Optimization of Extraction Condition of Antioxidants from Pigmented Rice Bran *cv.* Hom Nin Using Response Surface Methodology

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Abstract

Solvent extraction was implemented for antioxidants extraction from pigmented rice bran *cv.* Hom Nin using methanol, ethanol and ethylacetate as extractant. For food safety reason, ethanol was selected as extractant in the optimization experiment. The response surface methodology (RSM) was used to optimize the independent variables which were ethanol concentration (X_1), ratio of solvent and rice bran (X_2) and extraction time (X_3). The terms of X_1 and X_1^2 had significant effect on total phenolics (TPC), γ-oryzanol, α-tocopherol and anthocyanin contents. In addition, terms of X_2 and X_1X_2 had significant effect on TPC and anthocyanin contents. The optimal condition of antioxidants extraction was demonstrated as follows; 72.5% of ethanol, ratio of solvent and rice bran of 26.67 mL/g rice bran and extraction time of 120 min, achieving the contents of total phenolic content, γ-oryzanol, α-tocopherol, anthocyanin and extracts yield about 9,362.83 ±22.75 μgGAE/g, 253.34±7.73 μg/g, 9.02±0.01 μg/g, 1,859.15±71.53 μg/g and 17.8%, respectively. The total antioxidants in this extract were 49.10, 62.54 and 87.37% higher than the extracts from methanol, ethanol and ethylacetate extraction, respectively.

Keywords: Rice Bran, Antioxidants, Response Surface Methodology (RSM), Optimization.

1. Introduction

The natural antioxidants, the substances in cell wall and/or other part of general plants such as oryzanol, tocopherol, polyphenols and anthocyanin. These substances have been reported to be usable as radical scavengers, terminating the propagation of radical chain reaction by reacting with peroxy radicals and generating unreactive phenoxyl radicals as well as hydroperoxide products [1]. Many studies have proved the health benefits of antioxidants such as inhibition of low-density lipoprotein oxidation, cancer cell formation, platelet aggregation and inflammatory activity [2]-[4]. Moreover, antioxidants are required by food industry to inhibition and extend shelf life of food products. The potential synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used in food industry. However, many studies manifest their adverse effects such as carcinogenicity and toxicity [5]. Thus, the replacement of synthetic antioxidants by natural antioxidants is an essential choice to prevent the harmful risks for consumer health. Some studies reported the successful application of rice bran extract in food product to inhibit conjugated diene hydroperoxide in fish oil enriched mayonnaise and oil bulk system [5]-[6].

In general, rice bran is a rich source of important antioxidants such as vitamin E, phenolic compounds and γ-oryzanol which are found in the outer layers of rice bran kernels [7]-[8]. Moreover, pigmented rice bran also contained anthocyanin, the natural pigment antioxidants [9]-[10]. [11] Kong *et al*. reported that the black rice bran contained γ-oryzanol, anthocyanin and polyphenols approximately 3.45-3.94, 7.07-10.70 and 89.20-108.00 mg/g DM, respectively. In the previous data about their great bioactive benefits and antioxidant properties, there are many inventions for trying to extract antioxidants for commercially used. The most favorable extraction method is solvent extraction due to its simplicity and lower cost than others methods. Methanol, ethanol, hexane, isopropanol and ethylacetate are commonly used for extracting the low polarity antioxidants such as γ-oryzanol and α-tocopherol [6]-[8], [12]-[13]. However, these solvents are not suitable for extracting anthocyanin which is a polar molecule. Hence, the proceeding for both polar and non-polar antioxidants from pigmented rice bran in a single step is very interesting. Therefore, it is necessary to find an optimum extraction condition to obtain the extract with the highest amount of total phenolic content, anthocyanin, γ-oryzanol and α-tocopherol. The experiment was performed in respond surface methodology (RSM).

The first objective of this study was to investigate the effect of solvents to extract the antioxidant components in pigmented rice bran cv. Hom Nin including methanol, ethanol and ethylacetate. The second objective was to optimize

the extraction condition (ethanol concentration, ratio of solvent and rice bran, and extraction time) of total phenolic content, anthocyanin, γ-oryzanol and α-tocopherol in a single step with the highest amounts.

2. Materials and Methods

2.1 Materials

Rice grains cv. Hom Nin was purchased from Multiple Cropping Center (MCC), Faculty of Agriculture, Chiang Mai University, Chiang Mai, 50100, Thailand. The rice grains was milled with a laboratory miller, and then passed through a 20-mesh sieve and kept in poly propylene bag at -18 °C.

2.2 Extraction and selection of solvents

The extraction method was followed [1] with slightly modification. Rice bran (10 g) was extracted by using an electrical shaker for 3 hours at room temperature with 200 mL of methanol (MEOH), ethanol (ETOH) and ethylacetate (ETAC) followed by filtering through Whatman filter paper. The supernatants were evaporated to dry by rotary evaporator at 45 °C and weighed immediately after. The final volume of crude extracts was adjusted to 50 mL with methanol (HPLC Grade) and stored in brown glass bottles (covered by foil) at -18 °C for further analysis. The extraction was done in triplicate.

2.3 Optimization of extraction condition

An optimal condition for extraction was obtained from the response surface methodology (RSM) with central composite design (CCD). The effect of independent variables including X_1 (selected solvent concentration, %), X_2 (ratio of solvent and rice bran, mL/g) and X_3 (extraction time, min) at five variation levels as shown in Table 1.The complete design consisted of 20 experiment points including five replications of the center points (Table 2).

Table 1. Independent variables at five variation levels

Variables	code	Variation levels				
		-α	-1	0	1	α
Selected solvent concentration, %	X_1	13.0	30	50	80	97.1
Ratio of solvent and rice bran, mL/g	X_2	3.2	10	20	30	36.8
Extraction time, min	X_3	39.6	60	90	120	140

Table 2. The 20 experiment points including 5 replications of the center points

Std. order	Run order	Factors		
		X_1 (%)	X_2 (mL/g)	X_3 (min)
16	1	55.0	20	90
8	2	80.0	30	120
17	3	55.0	20	90
10	4	97.0	20	90
13	5	55.0	20	39.6
19	6	55.0	20	90
4	7	80.0	30	60
15	8	55.0	20	90
5	9	30.0	10	120
1	10	30.0	10	60
18	11	55.0	20	90
3	12	30.0	30	60
6	13	80.0	10	120
12	14	55.0	36.8	90
9	15	13.0	20	90
11	16	55.0	3.2	90
20	17	55.0	20	90
7	18	30.0	30	120
14	19	55.0	20	140
2	20	80.0	10	60

Experimental data were fitted to a second-order polynomial model and regression coefficients were obtained. The generalized second-order polynomial model used in the response surface analysis was as follows;

$$Y_n = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1X_2 + b_5X_2X_3 + b_6X_1X_3 + b_7X_1^2 + b_8X_2^2 + b_9X_3^2 \quad (1)$$

where Y_n is the response, X_{1-3} are the independent variables and b_{0-9} are the regression coefficients for the intercept, linear, quadratic and interaction terms, respectively. All of experiment

data were analyzed using Design Expert Software version 6.0.

2.4 Determination of total phenolic content

Folin–Ciocalteu reagent was diluted with distilled water 1:9 (v/v) followed by 1.25 mL of this reagent and 50 µL of crude extract were mixed. One milliliter of Na_2CO_3 (7.5%) was added. The mixture was incubated for 15 min at 50 °C. The absorbance at 760 nm was measured by using a UV-Vis spectrophotometer for 15 minutes. Gallic acid was used as a standard, and results were calculated as gallic acid equivalents (µg GAE/g of rice bran).

2.5 Determination of anthocyanin content

Anthocyanin content was determined by the pH differential method [9]. To measure the absorbance at pH 1.0 and 4.5 within 20-50 min after preparation, the crude extract was diluted about 20 times with pH 1.0 potassium chloride buffer and pH 4.5 sodium acetate buffer, respectively followed by mixing with vortex equipment. The absorbance of test portion was determined at both 513 and 700 nm. The diluted test portions are read and compared with a blank cell filled with distilled water. The concentration of monomeric anthocyanin pigment was calculated by the following equation:

$$\text{Monomeric anthocyanin pigment (mg/L)} = [A_{diff} \times MW \times DF \times 1000] / \square \quad (2)$$

Where MW represents molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, □ is molar absorptivity of cyanidin-3-glucoside (26,900 l/mol cm) and A_{diff} was calculated from the following equation:

$$A_{diff} = [A_{513} - A_{700}]_{pH1.0} - [A_{513} - A_{700}]_{pH4.5} \quad (3)$$

2.6 Determination of α-tocopherol and γ-oryzanol contents

α-tocopherol and γ-oryzanol were separated following method as described by Pacual *et al*. with slightly modification, using an analytical Shimadzu High performance liquid chromatography (HPLC) system equipped with photodiode array and a scanning fluorescence detector (Kyoto, Japan). Samples were injected onto a C18 column (250 mm×4.6 mm (i.d); Restec) using the manual sampler. The flow rate of elution is 1 ml/min. The initial composition of the mobile phase (45% acetonitrile, 45% methanol and 10% isopropanol) was held for 6 min, followed by a linear gradient to 25% acetonitrile, 70% methanol and 5% isopropanol in 10 min; the final composition was held for 12 min. α-tocopherol was monitored using the fluorescence detector at an excitation wavelength of 298 nm and an emission wavelength of 328 nm. γ-oryzanol was monitored with PDA detection at 325 nm.

2.7 Statistical analysis

All trials in solvent selection part were carried out in triplicate and all data were reported as means±SD (standard deviation). The statistics significance was evaluated using Duncan's New Multiple Range Test and $P<0.05$ was taken as significant.

All experimental results in optimization part were expressed as means±SD of three parallel measurements and all calculations were carried out with the help of Design Expert (Version 6.0). Analysis of variance (ANOVA) was performed. *P-values*<0.05 were regard as significant.

3. Result and Discussion

3.1 Selection of solvents

The highest TPC was observed for MEOH extracts (3,410.95±64.44µgGAE/g), which was significantly greater than the ETOH (2,196.63 ±30.88 µg GAE/g) and ETAC (378.38 ±15.25 µgGAE/g) extracts ($p<0.05$). Thus, the solubility of phenoics compound is affected by the polarity of solvents used. It was observed that the high polarity phenolics can be dissolved in polarity solvents both MEOH and ETOH. In contrast, ETAC which is a non-polarity solvent is not suitable for extracting the phenolics compound, because its solubility are not corresponding. Arab *et al*. also reported that the polar solvents both methanol and ethanol had shown higher extractability of the total phenolic content than non-polar solvents (ETAC) about 1-4 fold.

Anthocyanin was extracted with the highest amount by ETOH (312.34 ±12.72 µg/g), followed by MEOH (220.29 ±7.27 µg/g) and ETAC (32.89 ±1.12 µg/g), respectively. Polarity of solvent influences the extractability of anthocyanin. High polar molecules are more extracted by high polar solvent. However, diffusivity of solvent and mass transferring also affect the extractability [9]. Our result shows that anthocyanin in rice bran was extracted by ETOH in higher amount than MEOH, a higher polar solvent. The similar result has been reported by [14] who

found that the extraction of anthocyanin in fruit pulp of *Enterpe edulis* by ETOH yielded the higher content of anthocyanin than MEOH (21.6013.5 and 17.741.56 mg/100 g of fresh weight, respectively).

The concentrations of γ-oryzanol and α-tocopherol were also influenced by the extractant (Table 3). Among these solvents, MEOH extract contained the highest concentration of γ-oryzanol and α-tocopherol (2,153±13.38 μg/g and 60.28±2.08 μg/g). In the ETOH and ETAC extracts, γ-oryzanol contentswere 1,748.70±14.70 and 1,001.04±5.29 μg/g, respectively and α-tocopherol contents were 44.60±1.27 and 38.30±4.15 μg/g, respectively.

Table 3. Antioxidant contents and extract yields of Hom Nin rice bran with various solvents

Solvents	Antioxidant contents				Extracts yield (%)
	Total phenolic content (μg GAE/g)	Gamma-oryzanol (μg/g)	Alpha-tocopherol (μg/g)	Anthocyanin (μg/g)	
MEOH	3,410.95 ±64.44[a]	2,153.75 ±13.38[a]	60.28 ±2.08[a]	220.29 ±7.27[b]	12.9 ±0.03[c]
ETOH	2,196.63 ±30.88[b]	1,748.07 ±14.70[b]	44.60 ±1.27[b]	312.34 ±12.72[a]	14.7 ±0.03[b]
ETAC	378.38 ±15.25[c]	1,001.04 ±5.29[c]	38.30 ±4.15[c]	32.89 ±1.12[c]	18.9 ±0.06[a]

Table 4. Antioxidant contents and extract yields of Hom Nin rice bran

Standard order	Run order	Factors			Responds				
		X_1[a] (%)	X_2[a] (ml.)	X_3[a] (min)	Total phenolic content (μgGAE/g)[c]	Gamma-oryzanol (μg/g)[c]	Alpha-tocopherol (μg/kg)[c]	Antho-cynanin (μg/g)[c]	Extract yields (%)[c]
16	1	55.0	20	90.0	9,630.91 ±22.75	13.20 ±0.09	508.92 ±4.35	1,571.22 ±37.37	15.3
8	2	80.0	30	120.0	8,697.60 ±82.92	12.06 ±0.50	3,375.98 ±112.04	1,857.29 ±110.83	16.6
17	3	55.0	20	90.0	9,635.87 ±53.70	13.31 ±0.47	515.18 ±8.86	1,522.20 ±14.22	15.5
10	4	97.0	20	90.0	2,233.97 ±74.47	1401.7 ±12.28	43,781.58 ±458.17	229.60 ±49.16	16.0
13	5	55.0	20	39.5	9,849.34 ±34.39	1.41 ±0.04	237.40 ±1.99	1,594.80 ±54.25	8.4
19	6	55.0	20	90.0	9,695.44 ±14.89	13.43 ±0.72	500.41 ±5.64	1,564.40 ±65.59	15.3
4	7	80.0	30	60.0	8,921.00 ±89.36	4.82 ±0.83	1,369.74 ±33.18	1,715.81 ±83.43	16.5
15	8	55.0	20	90.0	9,675.59 ±22.75	13.38 ±0.74	502.58 ±4.36	1,592.94 ±19.11	15.1
5	9	30.0	10	120.0	9,253.61 ±8.60	ND[b]	24.81 ±0.71	1,098.37 ±87.66	19.3
1	10	30.0	10	60.0	7,580.62 ±89.36	ND[b]	12.73 ±0.80	1,059.27 ±47.50	13.0
18	11	55.0	20	90.0	9,675.59 ±22.75	13.36 ±0.49	507.25 ±3.47	1,558.81 ±49.58	15.1
3	12	30.0	30	60.0	9,918.84 ±64.92	ND[b]	12.20 ±0.74	1,576.81 ±54.85	39.0
6	13	80.0	10	120.0	8,161.45 ±59.57	79.60 ±1.04	8,327.36 ±183.30	1,249.16 ±35.52	10.9
12	14	55.0	36.8	90.0	10,117.42 ±37.48	36.66 ±0.67	835.22 ±18.46	2,004.98 ±91.83	16.6
9	15	13.0	20	90.0	9,491.90 ±37.00	ND[b]	24.68 ±1.61	1,632.04 ±3.88	20.1
11	16	55.0	3.18	90.0	5,867.90 ±74.47	ND[b]	69.33 ±3.36	753.65 ±13.04	6.2
20	17	55.0	20	90.0	9,680.55 ±39.40	13.41 ±0.73	505.46 ±5.87	1,580.53 ±17.95	15.1
7	18	30.0	30	120.0	10,176.99 ±67.16	ND[b]	55.61 ±3.37	1,684.78 ±53.02	26.3
14	19	55.0	20	140.5	9,804.66 ±70.38	19.40 ±0.41	488.91 ±17.14	2,109.86 ±94.01	15.8
2	20	80.0	10	60.0	7,828.84 ±31.00	54.21 ±0.62	3273.71 ±81.45	1,404.29 ±68.81	3.3

a ; X_1 = Ethanol concentration, X_2 = Ratio of solvent and rice bran, X_3 = Extraction time

b ; ND = Not detectable and c ; Calculated by dry basis weight

α-tocopherol is higher when increasing the polarity of the solvent. This observation highlights the better extractability of oryzanol and tocols extracted by MEOH and ETOH (polar solvent) than when extracted by ETAC (non-polar solvent). It could be attributed to the amphipathic characteristics of these antioxidants, involving the methyl groups on a chroman ring and the hydroxyl groups on an aromatic ring of vitamin E homologs, or the carboxylic group on a ferulated moiety, long side chain, and methyl groups on a hydropyrano moiety and sterol of oryzanols that might make these compounds more extractable in alcohol than in ETAC [1], [15].

The results confirm a previous study by Chen *et al.* and Zigoneanu *et al*., their results showed that polar solvents (methanol and isopropanol) had higher extractability of tocols and oryzanol than non-polar solvent (hexane).

The yield extracts significantly varied with the solvents applied, in the order of ETAC (18.9±0.06%), ETOH (14.7±0.03%) and MEOH (12.9±0.03%), respectively. It was observed that ETAC, the non-polar solvent could extract non-polar substances such as fat and lipids, which were contained in rice bran.

This study showed that MEOH is efficient and suitable solvent for analytical work, because it showed the highest extractability. Nevertheless, according to its toxicological issue, the use of MEOH in food product is concerned. Several studies [1], [18]-[20] showed that the use of MEOH as extractant has been avoided because it is not safe for consumers due to potential toxic effects from the residue solvents. In consequence, the more suitable solvent was selected for optimization of extraction condition in the further part is ETOH, because antioxidant contents are slightly different when compared with ETAC, it was usually used for the extraction of natural antioxidants for food products.

3.2 Model fitting and responses surface analysis

Ethanol was selected for solvent extraction of antioxidants. The extract was determined for TPC, γ-oryzanol, α-tocopherol and anthocyanin contents.

This study verified the effect of process variables including ethanol concentration (X_1), ratio of solvent and rice bran (X_2), extraction time (X_3) on the extraction efficiency, thus a response surface design was implemented. The results from the combination of the study variables are shown in Table 4.

The TPC, γ-oryzanol, α-tocopherol, anthocyanin contents and extracts yield ranged between 2,233.97±74.47-10 μg/g, 176.99±67.16 μg/g, 1.41 ±0.04 - 1,401.7±12.28 μg/g, 12.20±0.74 - 43,781.58 ±458.17μg/kg, 229.60±49.16 - 2,109.86±94.01 μg/g and 3.3-39%, respectively. The data were statistically analyzed at 95% confidence level. Each coefficient was determined by *F*-value and *p*-value (Table6). The corresponding *p* values suggest that among the test variables, ethanol concentration (X_1), ratio of solvent and rice bran (X_2) and the interaction between these variables were significant model terms ($p<0.05$) for the responses including TPC, anthocyanin and extract yields. In addition, the ethanol concentration (X_1) was significant model term for γ-oryzanol and α-tocopherol as well.

The quadratic term of ethanol concentration is a significant model term for all response except extract yields. However, the other terms such as extraction time (X_3), and the interactions related with this variable were insignificant for the responses (Table 5).

Table 5. Summary of the effects of independent variables on the responses

Effect	Responses				
	TPC	**γ-oryzanol**	**α-tocopherol**	**Antho-cynanin**	**Extract yields**
Linear					
X_1	-	+	+	-	-
X_2	+	n.s.	n.s.	+	+
X_3	n.s.	n.s.	n.s.	n.s.	n.s.
Quadratic					
X_1^2	-	+	+	-	n.s.
X_2^2	n.s.	n.s.	n.s.	n.s.	n.s.
X_3^2	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction					
X_1X_2	-	n.s.	n.s.	-	-
X_1X_3	n.s.	n.s.	n.s.	n.s.	n.s.
X_2X_3	n.s.	n.s.	n.s.	n.s.	n.s.

(+) : positive effect, (-) : negative effect, n.s. : effect not significant

The equation models number 1, 2, 3, 4 and 5 (Table 6) show the empirical code models related to responses of the TPC, γ-oryzanol, α-tocopherol, anthocyanin contents and extracts yield, respectively.

In addition, the values of R^2 for equation numbers 1 to 5 were 0.775, 0.613, 0.771, 0.703 and 0.790, respectively. At the same time, it also confirmed that the models were significant and indicated an adequate degree of correlation between the observed and predicted data.

However, the lack of fit of all equations were significant ($p<0.05$) indicating that there were other factors influencing the extraction of antioxidants in pigmented rice bran.

Table 6. Equation models and R^2 of each responses

No.	Response	Equation model	R^2
1	Total pheolic content	$9.64 - 1.14(X_1) + 0.89(X_2) - 1.13(X_1^2) - 0.20(X_1X_2)$	0.775
2	Gamma-oryzanol	$21.04 + 183.65(X_1) + 192.78(X_1^2)$	0.613
3	Alpha-tocopherol	$6.98 + 65.78(X_1) + 63.10(X_1^2)$	0.771
4	Anthocayanin	$1.56 - 0.11(X_1) + 0.30(X_2) - 0.21(X_1^2) – 0.021(X_1X_2)$	0.703
5	Extract yield	$1.50 - 0.42(X_1) + 0.51(X_2) - 0.18(X_1X_2)$	0.790

where X_1, X_2 and X_3 are coded variables.

Figure 2. A and B, show the similar tendency of the responses from γ-oryzanol and α-tocopherol which were showed as a function of the ethanol concentration when the value of extraction time was set at zero level (90 minutes). It can be observed that mass transferring and diffusivity of γ-oryzanol and α-tocopherol increased with increasing ethanol concentration. Diffusivity depends on both extractant and solute. The increase of ethanol concentration would have increased the dielectric constant of the solvent and thus increased the solubility of these molecules. The hydrogen bond acceptor and donor capability of the solvent mixture could also play a role [21]. Thus, the increase of solvent concentration would lead to an increase in the diffusion of the molecules by increasing of the interaction with the solvent. Moreover, the polarity ofthe extractant influences the extractability. The diffusivity and mass transfer are higher when the extractant and solute have similar polarity. γ-Oryzanol is a mixture of esters of ferulic acid with sterols and triterpene alcohol [8] which might cause a more extractable behavior of such compound with similar polarity of ethanol. Furthermore, α-tocopherol, which is a non-polar molecule and consists of hydroxyl group at the chroman rings of vitamin E homologs [6], [12] also showed similar extractability behavior to γ-oryzanol.

However, the less effect on the change of the ratio of solvent and rice bran was observed on γ-oryzanol and α-tocopherol extraction because of the relatively high ratio of solvent/rice bran had used in our study. Similarresult were reported by Lilitchan *et al*., the high ratio of solvent and rice bran more than 15:1, the extraction efficiency was not increased.

Anthocyanin and TPC, the high polar antioxidants molecules, showed similar behavior (Figure 2. C and D). Their tendencies were strongly influenced by the ethanol concentration, ratio of solvent/rice bran and their interaction. The extraction of anthocyanin from radish and red cabbage were studies by Patill *et al*. and Chandrasekhar *et al*., respectively. Their results show that the optimum concentration of ethanol ranged between 40 to 50%. Thus, the presence of water was required for the extraction of hydrophilic anthocyanin. In addition, [21] reported that the diffusion coefficient of anhocyanin and total phenolics contents were increased with an increasing of ethanol concentration from 39% to 67% and the diffusion coefficient were then decreased with further increasing of ethanol concentration for 67% to 95%. Spigno *et al*. found that increased water content of ethanol for phenolic extraction from grape was of statistical influence in improving TPC yield.

The using of ethanol containing different volumes of water about 30% to 50% was optimal range, while phenols concentration of extract decreased for water content above 50%. Therefore, it is possible to improve the extraction of high polar antioxidants with organic solvent by adding lower water content.

The ratio of solvent and rice bran including their interaction had some effect for anthocyanin and TPC because the driving force during mass transfer within the solid is considered to be the concentration gradient, which was greater when a higher ratio was used, resulting in an increase of the diffusion rate [21].

Figure. 2 E shows the interaction effect of ethanol concentration and ratio of solvent and rice bran on the extract yield. The extract yield was highly increased with respect to water content. However, it is possible that the higher yield as observed in this study are caused by better extractability of soluble compounds such as sugars and proteins which were high hydrophilicity [6].

3.3 Optimization of extraction variables and model validation

The highest amounts of TPC, γ-oryzanol, α-tocopherol, anthocyanin contents and extract yield are the goal of optimization. The suitability of the model equation for predicting the optimum responses values were carried out; ethanol concentration of 72.5%, extraction time of 120 mins and ratio of solvent and rice bran of 26.67 mL/g. The model predicted maximum responses of TPC, γ-oryzanol, α-tocopherol, anthocyanin and extract yield of 8,702.63 µg GAE/g, 173.05 µg/g, 7.01 µg/g, 1,771.64 µg/g and 15.3 %, respectively. To ensure these predicted values, verification experiment was performed, achieving the contents of TPC, γ-oryzanol, α-tocopherol, anthocyanin contents and extracts yield about 9,362.83±22.75 µg GAE/g, 253.34±7.73 µg/g, 9.02 ±0.01 µg/g, 1,859.15±71.53 µg/g and 17.8%, respectively. It was found that the observed values of all response were close to the predicted values with some positive errors. The total antioxidants content (sum of TPC, γ-oryzanol, α-tocopherol, anthocyanin in Table 3) extracted by optimal condition was higher than extracted by MEOH, ETOH and ETAC by 49.10, 62.54 and 87.37%, respectively. Although, the amounts of γ-oryzanol and α-tocopherol extracted from the optimal condition were lower than methanol extract, the amounts of TPC and anthocyanin were increased with about 76.54 and 83.20%, respectively, when compared with pure ethanol extraction. Hence, the results of analysis confirmed that the response model was adequate for reflecting the expect optimization.

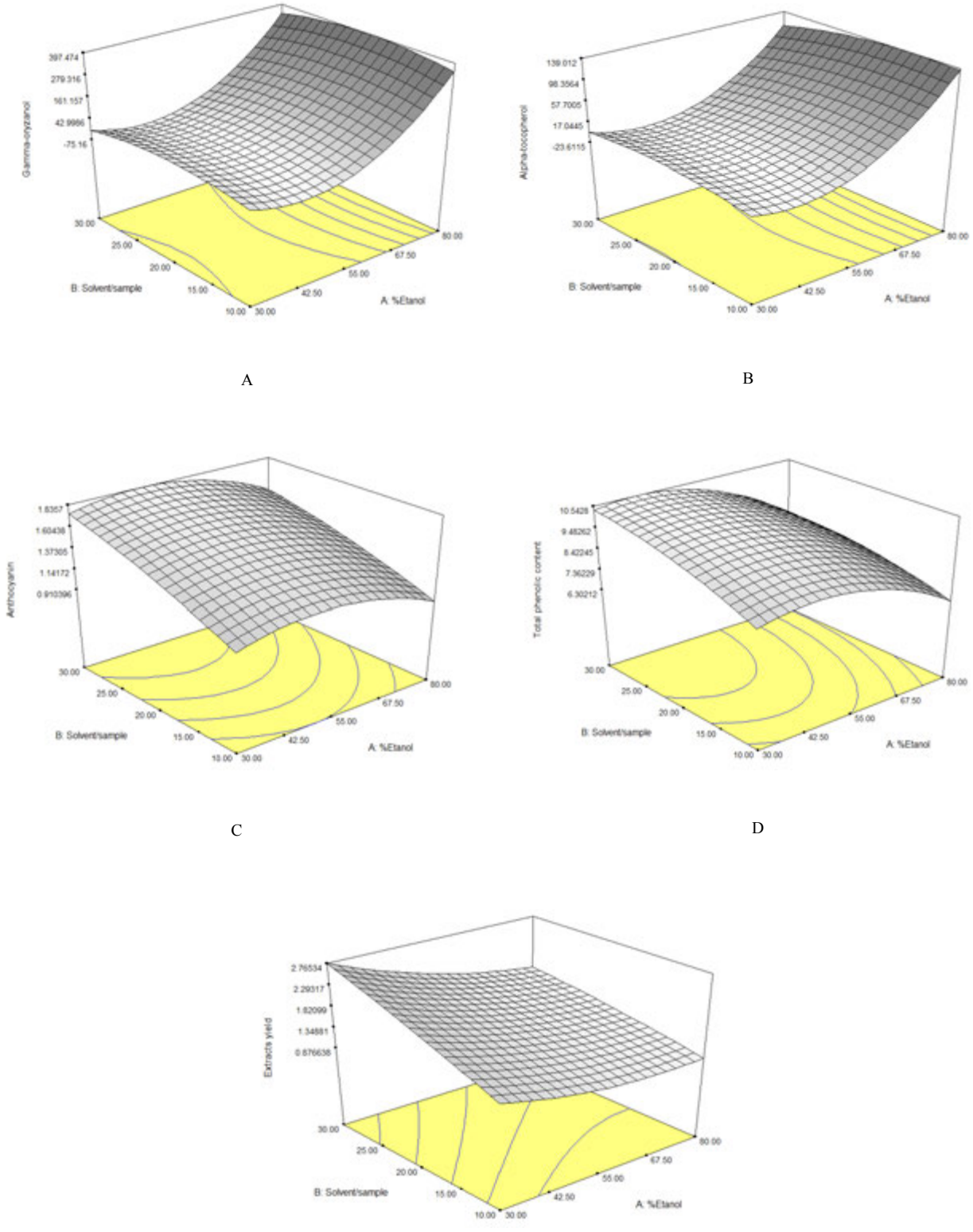

Fig 2. Response surface plot of ethanol concentration in water (%) and solvent/rice bran ratio (mL/g) and their mutual interaction with extraction time of 90 minutes on the yields of gamma-oryzanol (A), alpha-tocopherol (B), anthocyanin (C), total phenolic content (D) and extract yield (E), respectively.

4. Conclusion

Ethanol was selected for solvent extraction optimization of antioxidants from Hom Nin rice bran, because its extract showed a mounts of antioxidants close to methanol extract.

According to experimental design and response surface analysis, quadratic polynomial models can be used to predict the yield of extraction and extractability of antioxidants including TPC, γ-oryzanol, α-tocopherol and anthocyanin. Not only ethanol concentration had the strong impact on all responses, but also its quadratic term had the impact on the all responses which not including γ-oryzanol and α-tocopherol. The optimal extraction condition obtained the highest amounts of antioxidants was 72.5% of ethanol, extraction time of 120 min and ratio of solvent/rice bran of 26.67 mL/g, achieving the contents of TPC, γ-oryzanol, α-tocopherol, anthocyanin and extracts yields about 9,362.83 ±22.75 μg GAE/g, 253.34±7.73 μg/g, 9.02 ±0.01 μg/g, 1,859.15±71.53 μg/g and 17.8%, respectively. The total antioxidants were extracted by above condition were higher than extracted by MEOH, ETOH and ETAC about 49.10, 62.54 and 87.37%, respectively.

However, other factors such as temperature, stirring, new types of solvent and rice cultivar are needed to study in further research.

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