EXTRACTION OF FERULIC ACID FROM DEFATTED WHEAT BRAN FOR IMPROVING ANTIOXIDANT ACTIVITY

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Abstract

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is one of the most significant natural bioactive phenolic acids generally found in the seeds as well as leaves, both in its free form and covalently conjugated with the plant cell wall materials. The extraction of ferulic acid has been found much attention nowadays due to the fact that it exhibits a wide variety of biological activities. In the present study, the extraction of ferulic acid from wheat bran was carried out by alkaline hydrolysis using NaOH and later purified by precipitating hemicellulose and glucomannans with ethanol. Optimization of antioxidant activity in ferulic acid from defatted wheat bran was conducted using response surface methodology (RSM). The conditions investigated were 0.5, 1.0 and 1.5 M of NaOH concentration (x_1), 2, 4 and 6 hr of extraction time (x_2), and 40, 50 and 60 ˚C for the extraction temperature (x_3). Box-Behnken design indicated that the model can significantly ($p < 0.05$) express more than 80 % (> 0.80) of the response variation. The optimal extract condition for maximum antioxidant activity was 0.5 M of NaOH concentration, 2 hr of extraction time, and 50 ˚C for extraction temperature. The antioxidant activity of extracted ferulic acid was determined by DPPH (2, 2-diphenyl-1-picryl-hydrazyl) radical photometric scavenging method. According to these results, ferulic acid from wheat bran possesses high antioxidant activity.

Keywords: ferulic acid, wheat bran, extraction, response surface methodology, antioxidant activity

Introduction

Wheat bran is a by-product from the roller milling of the wheat grain to produce wheat flour (Apprich *et al*., 2014). One of the functional compounds extracted from agricultural by-product is ferulic acid, the most abundant hydroxycinnamic acid found in plant cell wall is covalently linked to polysaccharides and lignin (Masoomeh *et al*., 2008). Ferulic acid was exhibited several physiological benefits such as anti-microbial, anti-oxidants, anti-inflammatory and anti-cancer activities (Buranov & Mazza, 2009).

There are many different extraction techniques from natural plants. They are acid hydrolysis, alkaline hydrolysis and enzymatic hydrolysis. Acid hydrolysis can be used for the determination of bound phenolic acids, hydrolysable tannins or non-extractable proanthocyanidins (NEPA) remaining in the residue. White and coworkers (2010) described alkaline hydrolysis to be the best extraction option in order to release bound procyanidins from cranberry pomace matrix. Using this method there was a significant release of cell wall bound or insoluble ferulic acids. The treatment time, temperature, and the concentration of the base are parameters that were tested. They also found optimized conditions of 2 M NaOH, 60°C and 15 min, in order to release the highest amount of bound procyanidins from cranberry pomace (White *et al*., 2010). Enzymatic hydrolysis can also be performed by carbohydrases such as cellulases, amylases, hemicellulose or proteases. These are helpful in the release of cell wall bound phenolic acids or NEPA, because they break down the plant. Enzymes are specific, water-soluble, not toxic, biodegradable, but also expensive and rather unstable. Factors that can be optimized in order to increase the hydrolysis efficiency are the hydrolysis extraction time and the dosage of enzyme (Navarro *et al*., 2011). In order to optimize the extraction conditions, response surface

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methodology (RSM) has been widely used. The most common designs, such as Central Composite Design (CCD) and Box-Behnken design (BBD).

In present study, antioxidant capacity was considered as response value while extraction times, concentration of NaOH and extraction temperature were considered for optimization parameters. The experiments were done in triplicate. The results are given as mean standard deviation (SD). One-way Analysis of Variance (ANOVA) was used for comparison of more than two means. A difference was considered statistically significant when $p < 0.05$ (Barberousse *et al*., 2009).

Antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidants terminate these chain reactions by removing free radical intermediate and reducing other oxidative reactions (Ames *et al*., 1993 and Shenoy & Shirwaiker, 2002). Antioxidants are often reducing agents such as, thiols, ascorbic acid or polyphenols (Sies, 1997). The main function of antioxidants is to protect the body against the destructive effects of free radicals damage (Marques *et al*., 2014). Antioxidant activity of ferulic acid from wheat bran was measured by using the DPPH (2, 2-diphenyl-1-picryl-hydrazyl) radical photometric assay in a process guided by its discoloration (Xiong *et al*., 1996). The antioxidant activity of these extracts was compared with standard Ascorbic acid (Ascorbic acid Equivalent Antioxidant Capacity (AEAC). The main purpose of this research is to develop useful chemicals made from agricultural by-product.

Materials and Methods

Sample Collection

Raw material (Wheat Bran) was collected from Yaykyi Village, Patheingyi Township, Mandalay Region. The chemicals used for this research were n-hexane, sodium hydroxide, hydrochloric acid and 95% ethanol. These were purchased from Able and Golden Lady Chemical shops, Chan Aye Thar Zan Township, Mandalay Region.

Defatting of Wheat Bran

Wheat bran was initially cleaned and dried at room temperature for 24 hr. It was sieved on an 80 mesh screen for the removal of husk, grits and other impurities. Wheat bran was degreased with n-hexane in 1:3 ratio (w/v) at 50˚C for 3 hr on a water-bath shaker. The mixture was filtrated to remove n-hexane from wheat bran. Wet wheat bran was dried at about 105 ˚C for 2 hr in an oven. After drying, the wheat bran was sieved again on a 100 mesh screen. Finally, defatted wheat bran was obtained.

Extraction and Purification of Ferulic Acid

Extraction process was carried out according to the method described by Buranov and Mazza (2009). To optimize the extraction process, different extraction time, concentration of NaOH and extraction temperature were tested. 5 g of defatted wheat bran and 150 mL of sodium hydroxide were added in Erlenmeyer flask. The flask was kept on a water-bath shaker and boiled at 60 °C and 1.5 M NaOH concentration with different extraction time (2, 4 and 6 hr) at 200 rpm. The extraction was repeated as described above but at varying extraction temperature, namely

(40, 50 and 60˚C) and at different NaOH concentration (0.5, 1.0 and 1.5 M) for ensuring total hydrolysis. After cooling down, the hydrolysate was filtered and then neutralized by using 6 M hydrochloric acid. The hemicelluloses and glucomannans were precipitated by adding 95 % ethanol. The amount of added ethanol corresponded to three times of the original volume of wheat bran. The precipitate was separated by using a centrifuge machine. After decanting the supernatant extract, excess ethanol was removed from the extract by using a rotary vacuum evaporator. This led to the formation of a brown extract which contained ferulic acid. The extract was finally passed through a 0.45 µm filter before analysis. The yield percent of extracted ferulic acid was 6.95 ± 0.28 mg/g.

The extraction procedure of the experiment arranged by 'Box-Behnken design' was set up as follows; Three factors including sodium hydroxide concentration (x_1), extraction time (x_2) and extraction temperature (x_3) were chosen. The antioxidant activity of ferulic acid (y) was determined using optimization method Table 1. Each experiment was carried out in triplicate.

Experimental Design

A Box-Behnken experimental design was used to investigate the effects of three independent variables, namely NaOH concentration (x_1), extraction time (x_2) and extraction temperature (x_3). Three levels of each variable were coded as -1, 0 and 1 based on the results of preliminary single factor experiments according to the following equation.

$$x = (x_1 - x_0)/\Delta x$$

When x is the code value, x_1 is the corresponding actual value, x_0 is the actual value in the center of the domain and Δx is the increment of x_1 corresponding to a variable of 1 unit of x. The experimental design consists of 12 factorial experiments and three replicates of the central point that were given in Table 2. Ascorbic acid equivalent antioxidant capacity (AEAC) was selected as the responses for the combination of the independent variables using DPPH radical scavenging method. Experiment runs were randomized, to minimize the effects of unexpected variability in the observed responses.

Table 1: Coded and Actual Levels of Three Variables

Variables	Factors	Coded Levels of Variables		
		-1	0	1
Concentration of NaOH (M)	x_1	0.5	1	1.5
Extraction Time (hr)	x_2	2	4	6
Extraction Temperature (˚C)	x_3	40	50	60

Determination of Antioxidant Activity Using DPPH Radical Scavenging Method

The antioxidant activities of the samples were determined by the DPPH free radical scavenging assay according to (Lee *et al.*, 2004). The samples were diluted with 50% ethanol for various concentrations. Briefly, the reaction mixture containing 50 µL of diluted test sample of

various concentrations and 50 µL of DPPH (300 µmol) dissolved in methanol, was taken in a 96-well micro-titer plate and kept standing at 37 ˚C for 30 min. The absorbance was measured at 517 nm by using 96 well micro plate readers (Spectrostar Nano, BMG Labtech Microplate reader). Ascorbic acid was used as a standard. 50% ethanol was used as the control and added to the 96-well plate instead of the sample. Percent Radical Scavenging Activity (% RSA) was calculated by using the following formula:

$$\% \text{ RSA} = [(A_c - A_s) / A_c] \times 100$$

where,

% RSA= % Radical Scavenging Activity
A_c = Absorbance of Control
A_s = Absorbance of Sample

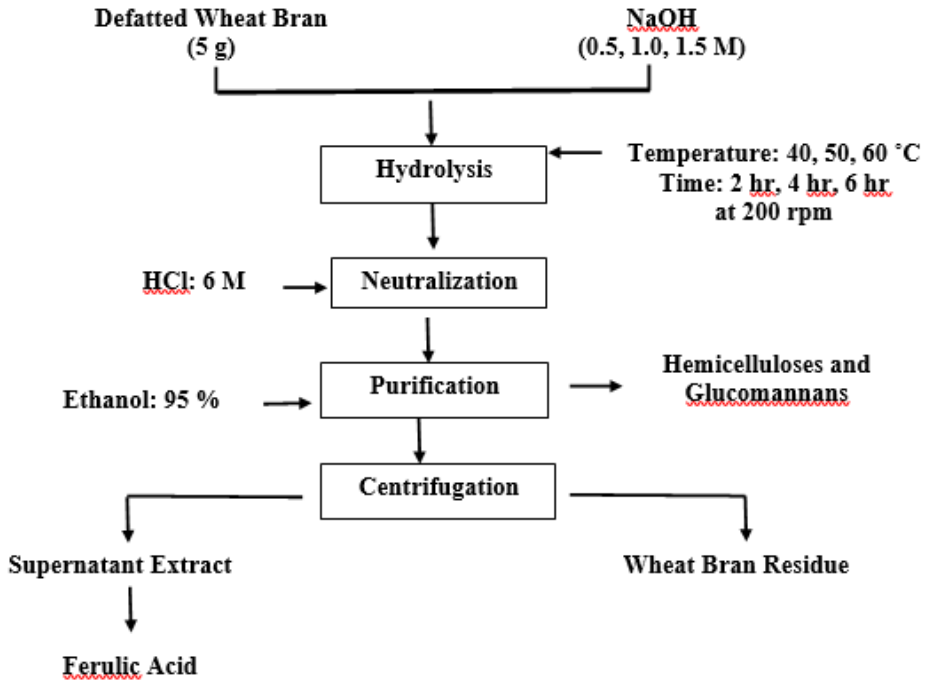

Figure 1: Process Flow Diagram for the Extraction of Ferulic Acid from Defatted Wheat Bran

Preparation of Whitening Scrub

Coffee powder (2 g), baking soda (1 g) and (2 mL) of coconut oil were placed in a cleaned, dried and weighed steel bowl. And then it was stirred at room temperature for about 15 min. When the ingredients had completely melted, (0.1 mL) of prepared extracted ferulic acid and (0.1 mL) of olive oil were added into the above mixture with constant stirring at room temperature for 15 min. The resulting mixture was poured into sterilized airtight container, labeled and stored in a cool and dry place. It was kept for up to 6 months.

Evaluation of Whitening Effect

In evaluation, seven women who were about fifty years old were tested with samples. Before testing with the samples, the face skin of tested women was initially photographed. And then, the face area was divided into two parts: the left part and the right part. The left part was no treatment (control) position. The right part was utilized with samples. After one week, face area

with samples of tested women was checked. The result was clear in these faces. And then two weeks later, their face became tight and very clear skin. In the last step, the right parts of seven women were totally white and got the sort of skin in three weeks.

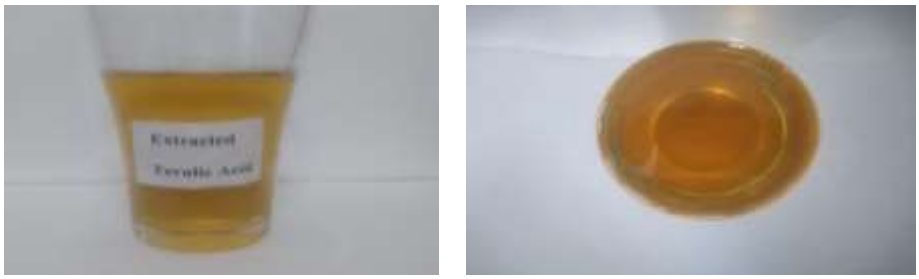

Figure 2: Extracted Ferulic Acid

Results and Discussion

In this research work, the extraction of ferulic acid from wheat bran was done by using sodium hydroxide. Hemicellulose was removed by using ethanol as a solvent. The waxy materials from the biomass were removed by using hexane prior to the NaOH extraction which enhances the quality of the extracted ferulic acid. Conventional solvent extraction in this study has produced extracts with high antioxidant activities.

The extraction conditions for improving antioxidant activity in ferulic acid were optimized through the RSM approach (Box-Behnken design). The coded and actual levels of the three variables as shown in Table 1 were chosen to optimize the antioxidant activities. Each experiment was carried out in triplicate. From the results of a single factor, the maximum antioxidant activity of each factor was defined as the center of domain (x_0). The actual level of -1, 0 and 1 were calculated as described in experimental part. Treatments with coded levels of variables and experimental results of antioxidant activity of ferulic acid from wheat bran are also represented in Table 2. The treatments with coded levels ranged from 37.39 ± 8.87 mg to 76.64 ± 3.88 mg AEAC. The highest AEAC value 76.64 ± 3.88 mg AEAC was obtained under experiment condition of 0.5 M of NaOH concentration and 2 hr extraction time and 50 ˚C extraction temperature. The process flow diagram for the extraction of ferulic acid from defatted wheat bran was indicated in Figure 1. The extracted ferulic acid was shown in Figure 2.

By applying multiple regression analysis on the experimental data, the response variable (antioxidant activity) and the test variables are related by the following polynomial equation (in terms of coded factors).

$$\% \text{ RSA} = 72.89 - 3.50875 X_1 - 5.1975 X_2 + 2.70875 X_3 - 0.665 X_1^2 X_2 + 0.225 X_2^2 X_3$$
$$+ 10.9425 X_1^2 X_3 - 0.40625 X_2^2 X_2 - 9.09875 X_1^2 X_1 - 11.5588 X_3^2 X_3$$

The analysis of variance (ANOVA) for the regression equation by Minitab 14 Software is presented in Table 3. The quality of fit to the second-order polynomial models was confirmed based on the coefficient of determination, $R^2 = 0.917$. The result indicated that models can significantly ($p < 0.05$) express more than 80 % (> 0.80) of the response variation. The lack of fit ($p < 0.05$) was significant suggesting that the model was suitable to represent the actual situation, reflecting the relationship between the antioxidant activity and extraction parameters. In addition, the obtained regression equation can predict well the extraction condition for high antioxidant

activity. The terms of square (p = 0.020), interaction (p=0.048) and regression model (p = 0.030) indicating that the relationship between response and the test variable were significant as shown in Table 3.

The DPPH radical has been extensively applied to assess the antioxidant potential of food items, such as vegetables, olive oils, fruits, juices and wines etc. Stable organic radical DPPH has been utilized in determination of the antioxidant activity of ferulic acid as well as purification compounds. The ability of antioxidants for DPPH radical scavenging is supposed to be free radicals due to their hydrogen donating property. After acceptance of an electron or a hydrogen atom, a stable diamagnetic molecule will emerge which will result in vanishing the absorption band at 517 nm. The radical scavenging activity of the sample corresponds to the remaining DPPH in an inverse manner. The antioxidant potential of wheat bran extracts to scavenge free radical varied from (37.39 ± 8.87 %) to (76.64 ± 3.88 %). Highest antioxidant potential of ferulic acid in present study (76.64 ± 3.88 %) was found in agreement with standard ferulic acid (78 %) (Alanon *et al*., 2011), however, it was lower than that reported for ascorbic acid (91.42 %). The DPPH assay for antioxidant activity of extracted ferulic acid is shown in Figure 3.

Three-dimensional response surface plots are presented in Figure 4-A to 4-C. These types of plots reflected the effects of two factors on the response at a constant agitation (200 rpm). As shown in Figure 4-A, the highest antioxidant activity of ferulic acid was observed at 1.0 M of NaOH concentration and 2 hr of extraction time, further increase of NaOH concentration with time significantly decreases the activity. The results as shown in Figure 4-B, the antioxidant activity of ferulic acid increases with increase in temperature. However, at 4 hr extraction time the activity decrease with increase in temperature. After 4 hr, the activity was significantly increase using highest extraction temperature. In Figure 4-C, the optimum condition was observed at mid-level of NaOH concentration and extraction temperature. By applying increased concentration with high temperature, the antioxidant activity will also decrease. The effects of extraction temperature had a more significant effect on the antioxidant activity than NaOH concentration and extraction time.

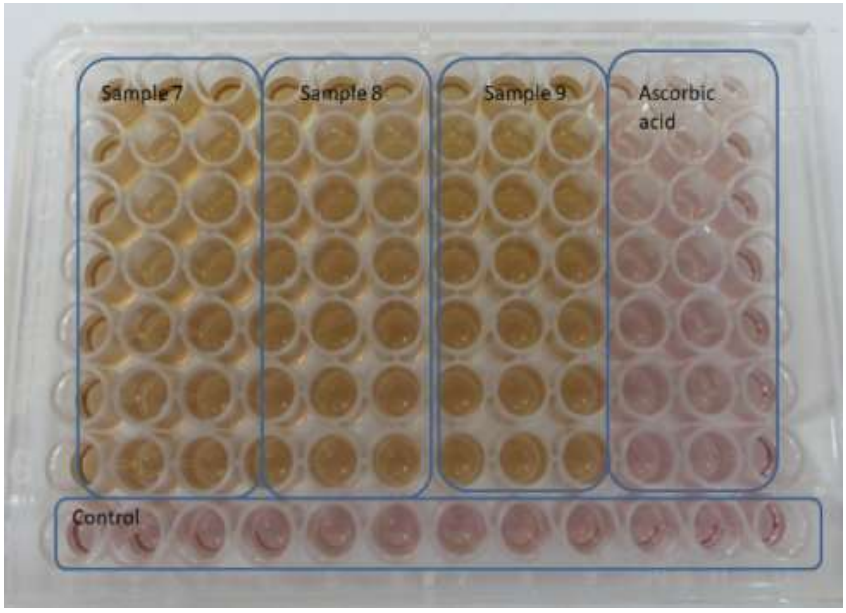

Figure 3: DPPH Assay for Antioxidant Activity of Extracted Ferulic Acid

Table 2: Experimental Designs Using Box-Behnken Design and Results

Run Order	Natural Variables			% RSA
	Concentration of NaOH (M)	Extraction Time (hr)	Extraction Temperature (˚C)	
1.	0.5	4	60	45.19±6.13
2.	1	2	60	65.708±3.59
3.	1	6	60	63.61±4.24
4.	0.5	4	40	63.71±10.97
5.	1	4	50	72.89±7.64
6.	1	2	40	58.69±5.47
7.	1	4	50	72.89±7.64
8.	1.5	4	40	37.39±8.87
9.	0.5	6	50	59.73±5.7
10.	0.5	2	50	76.64±3.88
11.	1.5	4	60	62.64±6.33
12.	1.5	2	50	68.37±3.90
13.	1.5	6	50	48.8±5.03
14.	1	6	40	55.59±5.72
15.	1	4	50	72.89±7.64

Table 3: Analysis of Variance (ANOVA) for the Regression Equation

SD	SS	DF	MS	F Value	P Value	S
Model	1602.14	9	178.015	6.14	0.030	*
Linear	373.30	3	124.434	4.30	0.075	-
Square	747.91	3	249.304	8.61	0.020	*
Interaction	480.92	3	160.308	5.53	0.048	*
Residual Error	144.85	5	28.97	-	-	-
Lack-of-Fit	144.85	3	48.28	-	-	-
Pure Error	0.00	2	0.000	-	-	-
Total	1746.99	14	-	-	-	-

Note: SD= source of deviation, SS= sum of square, DF= degree of freedom, MS= mean square, S= significant, p* = 0.05

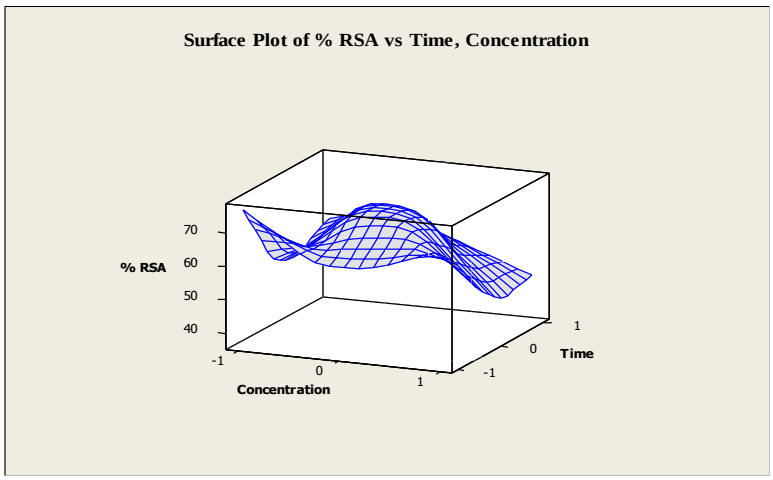

Figure 4-A: Three-dimensional Response Surface Plot of Antioxidant Activity of Ferulic Acid Showing the Influence of NaOH Concentration (x_1) vs. Extraction Time (x_2)

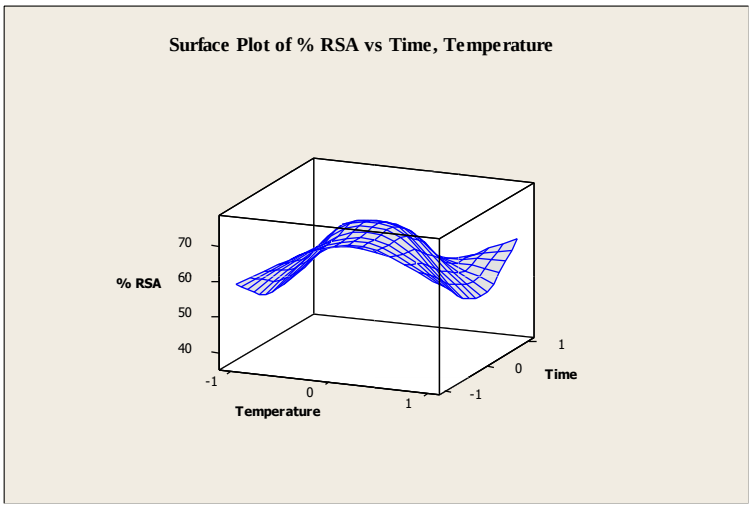

Figure 4-B: Three-dimensional Response Surface Plot of Antioxidant Activity of Ferulic Acid Showing the Influence of Extraction Time (x_2) vs. Extraction Temperature (x_3)

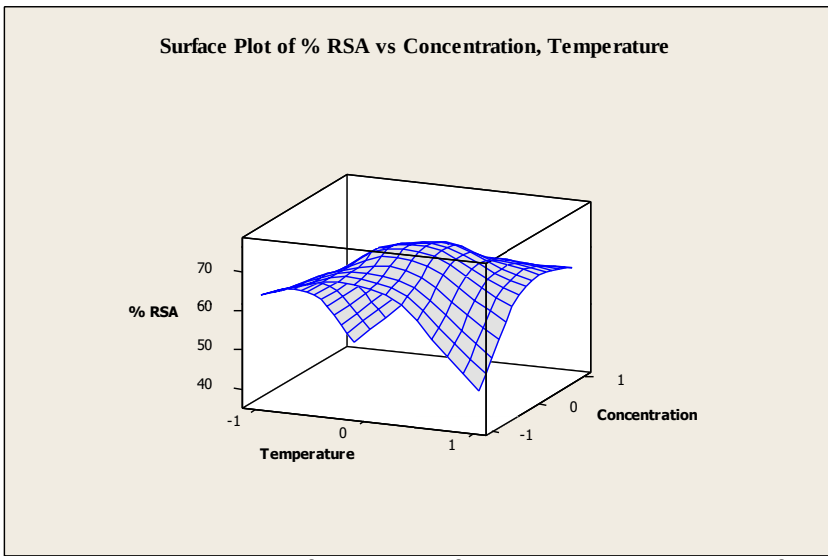

Figure 4-C: Three-dimensional Response Surface Plot of Antioxidant Activity of Ferulic Acid Showing the Influence of NaOH Concentration (x_1) vs. Extraction Temperature (x_3)

Conclusion

The response surface methodology (RSM) was used to optimize the extraction condition for antioxidant activity in ferulic acid from defatted wheat bran using Box-Benken design. According to the ANOVA analysis, the optimal condition was 0.5 M of sodium hydroxide concentration, 2 hr of extraction time and 50 ˚C for the extraction temperature. Moreover, p-value is less than the significant level. Therefore, it can be concluded that there is a statistically significant association between response variables and the terms. Conventional solvent extraction in this study has produced the extract having high antioxidant activities. Despite being agricultural by-product produced in the food supply chain, the enormous availability of ferulic acid could be beneficial for the production of value added products in line with green technology.

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