPLC analysis. A mixture of mobile phase (methanol:acetonitrile:isopropanol (5:4:1) and isocratic conditions at a wavelength of 327 nm. The flow rate is set at 1 ml / min..**Results**: The highest levels of γ-oryzanol was produced from the addition in KH_2PO_4 + **:** [Bmim] PF_6 by 0.26 ± 0.001 mg/g. The mechanism of salting-out effect of the compound due to the interaction of different types of interactions between solutes (ions of inorganic salts and ionic liquid) and solvent. **Conclusion**: The addition of inorganic salt increased the gamma oryzanol level. The addition of KH_2PO_4 on IL-MAE gave the highest level of gamma oryzanol.

Key words: [BMIM]PF_6, Ionic liquid, γ-oryzanol, IL-MAE, Inorganic salts.

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INTRODUCTION

Rice bran is a component of the raw rice obtained when it is separated from the endosperm during the rice milling process.[1] Rice bran is a by-product of rice milling process that has potential economic value due to rice bran oil content. Rice bran oil has a high nutrient and some bioactive compounds. Gamma oryzanol, tocopherols, tocotrienols, phytosterols, polyphenols, and squalene were found in rice bran oil.[2] Gamma oryzanol was reported to have some biological properties, such as antioxidant,[1] anti-inflammation,[3] cardioprotection,[4] and hypolipidemic.[5] One of the contents of rice bran oil is gamma oryzanol with a concentration concentration between 1.5 - 3%.[6]

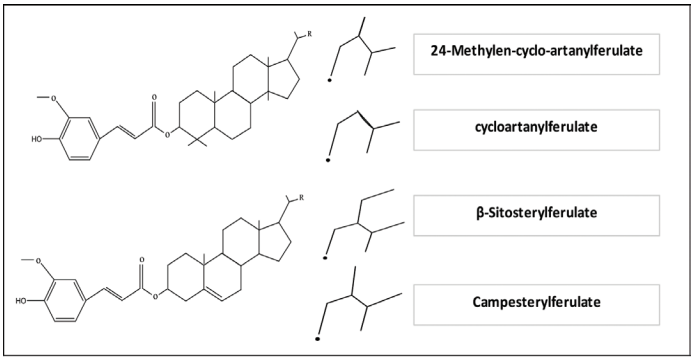
Gamma oryzanol is a chemical compound that is mostly composed of the complex ester trans-ferulate (*trans*-hydroxy cinnamic acid) with phytosterols (sterols and alcohol triterpene), including cycloartenol, β-sitosterol, 24-methylene cycloartenol ferulate and predominant campesterol.[7,8] Figure 1 shows the molecular structure of trans-ferulate of four phytosterols as the main content of gamma oryzanol.

Some researchers are beginning to switch to the use of environmentally friendly solvents such as ionic liquids[9,10] Besides that, IL can be utilized as a solvent in the extraction and separation of bioactive compounds from plants.[11] Ionic liquid are pure salt compound consisting of ionic components (cations and anions) that melt at a temperature below 100°C.[12] Application of ionic liquid increased total phenolic content and total flavonoid if compared to conventional solvent.[9] Tanshinone and crypto tanshinone were significantly extracted from *Salvia miltiorrhiza* Bunge by application of IL (C_{14}mimBr and C_{16}mimBr).[13]

Butylmethylimidazolium hexafluorophosphate ([BMIM] PF_6) was based on the solutes charged state or relative hydrophobicity. The length of IL alkyl chain indeed exerts a significant impact on the extraction yield of lipophilic compounds. Also the hydrophobic ion liquid phase exhibits a high affinity for ionic compounds when neutralized.[10] The ionic liquid [Bmim] PF_6 has [PF_6^-] hydrophobic anion as shown in Figure 2.

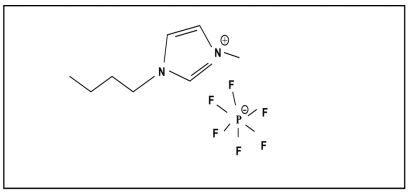
The problem of the use of IL was a suitable method to extract bioactive compounds from IL phase. The addition of salt inorganics such as KH_2PO_4, NaCl, $NaNO_3$ and Na_2CO_3 were reported to increase the level of the bioactive compound in extract via "salting-out" effect. The mechanism of effect of salting-out the complex due to the interaction of different types of interactions between solutes and solvent.[14] The addition of lithium salt on [Bmim]Br increased the concentration of syringin and oleuropein from *Syringa reticulata* var *mandshurica* significantly.[15]

MAE is an extraction method that utilizes microwave radiation to accelerate selective removal extraction by heating the solvent quickly and efficiently. Microwaves reduce enzymatic activity that damages the target compound.[16] Some of the advantages of the method MAE include the extraction time is shorter, the use of the volume of solventless, easily adapted to conditions ranging from the size of the sample, temperature,



[source: García, MJ Lerma et.al, 2000, it has been modified]

Figure 1: Chemical structure of main components in γ-oryzanol.



[source: Villemin, Didier; Didi, Mohamed Amine, 2013, it has been modified]

Figure 2: Chemical Structure of ILS BMIM [PF_6].

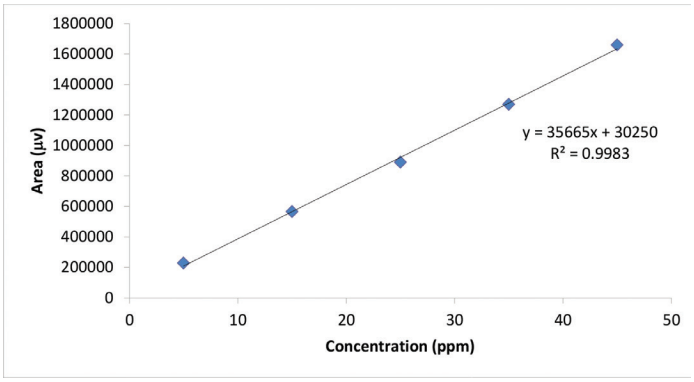


Figure 3: Calibration curve of standard γ-oryzanol.

pressure, amount of solvent, and the number of samples. Gamma oryzanol can be separated and quantified using high-performance liquid chromatography (HPLC). In this study will be conducted IL-MAE extraction method using a solvent ionic liquid [Bmim]PF_6 by optimizing the addition of some inorganic salts to separate gamma oryzanol from IL so that levels of gamma oryzanol in rice bran extract can increase.

MATERIALS AND METHODS

Instruments

Centrifuge (Heraeus-Christ GmbH, Osterode, Germany). Vortex (Wise-Mix VM-10, Daihan Scientific, Korea). Microwave (Modena MV-3002 with slight modification). HPLC system (Shimadzu, Japan). The column used is a type Zorbax Eclipse Plus C-18 Analytical 4.6 x 150 mm, 5µm (Agilent Technologies-USA).

Materials

Fresh rice bran varieties IR (*Oryza sativa* L.) were obtained from the milling of rice Bogor West Jawa. Standard gamma oryzanol was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). [Bmim]PF_6 1-(butyl-3-methylimidazolium hexafluorophosphate) (>99%) was purchased from Chengjie Chemical Co., Ltd., (Shanghai, China). Acetonitrile, isopropanol and methanol with HPLC grade were obtained from Merck.

Methods

Rice bran stabilization

The fresh rice bran was stabilized by heating in an oven at 110° C for 15 minutes, then cooled in a container of ± 30 minutes to reach room temperature. The stabilized rice bran was put in clear plastic and store at room temperature.

Preparation of [Bmim]PF_6

[Bmim]PF_6 (1M) was dissolved by the addition of 11% isopropanol (v/v) and then was sonicated for 10 minutes until homogeneous.

Preparation of inorganic salts

In this study the optimization of some inorganic salts solution such as KH_2PO_4, NaCl, $NaNO_3$ and Na_2CO_3 at a concentration of 0.01 mol.

Microwave assisted extraction (MAE)

Stabilized rice bran (1 g) was put in a flat-bottom flask, then added 10 mL of solvent ILs with the appropriate volume of experimental design 1:10 (b/v). After that the flask containing stabilized rice bran powder and IL concentration (1 M) was then put in the microwave that has modified with the same treatment includes variation extraction for 10 minutes and 30% microwave power. After the extraction process, the sample cooled at room temperature for 10 minutes. Then filtered using a filter paper to obtain a filtrate desired as a result of extraction MAE. Furthermore, the filtrate added N-hexane (1 ml) and 1 ml of inorganic salt. Then vortex for 10 seconds and was followed centrifuged at 3000 rpm for 15 minutes to separate the residue with the supernatant.

Determination of gamma oryzanol level with high-performance liquid chromatography

The supernatant (0.5 ml) was diluted by isopropanol and was put in a flask up to 10.0 ml. Then the solution of the sample (20 ml) was injected into the HPLC system (Shimadzu, Japan). The HPLC was equipped by a column type Zorbax Eclipse Plus C_{-18} Analytical 4.6 x 150 mm, 5µm (Agilent Technologies-USA). Methanol:acetonitrile:isopropanol (5:4:1) was used as mobile phase under isocratic conditions, with flow rate 1 ml/min, and monitored by UV detector wavelength set at 327 nm. Each sample measured with three repetitions.

RESULTS

Calibration curve of standard gamma oryzanol

Further calibration standard curve in the range of concentration of 1 ppm-45 ppm at wavelength 327 nm as shown in Figure 3 below. Figure 4 shows chromatogram of standard gamma oryzanol and sample after addition of KH_2PO_4 solution. Based on the chromatogram, four component of gamma oryzanol were detected, i.e: cycloartenol ferulate, 24-methylenecycloartenol ferulate, campesterol ferulate, and β-sitosteril ferulate.

The addition of various inorganic salts on ionic liquids

The sample preparation without the addition of salt showed levels of gamma oryzanol 0.026 ±0.001 mg/g. The addition of all used salts on the samples showed increasing of the gamma oryzanol level if was compared with that no salt addition. Figure 5 shows the effect of the addition of KH_2PO_4, $NaNO_3$, NaCl, and Na_2CO_3, respectively on the increase of gamma

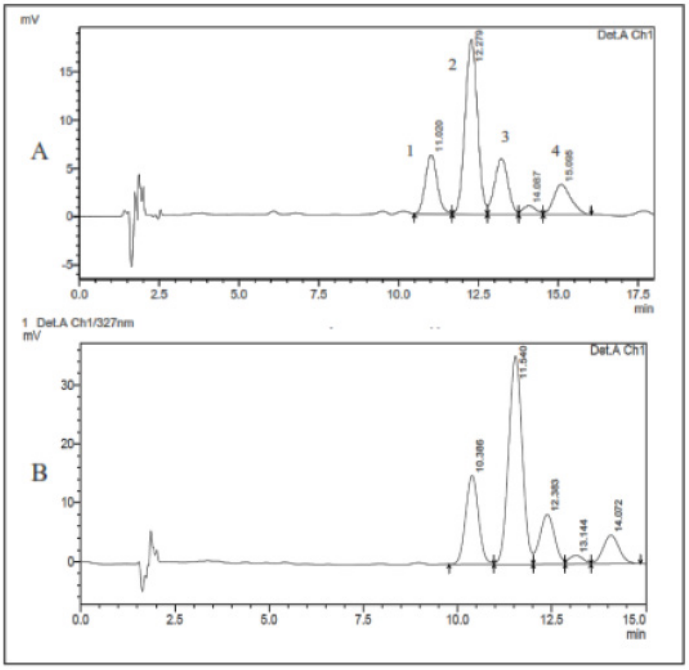


Figure 4: HPLC chromatograms of A: standard (45 ppm), B: Sample from ILs BMIM[PF_6] extraction after addition KH_2PO_4. HPLC condition: UV detector wavelength at 327 nm, the flow rate was set 1 ml/min. (1) cycloartenol ferulate, (2) 24-methylene cycloartenol ferulate, (3) campesterol ferulate, and (4) β-sitosteril ferulate.

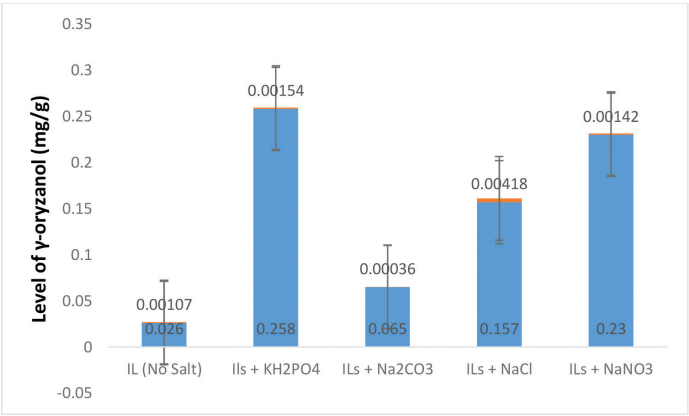


Figure 5: Effect of Addition of Inorganic Salts on Ionic Liquid.

oryzanol concentration. Based on the results, the order of strength of the salt solution on the increasing of gamma oryzanol level was as follow: KH_2PO_4> $NaNO_3$> NaCl> Na_2CO_3.

DISCUSSION

The methods of IL-MAE without the addition of salt obtainable levels of 0.026 mg/g while on the method IL-MAE by the addition of salts KH_2PO_4 provide levels of gamma oryzanol of 0.26 mg/g. This shows that the addition of KH_2PO4 salts increased levels of gamma oryzanol of 900%, which is caused by the interaction of solutes (ion of inorganic salts and ionic liquid) and solvents

The influence of the addition of inorganic salts on the solubility of the solute in an aqueous solution is very complicated, especially since a large number of different types of intermolecular interactions participate between ions and the solvent, ions and ionic solutes, and ions of the solute and solvent. Some theory of salting out the most commonly involves the concept of structure-forming (cosmotropic) salt, wherein when salt dissolved in an aqueous solution a process of hydration of ions are the ions are surrounded by a layer of water molecules, water molecules are moving so that its role as a solvent for molecular or other ions reduced. The addition of an inorganic salt to a solution of the ionic liquid causes to be "competitive" with each other for solvent molecules.[14-17,18,19]

The competition was won by inorganic ions as inorganic ions have a strong affinity and migration of solvent molecules to escape from the ion ionic liquid with salt inorganic to reducing the strength of ion hydration and cause the ionic liquid phase will separate from the rest of the solution. It can be concluded that the salting-out effects associated with different power ion hydration of inorganic salts.[14] The recent studies have demonstrated that either direct ion-macromolecule interactions or interactions with water molecules in the first hydration shell of the macromolecules govern the Hofmeister effect as well as phase separation in ATP systems (aqueous two phase system).[17]

In Figure 4 shows the results of HPLC chromatogram of gamma oryzanol obtained from an IL-MAE method with the addition of salt KH_2PO_4. In this study, the extract contained four components of the main content of gamma oryzanol referred to Lerma-Garcia *et al*, 2009 study.[20] 24-Methylene cycloartenol ferulate has the highest peak area compared to other peaks and this is considered to have the highest antioxidant activity compared cycloartenol ferulate, campesterol ferulate and β-sitosteril ferulate.[21]

In previous research that has been conducted by researchers with MAE method using isopropanol as conventional solvents and gamma oryzanol content of 0.25 mg /g. According to the Mandal *et al* 2007, this may be due to the high temperature and high power breakdown cell walls intensify.[22] Each microwave power influenced by extraction time and extraction temperature. An interaction between the solvent isopropanol and the matrix material so that the rice bran can absorb microwaves. Rapid heating of the cell causes cell breakdown and spending target compounds into the polar solvent efficiently. Results of the previous study by Duvernay (2005) shows an increase in rice bran oil was obtained from the temperature of 80°C to 120° C using microwave assisted extraction (MAE) with isopropanol as solvent extraction, where the high-temperature isopropanol as solvent proved to be significantly better than the rice bran oil by hexane extraction.[23] The stability of the γ-oryzanol in isopropanol solution was high enough at room temperature.[24] A solvent with medium or low volatility, such as isopropanol is more preferable, especially at room temperature.

CONCLUSION

The mechanism of salting-out effect of the compound due to the interaction of different types of interactions between solutes and solvent. The addition of various inorganic salts on the IL as an alternative for the recovery of active compounds from plants. The highest levels of gamma oryzanol (0.26 ± 0.001 mg/g) was resulted from the addition of salt KH_2PO_4 + [Bmim] PF_6.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATION USED

IL: ionic liquid; MAE: microwave-assisted extraction; [Bmim] PF_6: 1-butyl-3-methylimidazolium hexafluorophosphate; HPLC: High performance liquid chromatography.

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