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Original Research Article

Optimization of Extraction of Vitamin E and γ – Oryzanol from Thai Rice Germs Using Response Surface Methodology

Palita Wangsuntornpakdee [1] and Methavee Peanparkdee [1, 2]*

[1]Department of Food Science and Technology, Kasetsart University, Bangkok, 10900, Thailand
[2]Research Unit on Innovative Technologies for Production and Delivery of Functional Biomolecules, Kasetsart University Research and Development Institute (KURDI), 50 Ngam Wong Wan Road, Chatuchak, Bangkok 10900, Thailand

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ABSTRACT

The lipophilic antioxidants including vitamin E and γ–oryzanol contained in rice germs from three cultivars, namely Hommali Numnom (white rice), Tubtim Chumphae (red–brown rice) and Riceberry (purple rice) were evaluated. The objective of this research was to optimize the extraction conditions using Box–Behnken design (BBD) with response surface methodology (RSM) for achieving high efficiency extracts. The extraction process was conducted using ultrasonic–assisted extraction (UAE) with soybean oil as a green solvent. The effects of extraction temperature (30–50 °C), extraction time (30–50 min), and liquid–solid ratio (3–7, v/w) on vitamin E and γ–oryzanol contents were studied. The contents of vitamin E and γ–oryzanol from all rice germ cultivars using UV–vis spectrophotometer ranged from 2.510 to 63.605 µg/g extract and 0.230 to 8.482 µg/g extract, respectively. The antioxidant activity of extracts was determined by the ferric reducing antioxidant power (FRAP). FRAP varied from 17.76 to 305.35 µmol Trolox/g extract. Optimal extraction conditions for maximum vitamin E and γ–oryzanol contents were analyzed as 40 °C, 40 min, and liquid–solid ratio of 5 v/w. The results suggested that the RSM can be an appropriate approach for optimizing the extraction of vitamin E and γ–oryzanol from Thai rice germs. Furthermore, it is a green technology and has also great potential to be applied in functional food ingredients.

INTRODUCTION

Vitamin E and γ–oryzanol are lipophilic compounds, which have been known as antioxidant compounds (Ungurianu et al., 2021). They are generally found in wheat germ oil, soybean oil, peanuts, almonds, avocado, broccoli, and rice (Bonku & Yu, 2020; Chun et al., 2006; Ghafoor et al., 2017; Wu et al., 2022). Tocopherols and tocotrienols, the vitamin E derivatives, have been considered as the essential antioxidants for improving human health. γ–oryzanol is a mixture of ferulic acid esters of triterpene alcohols and phytosterols, which provide several beneficial effects

* *Corresponding author.* Tel.: +66-2562-5032
E-mail address: fagimvp@ku.th

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on human health, including lowering blood cholesterol, promoting anti–diabetic activity, cardiovascular disease, coronary artery disease, cancer prevention, immune enhancement (Devries, 2018; Rawal et al., 2018; Rychter et al., 2022; Wang et al., 2017).

Rice (*Oryza sativa*) is one of the most staple crops in Thailand. In the milling process, various by–product consisting of rice husk (20%), rice bran (8%), and rice germ (2%) are obtained, which are commonly used as animal feeds (Van Hoed et al., 2006). In particular, rice germ is containing high amounts of lipophilic compounds, including vitamin E and γ–oryzanol. The level of vitamin E in rice germ is 5 times higher than in rice bran (Mohd Esa & Ling, 2016). However, Yu et al. (2007) reported that the content of γ–oryzanol is 5 times lower than that in rice bran. Furthermore, extracts of vitamin E and γ–oryzanol from rice germ have been potential as a functional food ingredient and as a good source of healthy benefit.

The ultrasonic–assisted extraction (UAE) is a highly effective extraction method, since it can generate high power and break the cell wall of plant materials (Jalili et al., 2017). Ultrasonic technique is a substantiate green technology, due to simplicity, reduce operation time, less energy consumption, low cost, high quality, and high yield efficiency (Yusoff et al., 2022). In this study, soybean oil was used as an extraction solvent due to its environmentally friendly, safety, and low energy consumption (Chemat et al., 2012). Moreover, low viscosity of soybean oil (Diamante & Lan, 2014) can enhance extractability of compounds (Goula et al., 2017) compared to other vegetable oils.

The aim of this study was to optimize the extraction of vitamin E and γ–oryzanol from Thai rice germs using response surface methodology (RSM) with Box–Behnken design (BBD). The extraction factors, including temperature, time, and liquid–solid ratio, were examined in order to fine the proper conditions for obtaining high efficiency extracts.

MATERIALS AND METHODS

Materials

The three cultivars of rice germs (*Oryza sativa* L.) namely, Hommali Numnom (white rice; HM), Tubtim Chumphae (red–brown rice; TT), and Riceberry (purple rice; RB) were purchased in Thailand. Rice germs were grinded and passed through a 250 µm mesh sieve to gain fine particles. The obtained powders were stored in an aluminum bag (Al/PE) at –25 °C until further use.

Soybean oil (cook brand) was purchased from the local supermarket (Bangkok, Thailand) as a solvent for extraction. Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and 2,4,5-tri(2-pyridyl)-s-triazine (TPTZ) were obtained from Sigma–Aldrich®. All other chemicals and solvents were studied in analytical grade.

Extraction procedure

Ultrasonic–assisted extraction (UAE) was performed according to the method of Xu et al. (2016) with slight modifications. A 20 g of rice germ powder were blended with 100 ml of soybean oil in an ultrasonic bath (GT SONIC–D27, Guangdong, China) at constant ultrasonic frequency of 40 KHz and power effective of 80 W. The extraction conditions were varied as followed; temperature (30 – 50 °C), times (30 – 50 min), and liquid – solid ratio (3 – 7, v/w). The mixture was then centrifuged at 6000 rpm for 15 min at 25 °C (Biofuge Stratos, MA, US) to obtain rice germ extract (RGE). The supernatant was kept in brown bottle at –25 °C until further analysis.

Experimental design

Box–Behnken design (BBD) with response surface methodology (RSM) was used for optimization of vitamin E and γ–oryzanol extraction according to Alberti et al. (2014) with modifications. The experimental design was generated using Design – Expert software (Version 13, Stat-Ease Inc., Minneapolis, USA). The effects of the independent variables extraction temperature (X_1), time (X_2), and liquid–solid ratio (X_3) at three variation levels were performed in the extraction process (Table 1). The seventeen experiments were conducted to analyze response pattern and to establish models for extraction. Experimental data was fitted to a second – order polynomial model (quadratic model) and regression coefficients obtained. The generalized second – order polynomial used in the response surface analysis was explained by the following equation (1):

$$Y_k = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \beta_{ii} X_i^2 \qquad (1)$$

where Y_k is response variables (Y_1 = α – tocopherol, Y_2 = γ–oryzanol), β_0 , β_i , β_{ij} and β_{ii} are the constant coefficient for intercept, linear, interaction and quadratic terms, respectively and X_i and X_j are the independent variables.

Table 1. Coded and actual levels of three variables

Variables	Coded	Coded levels of variables		
		– 1	0	+ 1
Temperature (°C)	X_1	30	40	50
Time (min)	X_2	30	40	50
Liquid–solid (v/w)	X_3	3	5	7

Determination of antioxidant contents using UV–spectrophotometer

Vitamin E

The vitamin E content was determined according to the method described by Yilmaz et al. (2004) with some modifications. The samples (5 mg/mL) were added in ethanol and measured at 290 nm using UV–vis spectrophotometer (G10S UV–Vis, MA, US). The vitamin E content was expressed as microgram of α–tocopherol per gram of rice germ extract (µg α–tocopherol/g RGE).

γ–Oryzanol

The analysis of γ–oryzanol was carried out based on the method of Bucci et al. (2003) with slight modifications. The samples (5 mg/mL) were mixed with isopropanol and measured the absorbance at 325 nm. The γ–oryzanol content was expressed as microgram of γ–oryzanol per gram of rice germ extract (µg γ–oryzanol/g RGE).

Determination of antioxidant activity

Ferric Reducing Antioxidant Power (FRAP)

The antioxidant activity of extracts was determined using methods by Peanparkdee et al. (2017) with slight modifications. The extracts obtained were dissolved in hexane in ratio of 1:4 (v/v) before analysis. The FRAP was expressed as micromoles of Trolox equivalent per gram of rice germ extract (µmol Trolox/g RGE).

Statistical analysis

Response surface methodology was applied to optimized the experimental data using Design–Expert software (Version 13, Stat-Ease Inc., Minneapolis, USA). Statistical analysis of the models was performed using the one–way analysis of variance (ANOVA) by SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA). All experiments were conducted in triplicate (n = 3). Significant differences between treatments were considered by Duncan's Multiple Range Test at a level of $p < 0.05$.

RESULTS AND DISCUSSION

Optimization of extraction of vitamin E and γ - Oryzanol

Response surface methodology (RSM) with box – Behnken design (BBD) was used to optimized extraction conditions. The effect of three extraction variables including extraction temperature, time, and liquid–solid ratio was studied. Analysis of variance (ANOVA) represented by the quadratic polynomial model was significant with the *p*–value less than 0.05 and a highly significant with the *p*–value less than 0.0001 for vitamin E and γ–oryzanol contents. The experimental results of 17 runs for three species of rice germ are presented in Table 2.

Table 2. Box – Behnken design (BBD) and the response values for vitamin E and γ – oryzanol contents of extracts

Runs	Temperature (°C) X_1	Time (min) X_2	Liquid–solid ratio (v/w) X_3	HM		TT		RB	
				Vitamin E (µg/g extract)	γ – oryzanol (µg/g extract)	Vitamin E (µg/g extract)	γ – oryzanol (µg/g extract)	Vitamin E (µg/g extract)	γ – oryzanol (µg/g extract)
1	40	40	5	44.396	2.977	63.605	5.101	33.026	6.660
2	30	30	5	14.028	0.230	18.909	0.886	19.826	0.884
3	50	30	5	32.791	0.534	31.852	0.906	16.587	1.585
4	40	30	3	18.835	1.852	32.218	3.497	22.941	0.886
5	40	30	7	12.430	0.281	19.063	2.534	10.772	0.999
6	30	50	5	22.048	1.901	11.911	3.007	13.727	1.310
7	40	40	5	42.111	1.251	52.012	4.687	38.636	3.443
8	40	40	5	30.298	1.023	59.184	8.056	33.164	6.289
9	40	40	5	32.858	3.322	54.088	8.482	29.725	3.362
10	50	50	5	8.976	1.293	10.716	5.315	11.748	1.577
11	40	50	7	13.155	1.506	13.612	4.163	17.556	1.832
12	50	40	3	15.497	1.870	13.300	1.889	19.718	3.701
13	40	40	5	32.005	1.877	48.079	7.476	37.759	6.241
14	30	40	7	17.564	0.821	2.510	4.594	4.893	3.549
15	30	40	3	20.904	2.540	17.756	4.635	26.978	3.445
16	40	50	3	11.785	0.849	22.424	5.270	24.022	2.510
17	50	40	7	5.763	2.407	4.132	3.725	4.703	1.875

HM; Hommali Numnom, TT; Tubtim Chumphae, RB; Riceberry

Effect of variables on vitamin E contents

The quadratic model for contents of vitamin E from HM, TT, and RB showed significant linear and quadratic effects, which are shown the following equation (2).

$$Y_{1(HM)} = 36.33 - 1.44X_1 - 2.76X_2 - 3.36X_3 - 7.96X_1X_2 - 1.60X_1X_3 + 1.94X_2X_3 - 8.00X_1^2 - 8.88X_2^2 - 13.40X_3^2$$

$$Y_{1(TT)} = 55.39 + 1.11X_1 - 5.42X_2 - 5.80X_3 - 3.53X_1X_2 + 1.52X_1X_3 + 1.08X_2X_3 - 24.72X_1^2 - 12.32X_2^2 - 21.24X_3^2$$

$$Y_{1(RB)} = 34.46 - 1.58X_1 - 0.38X_2 - 6.97X_3 + 0.31X_1X_2 + 1.77X_1X_3 + 1.42X_2X_3 - 11.87X_1^2 - 7.12X_2^2 - 8.52X_3^2 \quad (2)$$

where Y is the response variable (contents of vitamin E), X_1 is temperature, X_2 is time, X_3 is liquid–solid ratio. X_1, X_2 and X_3 are linear terms of variables; X_1X_2, X_1X_3 and X_2X_3 are interaction terms of variables; X_1^2, X_2^2 and X_3^2 are quadratic terms of variables.

From Table 3, the interaction effects of temperature (X_1) and time (X_2) exhibited a significant effect at $p < 0.05$ on vitamin E contents in HM extract. For TT, the linear effects of time (X_2) and liquid–solid ratio (X_3) displayed a significant effect at $p < 0.05$, and the quadratic effects of time exhibited a significant at $p < 0.05$, temperature and liquid–solid ratio presented a highly significant ($p < 0.0001$) effect on vitamin E contents. For RB, the linear effect of liquid–solid ratio exhibited a significant effect at $p < 0.05$, and the quadratic effects of temperature, time, and liquid–solid ratio displayed a significant effect at $p < 0.05$ on vitamin E contents. The ANOVA analysis for the quadratic model of vitamin E contents was significant ($p < 0.05$) and the lack of fit was also not significant ($p < 0.05$). The results showed the coefficient of determination (R^2; 0.8837, 0.9694, and 0.9215 for HM, TT, and RB, respectively) and demonstrated vitamin E contents between 2.510 and 63.605 µg/g extract. The highest vitamin content for all rice germs (44.396, 63.605 and 38.636 µg/g extract of HM, TT and RB, respectively) was obtained at a temperature of 40 °C, time of 40 min and liquid–solid ratio of 5 v/w. Liquid–solid ratio was the most significant variable affecting the vitamin E contents for TT and RB.

Contour and response surface plots for vitamin E contents in relation to temperature – time (A), temperature – liquid–solid ratio (B), and time – liquid–solid ratio (°C) are shown in Figure 1 and 2. The results indicate that the ultrasonic treatment temperature of 40 °C at a time of 40 min at 5 v/w of liquid–solid ratio yielded high vitamin E content in all types of germs. In contrary, for TT and RB, the contents of vitamin E increased with decreasing extraction time (30–40 min) resulting in enhanced extractability.

Effect of variables on γ–oryzanol contents

The quadratic model for γ–oryzanol contents showed significant linear and quadratic effects, which are shown the following equation (3).

$$\begin{aligned} Y_{2(HM)} &= 2.09 + 0.08X_1 + 0.33X_2 - 0.26X_3 - 0.23X_1X_2 \\ &\quad + 0.56X_1X_3 + 0.56X_2X_3 - 0.15X_1^2 - 0.94X_2^2 \\ &\quad - 0.02X_3^2 \\ Y_{2(TT)} &= 6.76 - 0.16X_1 + 1.24X_2 - 0.03X_3 + 0.57X_1X_2 \\ &\quad + 0.47X_1X_3 - 0.04X_2X_3 - 2.19X_1^2 - 2.04X_2^2 \\ &\quad - 0.86X_3^2 \\ Y_{2(RB)} &= 5.20 - 0.06X_1 + 0.36X_2 - 0.28X_3 - 0.11X_1X_2 \\ &\quad - 0.48X_1X_3 - 0.20X_2X_3 - 1.14X_1^2 - 2.72X_2^2 \\ &\quad - 0.92X_3^2 \end{aligned} \qquad (3)$$

where Y is the response variable (γ–oryzanol contents), X_1 is temperature, X_2 is time, X_3 is liquid–solid ratio. X_1, X_2 and X_3 are linear terms of variables; X_1X_2, X_1X_3 and X_2X_3 are interaction terms of variables; X_1^2, X_2^2 and X_3^2 are quadratic terms of variables.

As shown in Table 3., for HM, the linear, interaction and quadratic effects were not significant on γ–oryzanol contents. For TT, the quadratic effects of temperature (X_1) and time (X_2) exhibited a significant effect at $p < 0.05$. For RB, the quadratic effect of time (X_2) exhibited a significant effect at $p < 0.05$. The coefficient of determination (R^2) was 0.6202, 0.7516 and 0.7910 for HM, TT and RB, respectively. The γ–oryzanol contents were between 0.230 and 8.482 µg/g extract. Non – significance ($p < 0.05$) of the linear and interaction effect of temperature (X_1), time (X_2) and liquid–solid ratio (X_3) of all rice germs was observed, since the temperature, time and liquid–solid ratio were not linear and interaction correlation with the γ–oryzanol contents. The highest γ–oryzanol content of 8.482 µg/g extract was obtained at temperature of 40 °C, time of 40 min, and liquid–solid ratio of 5 v/w. The ANOVA analysis for the quadratic model and lack of fit of γ–oryzanol contents was not significant ($p < 0.05$). The results in Table 3 indicate that the time and liquid–solid ratio were the most significant variables influencing the γ–oryzanol contents.

Contour and response surface plots for γ–oryzanol contents in relation to temperature – time (A), temperature – liquid–solid ratio (B), and time – liquid–solid ratio (C) are shown in Figure 1 and 2. The γ–oryzanol contents showed that extraction temperature of 40 °C at a time of 40 mins and liquid–solid ratio of 5 v/w.

Antioxidant activity of extracts

FRAP

From the results of antioxidant activity based on FRAP assay (Table 4), it was found that the highest antioxidant activity for all rice germs was 305.35, 218.52, and 257.54 µmol Trolox/g rice germ extract of HM, TT, and RB, respectively. Almost runs of HM provided high antioxidant activity in all rice germ extracts. The extraction condition of 40 °C, 40 mins, and 5 v/w of liquid–solid ratio gave higher antioxidant activity in all rice germs. For other conditions such as conditions 7 (50 °C, 50 mins, 5 v/w), the highest antioxidant activity of HM with no significant difference was detected. However, these conditions led to high energy consumption (higher temperature with longer time) and high solvent consumption.

Table 3. Analysis of variance (ANOVA) for the response variables (vitamin E and γ–oryzanol)

Vitamin E

	HM					TT					RB				
Variables	SS	df	MS	F value	*p*–value	SS	df	MS	F value	*p*–value	SS	df	MS	F value	*p*–value
Model	1902.92	9	211.44	5.91	0.0144*	6235.23	9	692.80	24.68	0.0002*	1665.28	9	185.03	9.13	0.0041*
X_1	16.58	1	16.58	0.4634	0.5179	9.93	1	9.93	0.3538	0.5707	20.06	1	20.06	0.9895	0.3530
X_2	61.16	1	61.16	1.71	0.2324	235.22	1	235.22	8.38	0.0232*	1.18	1	1.18	0.0582	0.8162
X_3	40.99	1	40.99	1.15	0.3200	268.90	1	268.90	9.58	0.0174*	388.30	1	388.30	19.15	0.0032*
X_1X_2	253.37	1	253.37	7.08	0.0324*	49.97	1	49.97	1.78	0.2239	0.3969	1	0.3969	0.0196	0.8927
X_1X_3	10.22	1	10.22	0.2857	0.6096	9.24	1	9.24	0.3289	0.5842	12.50	1	12.50	0.6164	0.4581
X_2X_3	15.11	1	15.11	0.4224	0.5365	4.72	1	4.72	0.1680	0.6942	8.13	1	8.13	0.4011	0.5467
X_1^2	269.21	1	269.21	7.52	0.0288*	2574.14	1	2574.14	91.69	< 0.0001*	593.24	1	593.24	29.26	0.0010*
X_2^2	331.78	1	331.78	9.27	0.0187*	639.18	1	639.18	22.77	0.0020*	213.46	1	213.46	10.53	0.0142*
X_3^2	756.67	1	756.67	21.15	0.0025*	1900.14	1	1900.14	67.68	< 0.0001*	305.58	1	305.58	15.07	0.0060*
Residual	250.46	7	35.78			196.53	7	28.08			141.91	7	20.27		
Lack of Fit	84.83	3	28.28	0.6829	0.6073	48.09	3	16.03	0.4320	0.7418	87.44	3	29.15	2.14	0.2379
Pure Error	165.63	4	41.41			148.44	4	37.11			54.48	4	13.62		
Cor Total	2153.38	16				6431.76	16				1807.20	16			
R^2	0.8837					0.9694					0.9215				
C.V.%	27.08					18.95					20.93				

Table 3. Analysis of variance (ANOVA) for the response variables (vitamin E and γ–oryzanol) (Continued)

γ – oryzanol

	HM					TT					RB				
Variables	SS	df	MS	F value	*p*–value	SS	df	MS	F value	*p*–value	SS	df	MS	F value	*p*–value
Model	8.16	9	0.9065	1.27	0.3848	59.63	9	6.63	2.35	0.1361	46.43	9	5.16	2.94	0.0842
X_1	0.0468	1	0.0468	0.0656	0.8052	0.2070	1	0.2070	0.0735	0.7941	0.0253	1	0.0253	0.0144	0.9077
X_2	0.8791	1	0.8791	1.23	0.3038	12.33	1	12.33	4.38	0.0747	1.03	1	1.03	0.5897	0.4677
X_3	0.5492	1	0.5492	0.7693	0.4095	0.0095	1	0.0095	0.0034	0.9554	0.6538	1	0.6538	0.3731	0.5606
X_1X_2	0.2079	1	0.2079	0.2913	0.6061	1.31	1	1.31	0.4647	0.5173	0.0471	1	0.0471	0.0269	0.8744
X_1X_3	1.27	1	1.27	1.78	0.2236	0.8808	1	0.8808	0.3128	0.5934	0.9312	1	0.9312	0.5315	0.4897
X_2X_3	1.24	1	1.24	1.74	0.2288	0.0052	1	0.0052	0.0018	0.9670	0.1564	1	0.1564	0.0893	0.7738
X_1^2	0.1031	1	0.1031	0.1445	0.7151	20.26	1	20.26	7.19	0.0314*	5.44	1	5.44	3.11	0.1213
X_2^2	3.75	1	3.75	5.26	0.0556	17.49	1	17.49	6.21	0.0414*	31.22	1	31.22	17.82	0.0039*
X_3^2	0.0024	1	0.0024	0.0034	0.9551	3.09	1	3.09	1.10	0.3300	3.56	1	3.56	2.03	0.1971
Residual	5.00	7	0.7138			19.71	7	2.82			12.27	7	1.75		
Lack of Fit	0.8044	3	0.2681	0.2558	0.8541	7.51	3	2.50	0.8198	0.5468	1.40	3	0.4662	0.1716	0.9103
Pure Error	4.19	4	1.05			12.21	4	3.05			10.87	4	2.72		
Cor Total	13.16	16				79.34	16				58.70	16			
R^2	0.6202					0.7516					0.7910				
C.V.%	54.13					38.44					44.87				

X_1; Temperature (°C), X_2; Time (min), X_3; liquid–solid ratio (v/w)

SS; Sum of Squares, df; degrees of freedom, MS; Mean squares, F value; Fischer test value, *p*–value; probability value

* $p < 0.05$

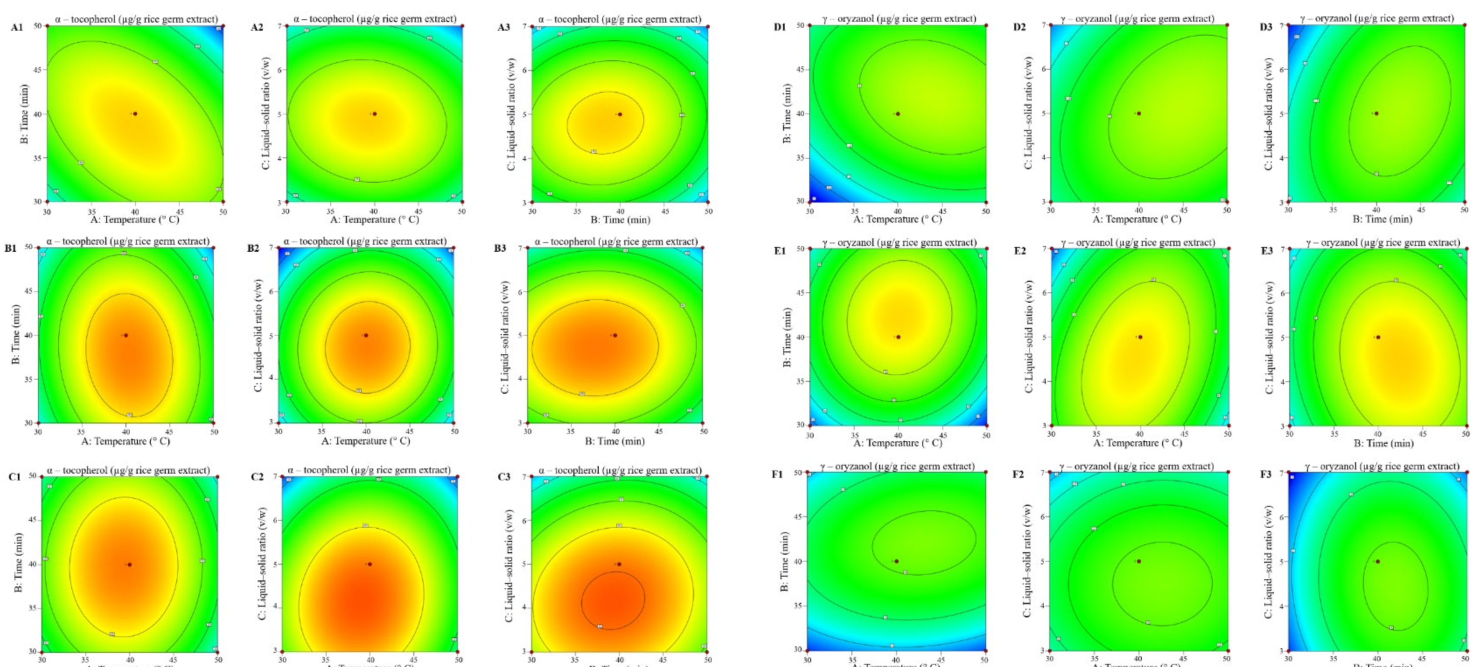

Figure 1. The contour plot of vitamin E (A1–C3) and γ–oryzanol contents (D1–F3) (A/D; Hommali Numnom, B/E; Tubtim Chumphae, C/F; Riceberry, respectively).

Table 4. Antioxidant activity of extracts from Thai rice germs

Conditions	Temperature (°C) X_1	Time (min) X_2	Liquid–solid ratio (v/w) X_3	FRAP (μmol Trolox/g rice germ extract)		
				HM	TT	RB
1	30	30	5	272.69 ± 5.79^{abcA}	92.24 ± 13.07^{deB}	78.50 ± 9.46^{cdefB}
2	50	30	5	102.35 ± 0.52^{jB}	132.80 ± 2.02^{bcdA}	53.74 ± 0.86^{defgC}
3	40	30	3	146.41 ± 1.25^{hiA}	50.60 ± 8.73^{fB}	149.21 ± 11.84^{bA}
4	40	30	7	250.68 ± 9.17^{bcdA}	134.34 ± 21.06^{bcdB}	80.54 ± 18.02^{cdefC}
5	30	50	5	182.06 ± 3.83^{fghA}	167.58 ± 0.84^{bB}	85.97 ± 2.91^{cdeB}
6	40	40	5	290.28 ± 39.08^{abA}	218.52 ± 15.02^{aA}	257.54 ± 58.50^{aA}
7	50	50	5	305.35 ± 4.54^{aA}	61.41 ± 11.38^{efB}	75.56 ± 1.76^{cdefB}
8	40	50	7	279.41 ± 12.90^{abA}	48.14 ± 4.08^{fB}	45.70 ± 0.88^{efgB}
9	50	40	3	172.09 ± 0.45^{ghiA}	112.00 ± 7.42^{dB}	60.71 ± 15.70^{cdefC}
10	30	40	7	302.69 ± 0.59^{aA}	216.00 ± 5.81^{aB}	17.76 ± 2.67^{gB}
11	30	40	3	223.32 ± 22.84^{defA}	ND	59.76 ± 11.84^{cdefB}
12	40	50	3	160.65 ± 37.66^{ghiA}	116.96 ± 10.81^{dAB}	68.33 ± 19.73^{cdB}
13	50	40	7	291.91 ± 27.20^{abA}	160.76 ± 3.15^{bcB}	96.08 ± 8.43^{cC}

Values within each column followed by different letters are significantly different ($p \leq 0.05$)
Values within each row followed by different capital letters are significantly different ($p \leq 0.05$)

CONCLUSIONS

In this work, the ultrasound–assisted extraction was applied to extract vitamin E and γ–oryzanol from Thai rice germs (HM, TT and RB). The experiment was performed with Box – Behnken Design using response surface methodology to optimized extraction conditions. The appropriate conditions for obtaining the extract with great contents of vitamin E and γ–oryzanol, and high value of antioxidant activity were 40 °C, 40 min with 5 v/w of liquid–solid ratio. Additionally, the enriched soybean oil has the potential to be applied as functional food ingredients or oil replacement in food products.

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