Enrichment of the Gamma Oryzanol Level from Rice Bran by Addition of Inorganic Salts on Ionic Liquid 1-Butyl-3-Methylimidazolium Hexafluorophosphate ([BMIM] PF₆) Extraction

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ABSTRACT

Objective: Rice bran oil has many health benefits. The biologically active compounds of rice bran oil are fatty acids, squalene, tocopherols, phytosterols, tocotrienols, oryanol, and polyphenols. Gamma oryanol is a combination of at least 10 components of ferulic acid esters and alcohols triterpene. Gamma oryanol has pharmacological activity includes cardiovascular disease, antioxidant, anticancer, antidiabetic, antiallergic, neuroprotective, and action immunomodulator. Ionic liquids are 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]PF₆ in increasing levels of gamma oryanol from rice bran. Method: In the experiments, IL [Bmim]PF₆ - MAE method was used to separated gamma oryanol compound, and then was partitioned with hexane after the addition of inorganic salts: KH₃PO₄, NaCl, NaNO₃, and Na₂CO₃, respectively. Gamma oryanol content was determined by HPLC analysis. A mixture of mobile phase (methanol:acetonitrile:isopropanol of (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 KH₃PO₄ + [Bmim]PF₆ 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 oryanol level. The addition of KH₃PO₄ on IL-MAE gave the highest level of gamma oryanol.

Key words: [BMIM]PF₆, Ionic liquid, γ-oryzanol, IL-MAE, Inorganic salts.

INTRODUCTION

Rice bran is a component of the raw rice obtained when it is separated from the endosperm during the rice milling process. 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 oryanol, tocopherols, tocotrienols, phytosterols, polyphenols, and squalene were found in rice bran oil. Gamma oryanol was reported to have some biological properties, such as antioxidant, anti-inflammatory, cardioprotection, and hypolipidemic. One of the contents of rice bran oil is gamma oryanol with a concentration between 1.5 - 3%. Gamma oryanol 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. Figure 1 shows the molecular structure of trans-ferulate of four phytosterols as the main content of gamma oryanol. Some researchers are beginning to switch to the use of environmentally friendly solvents such as ionic liquids. Besides that, IL can be utilized as a solvent in the extraction and separation of bioactive compounds from plants. Ionic liquid are pure salt compound consisting of ionic components (cations and anions) that melt at a temperature below 100°C. Application of ionic liquid increased total phenolic content and total flavonoid if compared to conventional solvent. Tanshinone and crypto tanshinone were significantly extracted from Salvia miltiorrhiza Bunge by application of IL (C₆ mimBr and C₆ mimBr). Butylimidazolium hexafluorophosphate ([BMIM] PF₆) 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. The ionic liquid [Bmim] PF₆ has [PF₆]⁻ 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 KH₃PO₄, NaCl, NaNO₃, and Na₂CO₃ 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. The addition of lithium salt on [Bmim]Br increased the concentration of syringin and oleuropein from Syringa reticulata var mandshurica significantly. 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. 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,
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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). 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 γ-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$_2$PO$_4$ solution. Based on the chromatogram, four component of gamma oryzanol were detected, i.e: cycloartenol ferulate, 24-methylenecycloartenol ferulate, campesterol ferulate, and β-sitosterol 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$_2$PO$_4$, NaNO$_3$, NaCl, and Na$_2$CO$_3$, respectively on the increase of gamma.
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.  

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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. 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).  

In Figure 4 shows the results of HPLC chromatogram of gamma oryzanol obtained from an IL-MAE method with the addition of salt KH2PO4. In this study, the extract contained four components of the main content of gamma oryzanol referred to Lerma-Garcia et al, 2009 study.  

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. 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. 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 80ºC to 120º 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. The stability of the γ-oryzanol in isopropanol solution was high enough at room temperature. A solvent with medium or low volatility, such as isopropanol is more preferable, especially at room temperature.

CONCLUSION

The method 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 KH2PO4 provide levels of gamma oryzanol of 0.26 mg/g. This shows that the addition of KH2PO4 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

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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<sub>6</sub>: 1-butyl-3-methylimidazolium hexafluorophosphate; HPLC: High performance liquid chromatography.

REFERENCES


