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Original research article

Optimization of Solid-Liquid Extraction of γ-oryzanol from Rice Bran Oil Soapstock using Soxhlet Extraction Method

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ABSTRACT

Rice bran oil soapstock (RBOS) is a by-product from the chemical refining process of rice bran oil production. It contains a large amount of γ-oryzanol. The main objective of this study was to investigate the amount of γ-oryzanol obtained by solvent extraction using soxhlet apparatus. RBOS was saponified and was then dehydrated and extracted with ethyl acetate. The optimum conditions were determined using response surface methodology (RSM) with a Box-Behnken experimental design (BBD). BBD was used to investigate the effects of three independent variables, namely solid to solvent ratio (w/v), extraction temperature (°C), and extraction time (h). The results showed that the most suitable conditions for the extraction of RBOS that can provide the highest yield of γ-oryzanol (9.04% dry basis) were 1:12 solid to solvent ratio, 70.1°C extraction temperature and 7.26 h extraction time.

Keywords:γ-oryzanol; Rice Bran Oil Soapstock; Soxhlet Extraction; Box-Benhken experimental design

1. Introduction

In the world's cereal grain production, rice (*Oryza sativa* L.) is the second largest cereal grain after wheat. The United States Department of Agriculture has estimated that the world rice has produced approximately 741.3 million metric tons in 2015/2016. Most of the rice production is cultivated in the countries in Asian countries. Thailand produces 30 million metric tons of paddy or 19.8 million tons of milled rice, divided between domestic consumption and exports [1]. Rice bran is a by-product of the rice mill process. Accordingly, most of rice bran is used as animal feed and raw material for rice bran oil (RBO) industry. The crude rice bran oil (CRBO) can be subjected to either chemical or physical refining. The chemical refining of CRBO produced rice bran oil soapstock

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(RBOS). RBOS generally consists of water 65-70% , soap 20-22% and 7-7. 5% unsaponified fraction [2, 3]. The unsaponified fraction contains 20% oryzanol. At present, most soapstock is used as animal feed, soap and detergent industries. It could be used as nutritional or functional ingredient in food industry. The utilizations of RBOS as noted can help adding value to agricultural products and also reducing pollution from rice bran oil industrial waste. The γ-oryzanol also is claimed to have a protective role in lipid peroxidation and thus finds applications in sunscreen agents, as an antioxidant and preservative in cosmetics and food preparations, in the treatment of atopic dermatitis, in senile xeroderma, and in the prevention of skin dryness [3] . Extraction of γ-oryzanol from RBO and RBOS such as solid-liquid extraction (leaching) with organic solvent, ultrasound-assisted aqueous extraction and supercritical fluid extraction with carbondioxide, have been studied [4, 5, 6, 7, 8, 9]. Furthermore, there are several works about extraction and purification of the RBOS as, Xu and Godber [10] reported a process for obtaining γ-oryzanol of 43% (w/ w) purity and 80% (w/w) recovery. Relate to Kaewboonnum et al., [7] have been studying the use of solvent extraction to extract γ-oryzanol obtain 55.71% (w/w) purity and 74.6% recovery. In addition, Jesus et al., [6] propose an improved technique for supercritical fluid extraction to obtain high γ-oryzanol content (3.2% w/w). However, soxhlet extraction is very simple methodology and it has been generalized for extraction in agricultural chemistry before becoming the most used tool for solid-liquid extraction in many fields like environment, foodstuffs and pharmaceutics. Although, there are many studies extraction process of γ-oryzanol but there were no reports that any suitable condition. The Box-Behnken is a good design for response surface methodology because it permits: (i) estimation of the parameters of the quadratic model; (ii) building of sequential designs; (iii) detection of lack of fit of the model; and (iv) use of blocks [8] . Response Surface Methodology (RSM) has been widely used for the optimization of extraction conditions such as temperature, extraction time and solid to solvent ratio. RSM consists of mathematical and statistical techniques used to develop an adequate functional relationship between a response of interest and some independent variable. The aim of this study was to optimize, using RSM with a Box-Benhken experimental design (BBD), the soxhlet extraction conditions for γ-oryzanol content from RBOS.

2. Materials and Methods

2.1 Materials

Rice bran oil soap stock (RBOS) was obtained from Kasisuri Co., Ltd, Thailand. γ-oryzanol standard (98% purity) was purchased from Spectrum, USA. Ethyl acetate (AR grade 99. 98%) , sodium hydroxide (AR grade 98%) , and sodium bicarbonate (AR grade 99. 9%) were purchased from APS Finechem, NSW, Australia. Methanol (AR grade 99. 98%) was purchased from Fisher Scientific, UK.

2.2 Preparation of RBOS

RBOS sample was stored in a refrigerator before use. RBOS was characterized by the following analyses: moisture content was determined by drying the sample to constant weight in a vacuum oven at 105 ± 2 °C. The sample was allowed to cool to room temperature in desiccator and weighed [11], fat content was measured by soxhlet extraction apparatus with petroleum ether. The extract was then weighted and calculated % fat in dried sample. The pH was used 5 g of soapstock and dissolved in 20 ml water. The mixture was then heated at 40 °C until the soapstock was completely dissolved. The pH of the mixture was measured using a pH meter [11], γ-oryzanol content describe in section 2.4 [7], and saponification value [12].

The saponification value is the number of mg of potassium hydroxide (KOH) to saponify the esters in 1 g of the sample and neutralize the free acids in 1 g of a sample. The saponification value was calculated from the following Eq. (2.1);

$$\text{Saponification value (mg KOH/1g sample)} = (a-b) \times 28.05 / \text{weight (g) of sample} \quad (2.1)$$

Where a = volume (ml) of 0.5 mol/l hydrochloric acid consumed in the blank test and b = volume (ml) of 0.5 mol/l hydrochloric acid consumed in the test.

RBOS was saponified according to Kaewboonnum et al., [7] with some modifications. Saponification process were added NaOH whose extract quantity require for the reaction can be calculated based on the saponification value determine using AOCS method [13, 14]. The reaction was carried out at 80 °C and constant stirring over the period of 2 h. After reaction was completed, the saponified RBOS were adjusted pH 9.5 and dehydrated by using hot air oven at 90 °C for 8 h.

2.3 Extraction Methods

The soxhlet extraction was performed according to Jesus et al., [6] with modifications. The extraction process were perform at different sample to solvent ratio (1:5, 1:10 and 1:15 w/v), extraction temperature (60, 70, and 80 °C), and extraction time (4, 6, and 8 h). The dehydrated saponified RBOS were grounded and weighed. Sample and solvent (ethyl acetate) were placed into a soxhlet apparatus. The yield and purity of γ-oryzanol were calculated from the following Eq. (2.2) and (2.3):

$$\%\text{yield of } \gamma\text{-oryzanol} = (\text{Amount of } \gamma\text{-oryzanol in extract / Amount of } \gamma\text{-oryzanol in raw material}) \times 100 \quad (2.2)$$

$$\%\text{ purity of } \gamma\text{-oryzanol} = (\text{Amount of } \gamma\text{-oryzanol in extract/ Weight of sample}) \times 100 \quad (2.3)$$

2.4 Determination purity of γ-oryzanol extracted by UV-spectrophotometric and HPLC

2.4.1 UV-spectrophotometric analysis

For quantification of γ-oryzanol content in the sample, UV-spectrophotometric analysis was used according to Zullaikh et al., [5] with some modifications. Absorbance of the sample solution in ethyl acetate was measured at the wavelength of 320 nm in 1-cm cell using UV-Visible spectrophotometer (T60, PG Instrument limited, Canada). The γ-oryzanol (98% purity, Spectrum, USA) was used as a standard.

2.4.2 High Performance Liquid Chromatography (HPLC)

HPLC analysis was carried out using the Agilent 1200 series equipped with an Agilent 1200 series diode-array detector (DAD) and autosampler. Data analysis was performed using Open LABCDS EZChrom software. Separation was achieved at 25 ºC on aPoroshell120 EC-C18, 3.0×45 mm, 2.7μm (Agilent Technologies, USA). The mobile phase consisted of isopropanol-methanol (30:70v/v) and was pumped at a flow rate of 1.0 mL/min. The injection volume was 20 μL. The quantitation wavelength was set at 325 nm.

2.5 Experimental design and statistical analysis

Box-behnken design (BBD) with three independent variables was used for optimization. The parameters sample to solvent ratio, extraction temperature and extraction time were chosen as key variable based on the results of preliminary experiments and designed as X_1, X_2, and X_3, respectively, as show in Table 1. The complete quadratic equation was used is as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_1 X^2_1 + \beta_2 X^2_2 + \beta_3 X^2_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon$$

Where, Y is the estimated response (% yield of γ-oryzanol), β_0 is the constant term, βi is the linear coefficient. (i = 1-3), βij is the quadratic coefficient. (i = 1-3 and j = 1-3), ε is the random error.

Box–Behnken design for three factors and three levels required 16 experiments corresponding to the mid-point of edge and the three replications at the

center points of the cube. All of 16 experiments with three independent factors and their three different levels and the responses were shown in Table 1.

Table 1. Uncoded and coded levels of independent variables in Box-Behnken experimental design and percent extracted γ-oryzanol

Treatment runs	Sample to solvent (w/v)		Extraction temperature (°C)		Extraction time(h)		Exp.γ-oryzanol (% dry basis)[a]
	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded	
1	-1	1:5	-1	60	0	6	7.31±0.05
2	1	1:15	-1	60	0	6	7.07±0.06
3	-1	1:5	1	80	0	6	7.59±0.11
4	1	1:15	1	80	0	6	6.38±0.22
5	-1	1:5	0	70	-1	4	8.39±0.10
6	1	1:15	0	70	-1	4	7.60±0.22
7	-1	1:5	0	70	1	8	8.59±0.08
8	1	1:15	0	70	1	8	8.58±0.17
9	0	1:10	-1	60	-1	4	7.99±0.06
10	0	1:10	1	80	-1	4	8.64±0.11
11	0	1:10	-1	60	1	8	7.18±0.04
12	0	1:10	1	80	1	8	7.72±0.07
13	0	1:10	0	70	0	6	8.57±0.12
14	0	1:10	0	70	0	6	8.20±0.06
15	0	1:10	0	70	0	6	7.05±0.15
16	0	1:10	0	70	0	6	8.08±0.17

[a] Means±standard deviation of triplicate analysis

After the multifactor analysis of variance and the 2nd order model prediction determinations, the optimal extraction conditions were obtained by the desirability function approach using Minitab statistical software v. 16 (Minitab Inc., USA). The response surface plots were developed using the Statistica program (Statistica version 7.0, Statsoft Inc., Tulsa, OK, USA) and represented a function of two independent variables while keeping the other two independent variables at optimal values.

3. Results and Discussion

3.1 Raw material characterization

The results of the rice bran oil soapstock (RBOS) in terms of pH, moisture content, saponification value, and γ-oryzanol content are shown in Table 2.

Table 2. Physical and chemical properties of RBOS

properties	Value[a]
pH	9.10±0.00
Moisture content	50.01±0.09%
Fat content	16.03±0.81%
Saponification Value	30. 24±3. 11(mg KOH/1g sample)
γ-oryzanol content	4.95±0.12%wet basis 9.92±0.24%dry basis

a = Mean±standard deviation of triplicate analysis.

3.2 Raw material pretreatment

The problems encountered during extraction of oryzanol are mainly due to variations in the compositions of RBOS, which include surface-active impurities such as soaps, phospholipids, waxes, and glycolipids [3]. To make the extraction process of γ-oryzanol easier, any remaining glycerides (fat content 16.03%) in the RBOS was converted into insoluble soap by saponification. The suitable quantity of NaOH could be estimated based on the saponification value, using a standard method [11], which was found to be 30.24 mg NaOH/g soapstock (or 3.02 wt%). Saponification can be reducing any remaining glycerides in the RBOS [7].

3.3 Optimization of extraction and response surface analysis

Regression analysis was employed to fit a full response surface model for every response investigated including all linear (X_1, X_2, X_3), interaction (X_1X_2, X_1X_3, X_2X_3), and quadratic terms (X_{12}, X_{22}, X_{32}). The coefficients for the quadratic equations

in term of coded units were shown in Table 3.

The regression coefficients for the 2nd order response surface model in terms of coded units are shown in Table 3. The predicted values calculated by these models are presented in Table 1. The examination of the fitted model was always necessary to ensure that it provided an adequate approximation to the true system [15, 16].

Table 3. Regression coefficients for the 2nd order response surface models in terms of γ-oryzanol (% dry basis)

Parameter	Term	γ-oryzanol (% dry basis)	
		Coefficient	p-value[a]
β_0	Intercept	8.594	0.000
β_1	X_1	0.478	0.001
β_2	X_2	-0.014	0.863
β_3	X_3	0.146	0.118
β_{11}	X_1^2	-0.618	0.002
β_{22}	X_2^2	-0.666	0.001
β_{33}	X_3^2	-0.290	0.043
β_{12}	X_1X_2	-0.323	0.029
β_{13}	X_1X_3	0.032	0.788
β_{23}	X_2X_3	-0.393	0.013

X_1(Solid to solvent ratio), X_2 (Extraction temperature, °C), X_3 (Extraction time, h)
The p-value more than 0.05 is not significantly a different at the 5% level.

To develop the fitted response surface model equations, all insignificant terms ($P > 0.05$) were eliminated and the fitted models are shown in Table 3. The coefficient of determination (R^2) for Y was 0.956. Thus, the models were suitable to represent the real relationships among the selected reaction parameters. The predicted values agreed well with the experimental ones that are obtained from the model.

The regression equation for the surface core material (Y) was followed:

$$Y = 8.954 + 0.478X_1 - 0.618X_{12} - 0.666X_{22} - 0.290X_{32} - 0.323X_1X_2 - 0.393X_2X_3$$
$$R^2 = 0.956$$

Optimum conditions for γ-oryzanol content (% dry basis) were provided from the calculation by the response optimizer in Minitab program. The goal of the total the γ-oryzanol content (%) was maximized. For target values, they were 8.7% dry basis of the γ-oryzanol content. Each parameter had the different level of important which were 0.35, 0.01, and 0.13 for solid to solvent ratio, extraction temperature, and extraction time, respectively. The results of the optimization and the predicted responses were shown in Table 4.

Table 4. Optimization solutions obtained using the response optimizer

a = Mean±standard deviation of triplicate analysis.

	Optimal solution			Predicted response	Experimented response
	X_1	X_2	X_3	γ-oryzanol content (% dry basis)	
Coded	0.35	0.01	0.13		
Uncoded	1:12	70.10 °C	7.26 h	9.04	8.74±0.05[a]

The predicted values of extraction yield were 9.04% and experimental values of extraction yield repeated three times determined by spectrophotometer and HPLC were 8.74±0.05 % and 8.65±0.04% (87% yield and 24% purity), respectively. The results indicated that soxhlet extraction was an effective method for leaching of γ-oryzanol from the dehydrated saponified soapstock as a portion of fresh solvent was repeatedly brought into contact with the sample, thereby mass transfer was enhanced. In addition, the high temperature of the system (at the boiling temperature of the solvent) could increase the extractability. This loss of γ-oryzanol could be due to the fact that, even though γ-oryzanol is often classified as an

unsaponifiable matter, the compound could undergo saponification and could be converted into ferulic acid and sterols [2]. Moreover, some γ-oryzanol loss could occur during the transfer of viscous material in the saponification process [7]. The problems encountered during extraction of γ-oryzanol are mainly due to variations in the compositions of RBOS, which include surface-active impurities such as soaps, phospholipids, waxes, and glycolipids [2].

3.4 Response surface plots

Figure 1 shows the estimated response function and the effects of the independent variables (X_1, X_2 and X_3) on the dependent variables (extraction yield). The effect of independent variables on percent γ-oryzanol yield (Y) are show in Figure 1 (A, B and C) when code values of the X_1 and X_2 independent variables were close to zero, extraction yield increased. Factor X_3 (extraction time) had higher impact on the yield which illustrated the increase of the extraction yield with an increase of extraction time.

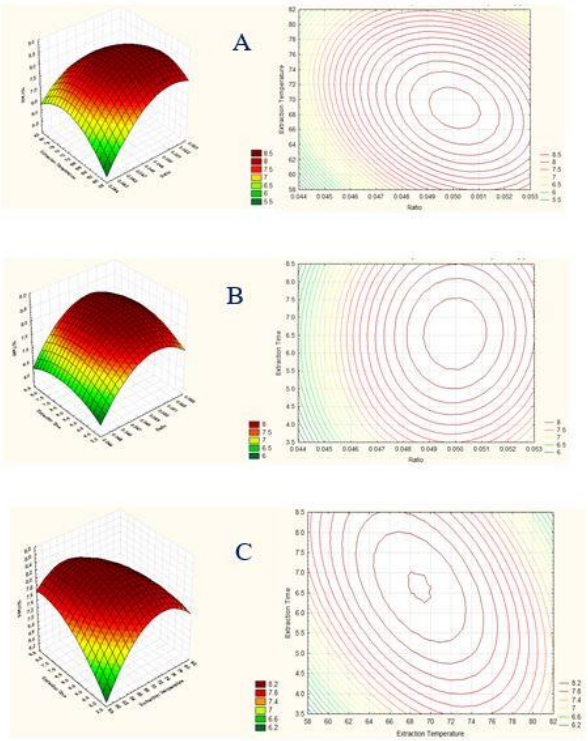

Fig. 1. Response surface plots of showing the interaction effects of extraction variables on the extracted yield; A = solid to solvent ratio-extraction temperature effect, B = solid to solvent ratio –extraction time effect, and C = extraction temperature -extraction time effect.

4. Conclusion

The characterization analyses confirmed the potential of the RBOS as an interesting γ-oryzanol source. The suitable procedures for isolation of γ-oryzanol from RBOS were examined. The process of separation starting saponification of the RBOS to convert the remaining glycerides into soap, adjust pH 9.5, and followed by extraction of γ-oryzanol from the dried matter with organic solvent (ethyl acetate). The best operational conditions for soxhlet extraction were found at 1: 12 solid to solvent ratio, 70. 1 °C extraction temperature, and 7.26 h extraction time gave the best result. The suitable condition provided the highest yield was 87% yield base on initial γ-oryzanol in RBOS and 24% purity. The γ-oryzanol obtained from the optimal conditions of soxhlet extraction need purifying before use as food ingredient in food industry.

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