

Removal of Bioactive Compound (γ -Oryzanol) from Rice Bran Oil-Based Biodiesel Using Deep Eutectic Solvent

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Rice bran oil-based biodiesel contains high level of unsaponifiable matters (4.0 - 5.4 %) including bioactive compounds, such as γ -oryzanol, which have antioxidant activity. To fulfil the standard biodiesel specification, crude biodiesel should be refined. Isolation and purification of bioactive compounds from rice bran oil-based biodiesel are attractive options to further reduce the production cost of biodiesel since bioactive compounds can be sold separately at high price. The objective of this work is to study the effect of extraction time on the separation of fatty acid methyl ester (FAME) and γ -oryzanol from rice bran oil-based biodiesel using deep eutectic solvent (DES). Acid catalysed methanolysis was employed to convert rice bran oil into biodiesel at the following operation conditions: T = 60 °C, t = 8 h, molar ratio of oil to methanol = 1 : 10, and H₂SO₄ = 1 % of oil. Crude biodiesel obtained contains 89.05 % of FAME, 2.59 % of γ -oryzanol and less than 0.05 % of free fatty acids (FFA). The DES was prepared by mixing of choline chloride and ethylene glycol at a molar ratio of 1 : 2. The extraction temperature was varied at 30 °C, 45 °C and 60 °C. The extraction time was varied from 15 min to 240 min at constant temperature 30 °C and molar ratio crude biodiesel to DES 1/2 and 1/4. The highest FAME content (96.60 %) and the lowest γ -oryzanol (1.18 %) was obtained after extraction time of 240 min at 30 °C and molar ratio 1 : 4. This work shows that DES has a potential to purify biodiesel and reduce unsaponifiable matter (γ -oryzanol) from rice bran oil-based biodiesel.

1. Introduction

Rice is a staple food in Indonesia. Rice bran is the main by-product of rice milling process and contains 10-26 % of rice bran oil (RBO) (Ju and Zullaikah, 2013). Zullaikah et al. (2009) reported isolation of γ -oryzanol from crude rice bran oil (RBO) by a two-step crystallisation process. In this work, we used rice bran oil-based biodiesel as raw material because RBO is potential low cost feedstock for biodiesel and new generation solvent (DES) to remove γ -oryzanol from crude biodiesel. Biodiesel produced from RBO contained impurities such as FFA, monoglycerides (MG), diglycerides (DG), triglycerides (TG) and bioactive compounds (γ -oryzanol, tocopherols, tocotrienols, phytosterol, polyphenols and squalene) (Kasim et al., 2007). Those impurities should be removed from biodiesel. Isolation of bioactive compounds from RBO-based biodiesel could be reducing the production cost of biodiesel (Ju and Zullaikah, 2013). Vegetable oils contain phytosterols of 1 - 5 g/kg, but the content of phytosterols in RBO was 30 g/kg, whereas γ -oryzanol content in RBO was 1 - 3 %. γ -Oryzanol is one of phenolic compounds which are defined as a group of ferulic acid esters of sterols and triterpene alcohol. It was an important substance that can be applied widely in the food, cosmetics and pharmaceuticals (Jesus et al., 2010). γ -oryzanol can be degraded at a rate which was lower than that of α -tocopherol at high temperature; therefore γ -oryzanol is an antioxidant that is promising for application at high temperature (Ju and Zullaikah, 2013). Other beneficial effects of γ -oryzanol were their activity against free radicals in the body, to lower cholesterol in the blood, liver, and inhibiting menopause. Isolation and purification of γ -oryzanol from RBO-based biodiesel is an attractive option to further reduce the production cost of biodiesel since γ -oryzanol can be sold separately at high price and increasing the purity of biodiesel to fulfill biodiesel standard. The most commonly method for purification of biodiesel are wet washing and dry washing. Wet washing using water or acid such as phosphoric acid, sulphuric acid and hydrochloric acid to removed impurities such as catalyst and soap. It's due to catalyst and soap were soluble in water and acid (Hayyan et al., 2010). Although water washing is efficient, it is associated with problems such as increase

in production time and cost of production. This method required 60 - 80 % of total production costs, and it was not suitable for the production of biodiesel in large capacity (Atadashi et al., 2011). Purification of crude biodiesel from RBO is more difficult than other vegetable oils because it was contained high amount of unsaponifiable matters. Novel methods had been developing for refining of crude biodiesel, such as using membrane separation technology, ionic liquids and DES. DES was a new generation solvent formed from a mixture of quaternary ammonium salts with hydrogen bond donor (HBD). DES has more advantages compare to organic solvent, such as non-volatile and non-flammable, making its storage easier. DES had a high polarity; it can be used to separate biodiesel from its impurities, such as glycerol, water, FFA, MG, DG and TG. DES is miscible in water but immiscible in biodiesel (Zhang et al., 2012). DES has been used to remove free and total glycerol from palm oil-based biodiesel by liquid-liquid extraction (Shahbaz et al., 2010). Kasim et al., (2007) was recovered of γ -oryzanol from RBO-based biodiesel using several steps, including liquid-liquid extraction, distillation and column chromatography. Separation of biodiesel and γ -oryzanol from RBO-based biodiesel using DES has not been reported. This study was investigated the separation of biodiesel and γ -oryzanol from RBO-based biodiesel using DES.

2. Methods

2.1 Materials

Rice bran (IR 64 rice variety) was donated by a rice mill located in Banyuwangi, Indonesia. Choline chloride (≥ 98 %) was obtained from Sigma Aldrich (China), Ethylene Glycol (99 %) was obtained from SAP Chemical (Indonesia), n-hexane, methanol and sulphuric acid (98 %) were obtained from ANHUI FULLTIME (Anhui, China). γ -oryzanol standard was obtained from Tsuno Rice Fine Chemicals Co. Ltd. (Wakayama, Japan). All other chemicals used were obtained from commercial sources.

2.2 Extraction of RBO

Rice bran (50 g) was extracted using n-hexane in a soxhlet extractor for 8 h. Crude RBO yield of 16.49 % and FFA content of 44.75 % can be achieved. The yield is expressed as percentage of crude RBO weight/rice bran. Crude RBO obtained was subjected to dewaxing/degumming process according to Kaimal et al. (2002). In this work, the molecular weight of RBO was determined by measuring saponification value (SV). SV for RBO after dewaxing/degumming process obtained amounted to 193.80 ± 1.53 mg KOH/g. The molecular weight of the RBO estimated from SV obtained was 868.40 g/mol.

2.3 Biodiesel Production from Dewaxed/Degummed RBO

RBO based-biodiesel in this work was produced according to Zullaikah et al. (2005). The acid-catalysed methanolysis reactions were carried out with 10 g of dewaxed/degummed RBO at 60 °C and atmospheric pressure under stirring at 300 rpm. Methanolysis reactions were performed in a 50 mL three-necked round-bottomed flask equipped with a reflux condenser, thermometer and rubber septum and placed in a water bath with a temperature controller and magnetic stirrer. Sulphuric acid (1 wt %) dissolved in methanol was injected into the reaction vessel through a septum after the RBO were heated to the required temperature (60 °C) under stirring. Crude biodiesel obtained contain 89.05 % of FAME with very low FFA content (0.05 %) and 2.59 % γ -oryzanol. The molecular weight of crude biodiesel is 291.99 g/mol, estimated from average molecular weight of methyl ester component at crude biodiesel after Gas Chromatography (GC) analysis.

2.4 Synthesis Of Deep Eutectic Solvent (DES)

DES was prepared by combination of choline chloride (ammonium quaternary salt) and ethylene glycol as HBD. Choline chloride and ethylene glycol with molar ratio of 1 : 2 was added into a stopper glass. The molecular weight of DES describe as average molecular weight of CHCl and ethylene glycol mixture, is 113.67 g/mol. The mixture was heat up to 60 °C under stirring condition (300 rpm) until homogenous transparent liquid was formed. Density of DES was measured by picnometer (IWAKI Pyrex).

2.5 Separation of γ -Oryzanol and Biodiesel Using Deep Eutectic Solvent

Separation of γ -oryzanol and biodiesel from RBO-based biodiesel will be carried out using DES, a mixture of choline chloride (ammonium quaternary salt) and ethylene glycol as HBD at molar ratio of 1 : 2. RBO-based biodiesel and DES are mixed in a stopper glass at molar ratio crude biodiesel to DES is 1 : 2. The mixture of biodiesel and DES was heated under stirring at 300 rpm at 30 °C, 45 °C and 60 °C to study the effect of temperature on extraction process. Afterwards, the mixture was settled for 2 h at ambient temperature, and 2 layers was formed. The upper layer is the biodiesel (FAME) rich phase and the bottom layer is the DES rich phase containing extracted compounds such as γ -oryzanol. Biodiesel (upper layer) was separated from DES using separator funnel then it was analysed using GC and spectrophotometer. The best temperature condition for removal of γ -oryzanol was used as temperature condition to learn the effect of extraction time and molar ratio in this work. Molar ratio crude biodiesel to DES 1 : 2 and 1 : 4 was used, the extraction time was varied

from 15 min to 240 min at constant temperature 30 °C. Figure 1 shows the Flowchart of γ -oryzanol removal from rice bran oil based biodiesel.

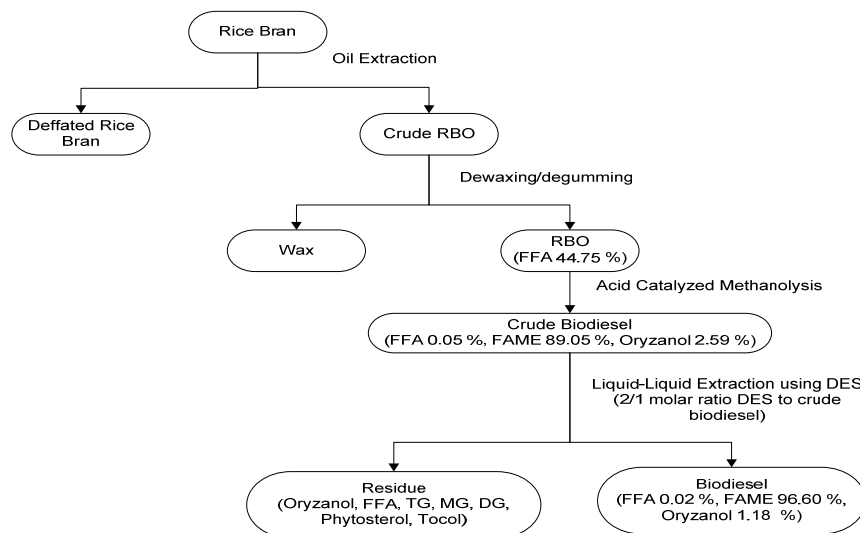


Figure 1: Flowchart of γ -oryzanol removal from rice bran oil based biodiesel

2.6 Determination of FAME, γ -oryzanol and FFA contents

FFA, FAME, MG, DG and TG content in biodiesel was determined using Shimadzu GC-2010 Plus (Kyoto, Japan) gas chromatograph equipped with a flame ionisation detector. Separations were carried out on a ZB-5HT (5 %-phenyl)-methylpolysiloxane non-polar column (15 m \times 0.32 mm i.d., 0.1 mm film thickness; Agilent Tech. Palo Alto, California). The temperatures of injector and detector were both set at 370 °C. Temperature of the column was started at 80 °C, increased to 365 °C at 15 °C /min, and maintained at 365 °C for 8 min. The split ratio is 1 : 50 and nitrogen as carrier gas. 20 mg sample was dissolved in 1.5 mL ethyl acetate, heated until clear solution was observed and 1.5 μ L samples was injected into the HTGC.

Chromatogram and non-chromatogram technology is commonly used to determine the content of γ -oryzanol. One example of a chromatogram technology is High Performance Liquid Chromatography (HPLC) and for non-chromatogram technology is Spectrophotometry Ultraviolet (UV Spectrophotometry) (Rogers et al., 1993). Each method has its own advantages and disadvantages, HPLC method are accurate and efficient, but it requires a high cost, while the UV Spectrophotometry an easier method, practical, yet low cost. UV-vis spectrophotometer method was chosen to determine the content of γ -oryzanol in this work. The use of UV-Vis spectrophotometry in this study is based on earlier journals such as the research of Bucci et al. (2003), using UV-Vis Perkin–Elmer 320 UV–visible spectrophotometer to determined γ -oryzanol content in rice bran oil with a set of wavelengths between 310 - 360 nm. It was found λ of oryzanol is 327 nm using isopropanol as solvent. The result is also compared with data using HPLC, Liquid chromatography was performed on a Thermo Quest Spectra Series. The results show that UV spectrophotometry, in its various aspects, is still a valid technique for the determination of analyte in real matrices (even rather complex), as it combines the simple and inexpensiveness of these methods, obtaining a great precision and accurate results. The previous research by Zullaikah et al. (2009) also used UV-vis Spectrophotometry Jasco V-550 from Japan. From the research, λ of oryzanol is 316 nm using n-hexane as a solvent. From previous journal that was mentioned above, it can be taken that the tool UV-Vis spectrophotometry can be used as a tool to analyse the content of oryzanol in biodiesel. γ -oryzanol content in this work was determined using UV-vis Spectrophotometer V-550. The calibration curve was obtained by using pure γ -oryzanol with a concentration range from 0 to 30 ppm. A straight line passing through the origin was obtained at a wavelength of 324 nm ($R^2 = 0.99$). To determine γ -oryzanol content, the sample was diluted in isopropanol in a 1 cm cuvette and the spectrophotometer was operated at 1 nm bandwidth and 1 nm data pitch.

3. Result and Discussion

3.1 Characteristic of Deep Eutectic Solvent (DES)

Characteristic of DES depend on the election of HBD, salt and the molar ratio of salt to HBD. Melting point of DES at molar ratio 1 : 2 of salt to HBD was 296.15 K (Zhao et al., 2011) and density of DES is 1.0217 g/mL. Ethylene glycol dimer as HBD was interacted with each other (CHCl/EG was 1 : 2) which forms hydrogen

bonds with the cyclic pattern with O-H bond, distance of 1.944 Å (Wagle et al., 2016). CHCl have 3 types of bonds, there is C-N, C-O and C-C with a distance within 0.01 Å of the bond lengths found in the crystal structure (Hjortas et al., 1971). The small distance of molecule bonding in CHCl caused difficulty in the formation of liquid phase at room temperature. Based on previous research by Wagle et al., (2016), DES from a mixture of CHCl with ethylene glycol (ethaline) form 3 CH-O interactions, i.e. the interaction between oxygen of ethylene glycol with methyl protons in CHCl. Distance of H-O is equal to 2.14 Å to 2.44 Å. There was also interaction between H⁺ on ethylene glycol with Cl⁻ in CHCl, H-Cl- bond formed is at a distance of 2.27 Å to 2.47 Å. Cl⁻ as anion in CHCl forms the centrepiece to interact with 5 hydroxyl groups, the hydroxyl groups of cations in choline and 4 of the 2 molecules of ethylene glycol. Hydrogen bonding interaction occurred because of dipole-dipole between electronegative atoms with hydrogen atoms which was bonded to other electronegative atoms, such as N, F, Cl and O. In this work, DES forms hydrogen bonding because of the interaction between H⁺ in HBD with Cl⁻, where Cl⁻ is the element in group VII A, the electronegativity was equal to 3.0. There was also a hydrogen bonding between the methyl protons (CH) in choline with oxygen from the HBD.

3.2 Effect of Temperature on Separation of γ -Oryzanol and Biodiesel

γ -oryzanol is one of bioactive compound in RBO-based biodiesel, removal of γ -oryzanol in this work was done using liquid-liquid extraction via DES. Based on Shahbaz et al. (2011), DES decreases MG and DG in crude biodiesel because it forms hydrogen bonding with DES, MG and DG that had hydroxyl group. In addition to MG and DG, biodiesel from RBO contains other impurities such as FFA, water, residual methanol and bioactive compound such as oryzanol. Such compounds have hydroxyl groups that forms hydrogen bonding with DES. Methyl ester compound as the main constituent of biodiesel was immiscible with DES. γ -oryzanol in rice bran oil based-biodiesel phase was transferred into DES phase because of the hydrogen bonding between DES and γ -oryzanol. The image of the materials are shown in Figure 2.

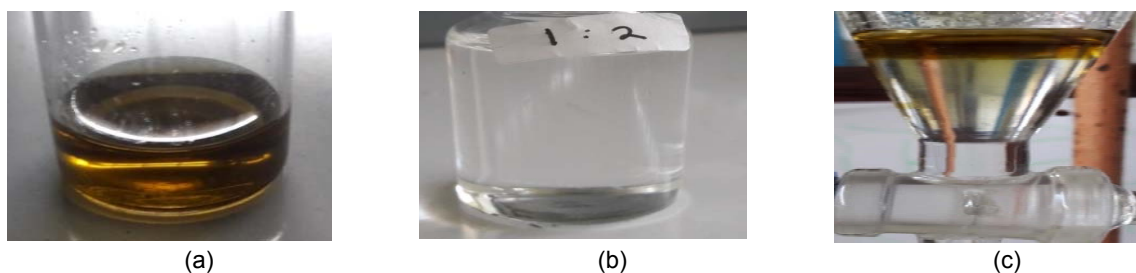


Figure 2: (a) Crude biodiesel; (b) DES; (c) mixture of crude biodiesel with DES at settling time

Temperature is one of the parameter that influences liquid-liquid extraction, in this work the highest of γ -oryzanol removal obtained at 30 °C. The content of γ -oryzanol is 1.47 % with FAME content of 96.55 %. This work shows that the best extraction temperature of γ -oryzanol is at low temperature. The previous research by Jiayou (2003) reported that higher adsorption temperature of γ -oryzanol (45 °C, 65 °C, 75 °C and 85 °C) decreases γ -oryzanol adsorption in silica gel. At high temperature, DES was removes FFA, MG and DG better than at low temperature. As DES molecule react with FFA, MG and DG to form hydrogen bonding. The effect of temperature on removal of γ -oryzanol from biodiesel is shown in Figure 3.

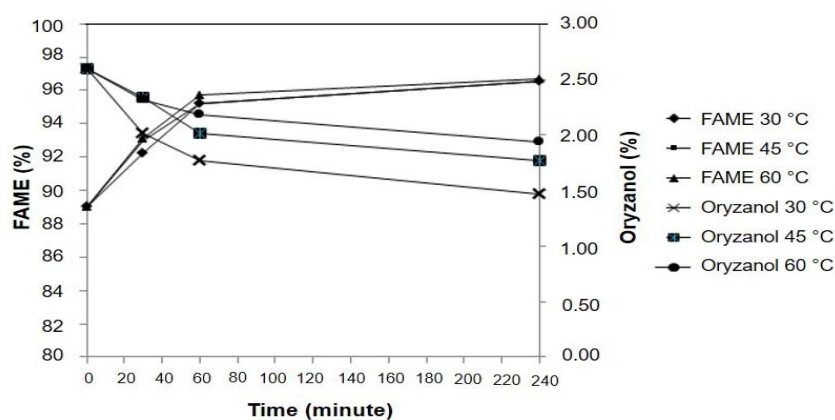


Figure 3: Effect of temperature on removal of γ -oryzanol from biodiesel (Crude biodiesel : DES = 1 : 2; CHCl : EG = 1 : 2)

3.3 Effect of Extraction Time and Molar Ratio Biodiesel/DES on Separation of γ -Oryzanol and Biodiesel

Extraction time is one of parameter that influences liquid-liquid extraction, the longer the extraction time, the more interaction between DES and γ -oryzanol. FFA content in biodiesel product is also undesirable because it will cause negative impact such as less oxidation stability and corrosion of vital engine components. Acid value of biodiesel product was obtained at 0.098 mg KOH/g. Acid value was lower than maximal acid value in SNI 7182 : 2012 (0.6 mg KOH/g) and EN 14214 : 2012 (0.5 mg KOH/g). The lowest FFA value at 240 min (molar ratio crude biodiesel to DES is 1/4) with the % FFA amounted to $0.02 \pm 0\%$, the acid value was 0.04 mg KOH/g. This value was lower than maximal acid value in biodiesel standard. This case shows that the extraction time can affect the extraction process of FFA in biodiesel. This work also shows that longer extraction time will produce purer biodiesel. It can be seen from the increasing of % FAME after purification in Figure 4.

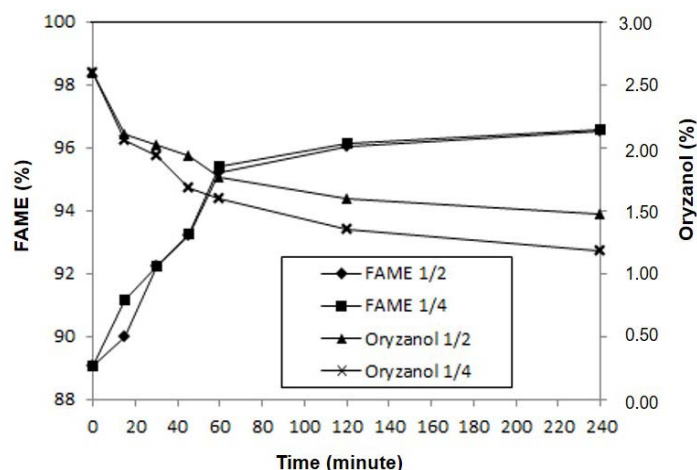


Figure 4: Effect of extraction time and molar ratio on removal of γ -oryzanol ($T = 30\text{ }^{\circ}\text{C}$; $\text{CHCL} : \text{EG} = 1 : 2$)

Figure 4 shows the content of γ -oryzanol in the upper layer; it decreased with time. The lowest γ -oryzanol content is at 240 min and molar ratio of crude biodiesel to DES 1/4, obtained 1.18 %. The reason of this phenomenon is the fact that the longer the extraction time, the more γ -oryzanol is moved from biodiesel to DES. The effect of molar ratio also influences the removal of γ -oryzanol. The higher molar ratio crude biodiesel to DES causes more DES molecule to be bonded with γ -oryzanol through hydrogen bonding. FAME content shows the opposite result, it increased with time. The highest FAME content at 240 min and molar ratio crude biodiesel to DES 1/4, obtained 96.60 %. Content of FAME increasing in biodiesel because of impurities such as γ -oryzanol were moved into bottom layer.

Beside γ -oryzanol, the content of FFA, MG and DG was also removed from crude biodiesel, increasing % FAME in rice bran oil based-biodiesel. The removal of TG was not significant because TG does not contain hydroxyl group, it was difficult to bond with DES. The highest % FAME (96.60 %) fulfils the Indonesia standard specification for biodiesel SNI 7182: 2012 and International standard EN 14214:2012 (96.5 % FAME). The decreasing of FFA, MG and DG based on GC analysis can be seen at Table 1:

Table 1: Composition of Upper Layer

| Time (min) | FFA (wt%) | MG (wt%) | DG (wt%) | TG (wt%) | FAME (%) | Others (wt%) |
|------------|-----------------|----------|----------|----------|----------|--------------|
| 0 | 0.05 \pm 0.00 | 0.30 | 4.01 | 4.03 | 89.05 | 2.55 |
| 15 | 0.05 \pm 0.00 | 0.30 | 2.86 | 4.03 | 91.13 | 1.62 |
| 30 | 0.04 \pm 0.00 | 0.26 | 2.37 | 3.61 | 92.25 | 1.47 |
| 45 | 0.04 \pm 0.00 | 0.24 | 1.78 | 3.61 | 93.28 | 1.06 |
| 60 | 0.04 \pm 0.00 | 0.09 | 0.69 | 3.36 | 95.41 | 0.41 |
| 120 | 0.03 \pm 0.00 | 0.03 | 0.38 | 3.06 | 96.11 | 0.39 |
| 240 | 0.02 \pm 0.00 | 0.02 | 0.25 | 2.92 | 96.60 | 0.19 |

(Extraction at $T = 30\text{ }^{\circ}\text{C}$; CBD : DES = 1 : 4; CHCL : EG = 1 : 2)

4. Conclusion

γ -oryzanol content in rice bran oil based-biodiesel decreased with time and the lowest γ -oryzanol content of 1.18 % and % FAME 96.60 % was obtained after 240 min, 30 °C at molar ratio 1/4 (crude biodiesel to DES). FAME content is over the Indonesia standard specification for biodiesel SNI 7182 : 2012 and the International standard EN 14214 : 2012 (96.5 % FAME). FFA, MG and DG reduced throughout the extraction time using DES. This work shows that DES has potential to remove bioactive compound (γ -oryzanol) from rice bran oil-based biodiesel.

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