Wet Extraction of $\gamma$-Oryzanol From Rice Bran

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Abstract. $\gamma$-Oryzanol is unique to rice bran oil (RBO) and it is known to be a potent hypocholesterolemic agent. Separation and isolation of $\gamma$-oryzanol from crude RBO is difficult due to complexity of components in crude RBO. Converting acylglycerides and FFA in RBO into FAME will concentrate bioactive compounds in the residue after FAME was removed from the RBO-based biodiesel. In this study, wet-extraction of bioactive compound from rice bran, $\gamma$-oryzanol, has been carried out using subcritical water-methanol mixture. The effects of extraction temperature, extraction time, water-methanol volume ratio and pressurizing gas type on the content and recovery of $\gamma$-oryzanol in crude RBO-based biodiesel have been investigated. It was found that the content and recovery of $\gamma$-oryzanol in RBO-based biodiesel increased with higher extraction temperature, longer extraction time and lower water concentration. The content of $\gamma$-oryzanol in RBO-based biodiesel was higher under N$_2$ atmosphere since CO$_2$ can acidify the solvent leading to hydrolysis of $\gamma$-oryzanol. The maximum $\gamma$-oryzanol content in RBO-based biodiesel was 2.16% (corresponding to 2750 mg $\gamma$-oryzanol/kg rice bran) obtained after extraction for 7 h at 200 °C and 40 bar with water concentration of 50% (v/v) under N$_2$ atmosphere.

1. Introduction

Indonesia is the third largest rice producer after China and India. Rice bran is produced during the milling of husked rice as a by-product and rice bran contains about 15 - 23 % oil [1,2]. Rice bran oil (RBO) is one of the most nutritious oil because of its favorable fatty acid composition and a unique combination of naturally occurring biologically active and antioxidant compounds. Crude RBO is extremely rich in micronutrients accounting for 4 - 6 % of the oil [1,3]. Among the micronutrients, $\gamma$-oryzanol, a mixture of ferulic acid esters and triterpenoid alcohols, accounts for 1 - 3 % of the total oil, and is unique to RBO [3 – 8]. The amount of $\gamma$-oryzanol in rice bran is ten times higher than that of vitamin E, therefore, $\gamma$-oryzanol is the main antioxidant in rice bran [9]. $\gamma$-oryzanol in RBO has been shown as an antioxidant against oxidation of linoleic acid in a certain molar ratios. $\gamma$-oryzanol can be degraded at a lower rate than that of $\alpha$-tocopherol at elevated temperatures; its make $\gamma$-oryzanol is a promising antioxidant to be applied at high temperatures [3]. $\gamma$-oryzanol in RBO was responsible as a hypocholesterolemic agent. RBO containing $\gamma$-oryzanol was added to the rat diets was shown to lower plasma cholesterol levels compared to rats given a diet containing RBO devoid of $\gamma$-oryzanol [10 – 11]. Other beneficial effect of $\gamma$-oryzanol is their effectiveness in reducing menopause symptoms [12]. Das 1998 and Narayan 2005 have problems encountered during the extraction of $\gamma$-oryzanol from RBO soapstock, and the problem was mainly arises from variations in the compositions of RBO soapstock. Xu and Godber 2000 was investigated the extraction of $\gamma$-oryzanol from rice bran using a supercritical fluid at low temperature (T = 50 °C) and high pressure (P = 680 atm) for 25 min. They found that the yield of $\gamma$-oryzanol was four times higher than that obtained using solvent extraction. Zullaikah 2009 reported the isolation of $\gamma$-oryzanol from RBO by a two-step crystallization process. In the first
crystallization step, triglycerides (TG) and steryl esters were mainly removed, while γ-oryzanol remained in the liquid phase along with free fatty acids (FFA), diglycerides (DG), monoglycerides (MG), squalene, tocols, and phytosterols. In the second crystallization step, γ-oryzanol was precipitated by adding n-hexane as anti-solvent to the liquid phase (γ-oryzanol-rich product) and keeping the mixture at 5 ± 1 °C for 48 h. This two-step crystallization process was able to produce 93 - 95 % pure γ-oryzanol, but the total recovery rate was only 59 %. Due to the difficulty on separation of γ-oryzanol from crude RBO or RBO soapstock, converting neutral oils into fatty acid methyl esters (FAME) will be concentrated γ-oryzanol in the bottom product after purification of RBO-based biodiesel through distillation. Purification of RBO-based biodiesel (initial FAME content of 89.05 %) to fulfill the standard biodiesel specification with FAME content more than 96.5 % was successfully carried out using green solvent (deep eutectic solvents, DES) [13]. In their work, FAME was immiscible in DES, while γ-oryzanol in RBO-based biodiesel was extracted into DES. Separation of extracted compounds which is rich of γ-oryzanol from DES will make the further process in the separation and purification of γ-oryzanol will be easier. Therefore, in this study, wet extraction of bioactive compound from rice bran, γ-oryzanol, has been carried out using subcritical water-methanol mixture. The effects of extraction temperature, extraction time, water-methanol volume ratio and pressurizing gas type on the content and recovery of γ-oryzanol in crude RBO-based biodiesel have been investigated.

2. Methods

2.1. Materials

Rice bran IR-64 type was obtained from Lamongan, East Java, Indonesia. Rice bran was filtered to remove any impurities and then kept in a refrigerator to minimize further formation of FFA. Standard of γ-oryzanol was obtained from Wako, Japan. Analytical grade solvents and pressurizing gases (CO2 and N2) were purchased from commercial source in Indonesia.

2.2. Soxhlet Extraction of RBO

Rice bran (20 g) was extracted using n-hexane as solvent in a soxhlet extractor for 12 h. In this work, it was assumed that soxhlet extraction can recover all RBO in rice bran. Yield of RBO (17.71 %) with high FFA content (37.71 %) was achieved using soxhlet extraction method, however the γ-oryzanol content in the RBO was quite low (1.33 %). The yield is expressed as percentage of RBO weight/rice bran weight.

2.3. Formatting author affiliations

The experiment setup for wet extraction of γ-oryzanol from rice bran extraction using subcritical water-methanol mixture was shown in Fig. 1. Rice bran (5 g) was mixed with 40 ml solvent (water-methanol mixture at different volume ratio) in a high pressure reactor (volume = 86 ml). The reactor was made of stainless steel and all fittings were purchased from Swagelok, Taiwan. The reactor was equipped with an external electrical heater and a thermocouple was inserted into the reactor to monitor the temperature. The extraction time was counted after a predetermined temperature was reached. The extraction process was investigated at different temperatures (T = 160 – 215 °C), extraction times (t = 1 – 8 h) and water-methanol volume ratios (5/35 – 35/5, ml/ml). The pressure was kept constant at 40 bar by using either CO2 or N2 as compressing gas. After a pre determined extraction time, the reactor was rapidly quenched and, as a consequence, the extraction and reaction inside the reactor was stopped. Product in the reactor were collected and mixed with n-hexane (50 ml). The mixture was stirred at 300 rpm for thirty minutes. The upper phase containing oil (RBO-based biodiesel) was collected and the bottom phase was mixed again with n-hexane (50 ml). This procedure was repeated three times. n- Hexane was then removed by using a rotary evaporator. The mass of the RBO-based biodiesel was determined and the content of γ-oryzanol was analysed.
2.4. Soxhlet Extraction of RBO

\gamma\text{-oryzanol} content in the sample was determined by UV-vis Spectrophotometer V-550. The calibration curve was obtained by using standard of \gamma\text{-oryzanol} with a concentration range from 0 to 97 ppm. A straight line passing through the origin was obtained at a wavelength of 316 nm \((R^2 = 0.99)\). To determine \gamma\text{-oryzanol} content, the sample was diluted in n-hexane in a 1 cm cuvet and the spectrophotometer was operated at 1 nm bandwith and 1 nm data pitch.

3. Results and Discussion

The process of wet extraction of \gamma\text{-oryzanol} from rice bran was carried out under subcritical condition of water-methanol mixture. Subcritical water was widely applied in the extraction, hydrolysis and wet oxidation to allow \gamma\text{-oryzanol} extracted in the oil phase \([14]\). The addition of methanol may increase the acquisition of \gamma\text{-oryzanol} \([15]\). Since \gamma\text{-oryzanol} is more easily dissolved into polar solvents such as isopropanol, ethyl acetate, and methanol. Dissolved CO\textsubscript{2} in subcritical water will form of carbonic acid, in other words CO\textsubscript{2} can be used as an acid catalyst \([16]\). Under subcritical condition, water may act as an efficient acid-base catalyst because it has a high dissociation constant. Subcritical water can also be utilized as solvent extraction of non-polar compounds. This is because the dielectric constant of water drops to 27 when in a subcritical state. This dielectric constant value is almost the same as methanol in ambient conditions \([17]\).

![Figure 1. Experimental setup for extraction of \gamma\text{-oryzanol} using subcritical water-methanol mixture](image)

3.1. Effect of Extraction Time

\gamma\text{-oryzanol} began to degrade at temperatures over 120 °C for 10 h \([18]\). While according to Nystrom 2007, \gamma\text{-oryzanol} begin degraded at 180 °C for 6 hours. In this study \gamma\text{-oryzanol} was slightly degraded at 200 °C for 8 h. From figure 2, \gamma\text{-oryzanol} content increased for both types of pressurizing gases used (\text{N}_2 and CO\textsubscript{2}) along with increasing of extraction time. Longer extraction time was required to extract overall \gamma\text{-oryzanol}. The use of N\textsubscript{2} as a pressurizing gas obtained a higher yield of \gamma\text{-oryzanol} than that of CO\textsubscript{2}, because \gamma\text{-oryzanol} is easily degraded under acidic conditions \([19]\). \text{N}_2 was an inert gas; therefore \gamma\text{-oryzanol} in RBO-based biodiesel obtained using \text{N}_2 as a pressurizing gas was quite stable and tend to increase \([3]\). The highest \gamma\text{-oryzanol} in RBO-based biodiesel using CO\textsubscript{2} as a pressurizing gas for 7 h of extraction time was 2.03 % ± 0.19, and then decreased after 8 h of extraction time (1.54 % ± 0.29). Both CO\textsubscript{2} and \text{N}_2 gases had shown the best conditions at 200 °C for 7 h of extraction time to extract \gamma\text{-oryzanol} of about 2 %. The decrease in yield and recovery is due to a greater degradation rate compared to the
extraction rate, the degradation rate may actually have occurred at 1 - 5 h extraction time, but the extraction rate is greater than the degradation rate. Zullaikah 2013 in the research said that “Decreased of yield and recovery of $\gamma$-oryzanol after 8 h extraction time, it’s because $\gamma$-oryzanol start degraded to phytosterol”.

Figure 2. Effect of extraction time on yield and recovery of $\gamma$-oryzanol in RBO-based biodiesel. Operation conditions: T= 200 °C, water-methanol volume ratio = 20/20 (mL/mL) and P = 40 bar

3.2. Effect of Extraction Time
Rising temperature will increase the rate of diffusivity and decreased the surface tension and viscosity. The higher water temperature accordingly lowers of water dielectric constant. As the dielectric constant increases, the solubility of non-polar compounds in water will be greater [20]. This makes the water as a solvent of non-polar compounds, and $\gamma$-oryzanol compounds are quite resistant to high temperatures when compared to other antioxidants in RBO such as tocopherols and tocotrienols [21]. Figure 3 show that under the influence of CO$_2$ atmosphere, $\gamma$-oryzanol content increased from 1.44 % ± 0.01 to 2.02 % ± 0.18 when the temperature was increased from 160 °C to 200 °C. If extraction temperature was raised to 215 °C the content of $\gamma$-oryzanol decreased dramatically to 1.41 % ± 0.05. Using N$_2$ as a pressurizing gas, $\gamma$-oryzanol content increased from 1.27 % ± 0.05 to 2.15 % ± 0.11 when the temperature was increased from 160 °C to 200 °C. If extraction temperature was raised to 215 °C the content of $\gamma$-oryzanol decreased slightly to 2.12 % ± 0.11. Both CO$_2$ and N$_2$ gases obtain the best conditions at 200 °C for 7 hours to reach $\gamma$-oryzanol content of about 2%. Increased extraction temperature has a positive effect on the content of extracted $\gamma$-oryzanol. At high temperatures, the physical properties of the bran are more easily penetrated by solvents thereby causing $\gamma$-oryzanol to be easily released from the matrix [22].
Figure 3. Effect of extraction temperature on yield and recovery of γ-oryzanol in RBO-based biodiesel. Operation conditions: Water-methanol volume ratio = 20/20 (mL/mL), t = 7 h and P = 40 bar

3.3. Effect of Water-Methanol Volume Ratio

Decreased water-methanol volume ratio has a positive effect on the extraction of γ-oryzanol. γ-oryzanol components were more dissolved in polar solvents such as isopropanol, ethyl acetate, and methanol [22]. Chen and Chiu, 2011 also used methanol as solvent to obtain 10 times more γ-oryzanol yields than that of using ethyl acetate. However, the addition of excess methanol does not increase the percentage of γ-oryzanol content. The use of methanol over the optimum amount will cause a reduction in the product, because methanol tends to extract more polar components such as carbohydrates. From the figure 4, it shown that N₂ as a pressurizing gas gives a positive effect on the content of extracted γ-oryzanol. The content of γ-oryzanol increased from 1.78 % ± 0.02 (methanol-water volume ratio = 5/35, mL/mL) to 2.15 % ± 0.11 (methanol-water volume ratio = 20/20, mL/mL). Then finally going down using methanol-water volume ratio of 35/5 (mL/mL) to 1.9 % ± 0.03. The use of CO₂ as a pressurizing gas also increased γ-oryzanol content from 1.6 % ± 0.08 to 2.03 % ± 0.19 when the methanol-water volume ratio was increased from 5/35 (mL/mL) to 20/20 (mL/mL) before finally decreasing to 1.65 % ± 0.07 at a methanol-water volume ratio of 35/5 (mL/mL). The presence of CO₂ as a pressurizing gas gives the acid state on the medium; therefore, γ-oryzanol was prone to degrade under CO₂ atmosphere [3, 19]. γ-oryzanol obtained in this study was 2.15 % or 2.75 mg of γ-oryzanol / g bran under N₂ atmosphere and 2.03 % or 2.5 mg γ-oryzanol / g bran under CO₂ atmosphere, and those results obtained were better in comparison with Xu and Godber's resulted in 2000 with Supercritical Fluid Extraction (SFE) at 680 atm, extraction time for 15-20 min at 50 °C resulting in γ-oryzanol of 1.11 ± 0.07 mg / g bran.
Figure 4. Effect of methanol-water volume ratio on yield and recovery of γ-oryzanol in RBO-based biodiesel. Operation conditions: T = 200 °C, t = 7 h and P = 40 bar

4. Conclusion
Wet extraction of γ-oryzanol from rice bran was found that the content and recovery of γ-oryzanol in RBO-based biodiesel increased with higher extraction temperature, longer extraction time and lower water concentration. The content of γ-oryzanol in RBO-based biodiesel was higher under N₂ atmosphere since CO₂ can acidify the solvent leading to hydrolysis of γ-oryzanol. The maximum γ-oryzanol content in RBO-based biodiesel was 2.16 % (corresponding to 2750 mg γ-oryzanol / kg rice bran) obtained after extraction for 7 h at 200 °C and 40 bar with water concentration of 50 % (v/v) under N₂ atmosphere. And the use of subcritical technology is far more effective and efficient for extracting γ-oryzanol (7 h) comparing with soxhlet extraction (12 h). The content of γ-oryzanol in RBO-based biodiesel was higher under N₂ atmosphere since CO₂ can acidify the solvent leading to hydrolysis of γ-oryzanol.

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