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Original research article

Analysis of Chemical Properties and Gamma - Oryzanol Content in Luem Pua Rice Bran Oil (*Oryza sativa* L.)

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ABSTRACT

The study investigated the soxhlet extraction of Leum Pua rice bran oil (*Oryza sativa* L.) using 12 hours of extraction time, with comparison of product with hexane and ethanol as solvent. The chemical properties and gamma-oryzanol content in the rice bran oil were investigated. The results showed that the highest yield of rice bran oil was obtained from extraction using 70:30 %v/v ethanol:water solvent with percent yield of 21.89±0.35 g (mean±SD)/100g of dry rice bran) and maximum amount of gamma-oryzanol of 0.4647 mg/L of rice bran oil. The chemical properties and gamma-oryzanol content of Leum Pua rice bran oil indicates acceptable quality of oil when stored for a long time causing foul odour due to lipid peroxidation.

Keywords: Rice bran oil; Leum pua rice; Gamma-oryzanol; Peroxide value; Acid value

1. Introduction

Rice bran oil is extracted from the hard outer brown layer of rice husk in the rice bran milling process. Rice bran oil is an edible oil that is popular in Asian countries such as Japan, India, Korea, China, and Indonesia as a cooking oil [1]. It has been found that rice bran oil is suitable for cooking due to its high smoke point and mild flavor. Rice bran oil has consistency of several phytochemical compounds such as tocotrienols, tocopherols, phytosterols, polyphenols, squalene, and gamma-oryzanol that are reported to have beneficial effects to human health [2]. In recent years, research has been done to develop various methods for extracting rice bran oil from rice bran. Gamma-oryzanol is a potent antioxidant component present only in rice bran oil that has been reported to have positive biological

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effects, such as decreasing the level of lowdensity lipoprotein (LDL), lowering cholesterol level, reducing blood pressure, and preventing colorectal cancer, and can beimproved with pharmaceutical dosage forms, cosmetic formulations, or food products [3]. The gamma-oryzanol value demonstrated is dependent on species of rice, cultivation area and the method of extraction [4]. Massarolo et al. evaluated the effect of the solid state cultivation time of rice bran by *Rhizopus oryzae* on gamma-oryzanol recovery and its antioxidant properties. They found that the solid state cultivation leads to an increase in the gamma-oryzanol recovery followed by improvement in the functional properties of rice bran [5]. Leum Pua is native Thai sticky rice that contains antioxidants higher than white rice and other colored rice. Pornputtapitak et al. found that the lipid components in Leum Pua rice bran oil consisted of oleic acid, linoleic acid, palmitic acid and gamma-oryzanol [6]. Moreover, Srisuwan et al. suggested that Luem Pua rice might be developed as a nutraceutical for improving mood and brain function, especially learning and memory [7].

Thus, the aim of this research was to compare the three solvent extraction of Luem Pua rice bran from Phetchabun province. The chemical properties and gamma-oryzanol of extracted Luem Pua rice bran oil were investigated.

2. Materials and Methods

2.1 Material

Leum Pua rice bran (*Oryza sativa* L.), provided by a local rice mill from Amphoe Khao Kho, Phetchabun Province, Thailand, was used as a raw material for the oil extraction and gamma oryzanol analysis. Gamma-Olyzanol standard was purchased from Sigma-Aldrich (Japan). Hexane and ethanol were purchased from Labscan (Thailand).

2.2 Oil extraction of Leum Pua rice bran

The 25 g on a dry weight basis of Leum Pua rice bran was extracted with 200 ml of different solvents, hexane, ethanol and 70:30 %v/v ethanol:water by using Soxhlet apparatus for 12 hr; after that the solvent was evaporated under vacuum. Then, the yield of extracted oil was calculated by using Eq 1. The dried material was sampled in triplicate.

$$\% \text{ yield} = \frac{\text{weight of extracted oil (g)}}{\text{weight of dried seed (g)}} \times 100 \quad ...(1)$$

2.3 Characterization of Leum Pua rice bran oil

Acid value (AV), free fatty acid (FFA) content, and peroxide value (PV) were determined following AOCS official methods (AOCS, 2004). FFA was calculated as oleic acid and expressed as percentage of the total lipids. Gamma-oryzanol content in rice bran oil was determined by using a spectrophotometer (Specord, USA) that was slightly modified following the method of Thanonkaew et al. [8]. Consecutively, rice bran oil samples were diluted in iso-propanol. The content of gamma-oryzanol was determined by measurement of UV absorption at 320 nm and was quantified against the standard curve. The calibration curve was obtained with gamma-oryzanol standard in a concentration range of 1-5 mg/L which was adjusted to 10 ml in iso-propanol.

The data were presented as the mean values and standard deviation (SD).

3. Results and Discussion

3.1 Oil content

Previous research reported the commercial rice bran contains 15–20% of oil and also an endogenous lipase which degrades the oil and produces free fatty acids [9]. In this study, we used the hexane, ethanol and 70:30 %v/v ethanol:water as the extraction solvents. The results are shown in Table 1.

Table 1. Oil content of Leum Pua rice bran extracted oil.

Solvents	Extracted oil (g)	%Yield (g/100 g of dry basis)
Hexane	3.81±0.02	15.25±0.08
Ethanol	5.23±0.28	20.94±1.12
70:30 %v/v ethanol:water	5.47±0.09	21.89±0.35

Table 1 shows that the extracted oil content ranged from 15.25±0.08 to 21.89±0.35% by weight. Extraction using 70:30 %v/v ethanol:water yielded the highest oil content. Azrina et al. studied the rice bran oil extraction using 3:2 chloroform:methanol mixture obtaining result of 16.4% of yield [10]. The heating may disrupt the cellular wall, which results in greater porosity when the cell membrane ruptures, and improves the efficiency of the pressing extraction of oil from materials. In another research, the extraction yield of cold-pressed rice bran oil was 3.29-5.53% of yield [8].

3.2 Chemical properties of Leum Pua rice bran oil

Acid value (AV), free fatty acid (FFA) and peroxide value (PV) were the parameters used for determination of chemical quality of the extracted rice bran oil. Generally, the content of AV, FFA and PV was positively related to the activity of enzyme lipase. Acid value can be used for purity check of oil whereby decomposition reactions may have already progressed. The free fatty acids were lower than 5% in rice bran oil that was used for edible production. The high free fatty acid content of 10-20% was used in industrial grade of productions which could be present at considerate amounts in crude oils [11].

Table 2 shows the acid value, free fatty acid and peroxide value in this study. The results show that the lowest value of the chemical properties was determined by using the 70:30 %v/v ethanol:water. Using hexane and ethanol as solvent extraction resulted in a slightly different value of the chemical properties. Furthermore, the 70:30 %v/v ethanol:water should be used as a solvent because it is less toxic to the environment than other solvents.

Raja Rajan and Gopala Krishina reported the refined rice bran oil had the free fatty acid in range 0.4-4.8% and that result was supported in this study [9]. The peroxide value indicated the quantity of peroxides in the oil which were important intermediates of oxidative reactions. The temperature of thermal extraction causes decomposition and free radicals formation. In table 2, the highest peroxide values are shown when the solvent extraction occurs at high temperature. Thanonkaew et al. reported the 11.72-12.13 meq Eqv O_2/kg oil of the peroxide value when using cold-pressed rice bran oil [8].

Table 2. The Chemical properties of Leum Pua rice bran extracted oil.

Solvents	Chemical properties of Leum Pua rice bran extracted oil		
	Acid value (mg KOH/ g oil)	Free fatty acid (%)	Peroxide value (meq Eqv O_2/ kg oil)
Hexane	4.97±0.68	2.49±0.34	21.50±0.50
Ethanol	4.85±0.31	2.42±0.16	19.83±0.58
70:30 %v/v ethanol:water	4.55±0.37	2.27±0.19	18.50±0.50

3.3 Gamma - oryzanol content of Leum Pua rice bran oil

Gamma-oryzanol can be used in nutraceutical, pharmaceutical and cosmoceutical productions [12]. In this research, the 5 ppm of gamma-oryzanol standard solution was used in scanning at the maximum wavelength (λmax) which showed at 320 nm. The result found that the 0.4647 mg/L of the gamma-oryzanol content of extracted oil using 70:30 %v/v ethanol:water was the highest value shown in Table 3.

Table 3. Gamma - oryzanol content of Leum Pua rice bran oil.

Solvents	Gamma -oryzanol content (mg/L)
Hexane	0.0451±0.01
Ethanol	0.3872±0.04
Ethanol:Water (70:30)	0.4647±0.03

Thanonkaew et al. reported the 2.03 ±0.05 g/100g oil of the gamma-oryzanol when using cold-pressed rice bran oil [8]. Srikaeo found the concentration of gamma-oryzanol in RBO ranges from 115 to 780 ppm [13]. However, gamma-oryzanol content in rice bran oil is dependent on the temperature and method of processing [14].

4. Conclusion

The oil of Leum Pua rice bran was extracted by soxhlet extraction using hexane, ethanol and 70:30 %v/v ethanol:water as the solvents for 12 hr. The results found that the highest oil content 21.89±0.35 g/100g of dry basis was extracted by ethanol to water ratio 70:30. Acid value, free fatty acid and peroxide value of extracted oil were the lowest in oil extracted using ethanol to water ratio 70:30 as a solvent at 4.55±0.37 mgKOH/goil, 2.27±0.19% and 18.50±0.50 meq Eqv O_2/kg oil, respectively. The highest of the gamma-oryzanol content was 0.4647 mg/L of the extracted oil using ethanol to water ratio 70:30. Furthermore, the chemical properties could be indicative of the oil quality, which is the major cause of odour due to lipid peroxidation.

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