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Research paper



Optimization of Solid State Fermentation Condition to Increase Total Phenolic Content and Antioxidant Activity in Seaweed (Kappaphycus Alvarezii) Extract

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Abstract

Conventional techniques such as solvent extraction can be effectively used to extract free phenolic compounds in plants. However, this method is inefficient to extract bound phenolics. Solid state fermentation (SSF) approach with *Aspergillus oryzae* was used to enhance bioavailability of polyphenols in *Kappaphycus alvarezii*. A set of experiment was computed by face centered central composite design (FCCCD) to optimize the fermentation parameters based on maximum phenolic content and antioxidant activity. Four independent variables namely: time (0, 4 and 8 days), temperature (28, 30 and 32 °C), initial moisture content (60, 70 and 80 %) and inoculum level (10, 20 and 30 % (v/v)) were investigated. The experimental results for both TPC and DPPH were 9.449 ± 0.198 mg GAE/g and 87.135 ± 0.857 % of scavenging activity, respectively; where both responses were in good agreement with RSM model prediction. The RSM design used has been proven to successfully predict the total phenolic content and antioxidant activity. Fermentation condition with 70% initial moisture content, 10% (v/v) inoculum level, performed at 30°C for 4 days was found to produce maximum TPC and DPPH radical scavenging activity of *Kappaphycus alvarezii*.

Keywords: *Aspergillus oryzae, DPPH radical scavenging activity, Kappaphycus alvarezii, response surface methodology, total phenolic content*

1. Introduction

Phenolic compounds are biologically active secondary metabolites produced in plants by shikimic acid pathway. It can act as antioxidants, anticarcinogenic, anti –inflammatory properties which are mostly related to the health promoting benefits against human health risks such as hypertension, obesity, cardiovascular diseases, diabetes and cancer. Most of phenolic compounds in plants occur in bound form as conjugate with sugars, fatty acids or proteins. In the past recent years, solid state fermentation with GRAS filamentous fungi, yeasts and lactic acid bacteria has gained interest for the enhancement of phenolics content and antioxidant activity.

Macroalgae or seaweed represents one of the richest sources of natural antioxidants and antimicrobials among the marine organisms. Seaweed also reported to be low in calorie content, vitamins, trace elements and a wide variety of secondary metabolites [1]. *Kappaphycus alvarezii* is a widely cultivated red algae in the world and it is highly demanded as the main source of raw material for carrageenan industry.

Fermentation is an ancient method of food processing aimed at extending shelf-life, improving palatibilty and product properties. It may also improve digestibility and enhance nutritional value of food and feed. Total extractable phenolics, anthocyanins and antioxidative activity was increased when legumes was fermented with GRAS filamentious fungi [2]. In addition, total phenolic content of sea tangle extract was enhanced by fermentation [3].

In order to maximize the phenolic compounds via fermentation apprroach, an appropriate experimental design is essential. This will evaluate effects of the major parameters involved in the fermentation process and their probable interactions, through the minimum number of experiments. Generally, optimization process could be achieved by either empirical or statistical methods. In classical optimization method, only one factor is variable at a time and other parameters are kept constant. The drawback of classical method including inability to determine interactions between the variables, time consuming, expensive, tedious and ineffective [4].

On contrary, response surface methodology (RSM) which can conduct several experiments silmutaneously is able to evaluate several factors at one-time. RSM is an empirical modeling approach for defining the relationship between various process parameters and responses with the various desired criteria.

One of the advantages is it requires fewer experimental run as compared to conventional optimization method while providing statistical results [5]. The use of RSM optimization in food processing was reported in production of seaweed paste [6], extraction [7] and fermentation [8].

This study was conducted based on RSM approach to optimize the solid state fermentation parameters, namely: initial moisture content, fermentation time, temperature and inoculum level for enhancement of total phenolic content and antioxidant activity in *Kappaphycus alvarezii* by using *Aspergillus oryzae*.

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2. Materials and method

2.1 Sample preparation

Seaweed *K. alvarezii* used in this study was purchased from Tawau, Sabah, Malaysia. Sample preparation was adapted from [9] with some modifications. Samples were rinsed with tap water, soaked in water with ratio 1:2 (g : ml) for 1 h and were then cut into smaller size. It was dried at room temperatures for five days and then ground by using Waring blender. Samples were kept in air-tight bottles until needed for future analysis.

2.2 Inoculum preparation

Aspergillus oryzae was obtained from MARDI culture collection, Serdang, Selangor. The strain was cultured on PDA plates for 4 days at 30°C. A hockey stick was used to collect the spores by evenly poured 100 ml of sterile distilled water on 4 PDA plates containing 4 days old culture. Then the suspended fungal cultures were filtered using Whatman filter paper No. 1 and the filtrate was used as inoculum for solid state fermentation. The inoculum was stored at 4°C and used within one month of storage.

2.3 Experimental design

Four independent variables or parameters, namely: temperature (28-32°C), time (0-8 days), initial moisture content (60-80%) and inoculum level (10-30%) were examined based on RSM design. It was aimed to determine the effects of each factor on total phenolic contents and antioxidant activity responses. The total of 30-run was computed using face centered central composite design (FCCCD) with six center points of replicates and the alpha value is equal to 1.0 [10]. The ranges and the designed levels of process variables are given in Table 1.

Table 1 : Uncoded value of parameters

Factors	Low level, (-1)	High level, (+1)
Time, days	0	8
Temperature, ^{0}C	28	32
Initial moisture content, %	60	80
Inoculum level, % (v/v)	10	30

The quadratic model equation for predicting the optimal point is expressed by Eq. (1) as followed:

$$Y = \beta 0 + \Sigma \beta iXi + \Sigma \beta iiXi^2 + \Sigma \beta ijXiXj + \mathcal{E} \quad (1)$$

where Y is the predicted response, β0 is constant coefficient, βi, βii and βij are coefficients for the linear, quadratic and interaction effects, Xi and Xj are factors (independent variables) while Ɛ is the error.

The Design Expert software version 6.0.4 was used for regression and graphical analyses of the attained data. Generally, a full second order model with linear, quadratic and interaction term was fitted in order to conclude the optimum combinations of factors level [11]. Meanwhile, analysis of variance (ANOVA) was used to statistically analyse the main and interaction effects of model term. The quality of fit for the second order polynomial model equation was expressed via the determination coefficient (R^2) and the adjusted R^2. The fitted polynomial equation was then expressed in the form of three dimensional surface plots as to illustrate the relationship between the responses and the experimental levels of each variable investigated in the present study.

2.4 Solid state fermentation

Static fermentation was conducted in 250 ml Erlenmeyer flasks containing 10g of working volume. The total of 30-run were conducted based on FCCCD and different process parameters (time, days; temperature,°C; initial moisture content, % and inoculum level, % (v/v)) were applied accordingly. At the end of fermentation, 100 ml of distilled water was added to the flasks containing the biomass and the whole content was agitated thoroughly on a rotary shaker for one hour at 180 rpm. The whole content was then centrifuged at 8000 rpm for 10 minutes. The supernatant obtained was further filtered by using Whatman filter paper no 1. The filtrate obtained was used as the crude fermented seaweed extract.

2.5 Total phenolic content determination

Total phenolic content of fermented seaweed extracts were determined using Folin-Ciocalteu (FC) reagent following Ganesan et al., [12] with minor modifications. Briefly, 100 µl of sample was mixed with 2 ml of 2% Na_2CO_3 and the mixture was left at room temperature for two minutes. Then, 100 µl of 50% Folin-Ciocalteu's reagent was added. The reaction mixture was mixed thoroughly and incubated at room temperature for 30 minutes in the darkness. Subsequently, absorbance of the sample was measured at 720 nm using spectrophotometer. The amount of total phenolic content was calculated as Gallic acid equivalent from the standard calibration curve of gallic acid and expressed as mg gallic acid equivalent per gram (mg GAE/g) of dried sample.

2.6 DPPH radical scavenging assay

The radical scavenging capacity of fermented seaweed extracts was measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) according to Bhuiyan et al., [13] with slight modifications. DPPH assay was chosen because it provides an easy, efficient and rapid way to evaluate antioxidants by spectrophotometry based on electron transfer. Initially, 1 mL of 0.1 mM methanolic solution of DPPH was added to 1 mL of sample. The mixture was shaken well and incubated at room temperature in the darkness for 30 minutes. The absorbance of sample was measured at 517 nm. Ascorbic acid was used as positive reference and was prepared in distilled water with different concentration ranged from 0 to 0.7 mg/ml. At the same time, a control was prepared by mixing 1 mL of methanol and 1 mL of 0.1 mM DPPH solution. The radical scavenging activity was expressed as radical scavenging activity percentage using the following equation;

$$\% \text{ Scavenging activity} = [(A_{control} - A_{sample})/ A_{control}] \times 100\%$$

where $A_{control}$ = absorbance of control (DPPH solution),
A_{sample} = absorbance of the sample solution

2.7 Validation of RSM prediction points

The optimization predicted points by the RSM software were tested in subsequent confirmation run in order to validate the RSM prediction points. The confirmation run was conducted in triplicate.

3. Results and discussion

3.1 Optimization of Total Phenolic Content (TPC) and DPPH

In the present study, a total of 30-set run which divided into 3 blocks was computed based on face centered central composite design (FCCCD) protocol as shown in Table 2. In general, total phenolic content and % of radical scavenging activity were initially low at 0 day of fermentation and then increased at day 4 before subsequently decreased at day 8 of fermentation. Referring to Table 2, the highest TPC i.e. 9.93 mg GAE/g and % of radical scavenging activity i.e. 88.59 % was obtained when the experimental conditions were set to time, 4 days; temperature, 30°C;

initial moisture content, 70% and inoculum level, 10% (v/v), respectively. The lowest TPC (2.53 mg GAE/g) and % of radical scavenging activity (10.10%) was obtained at 0 days; temperature, 32°C; initial moisture content, 80% and inoculum level, 10% (v/v), respectively.

Table 2: RSM design of experiment, experimental and predicted value

R	A	B	C	D	Response, TPC		Response, DPPH	
					Exp.	Pred.	Exp.	Pred.
1	4	30	70	20	8.13	8.16	82.99	82.88
2	8	28	60	10	4.57	4.77	46.76	47.62
3	8	32	80	10	4.43	4.51	43.45	42.03
4	8	28	80	30	4.17	4.02	33.66	35.90
5	4	30	70	20	7.99	8.16	81.99	82.88
6	0	28	80	10	3.40	3.07	26.73	25.57
7	0	32	80	30	3.35	3.10	23.66	22.61
8	0	32	60	10	3.10	3.20	15.03	12.60
9	0	28	60	30	2.69	2.56	12.55	13.77
10	8	32	60	30	4.67	4.95	51.79	52.76
11	8	32	60	10	5.76	5.83	52.80	55.42
12	0	28	60	10	3.27	2.24	21.79	21.59
13	0	32	60	30	3.22	3.18	17.25	17.60
14	0	32	80	10	2.53	2.82	10.10	13.11
15	4	30	70	20	8.81	8.35	85.60	84.02
16	0	28	80	30	3.45	3.49	29.03	26.84
17	8	28	60	30	4.21	4.07	39.31	36.73
18	8	32	80	30	4.59	4.73	47.83	48.46
19	8	28	80	10	4.33	4.47	42.21	42.29
20	4	30	70	20	8.30	8.35	84.17	84.02
21	4	28	70	20	7.79	8.25	81.05	82.79
22	0	30	70	20	3.37	3.72	22.27	24.73
23	4	30	70	30	8.88	9.19	86.11	86.53
24	4	30	70	10	9.93	9.42	88.59	87.22
25	4	30	80	20	7.27	7.32	80.02	79.87
26	4	30	70	20	8.03	8.51	82.11	84.87
27	4	30	70	20	8.16	8.51	83.82	84.87
28	4	32	70	20	9.25	8.58	87.25	84.57
29	8	30	70	20	5.85	5.30	54.07	50.67
30	4	30	60	20	7.77	7.51	80.83	80.03

Note: A= Time; B =Temperature; C= Initial moisture content and D= inoculum level; R= Run; Exp. = Experimental; Pred. = Predicted.

Initial moisture content between 50 – 75% is the best condition to increase phenolic compounds in coffee residues when fermented with *Penicillium purpurogen* [14]. Previous researchers reported that antioxidant activity was correlated well with total phenolic content, suggesting that polyphenols have a major influence in the antioxidant activity [15; 16]. Several studies reported that there were significant correlation between the total phenolic content and antioxidant activity [17; 18].

The ANOVA for TPC and DPPH responses in this study were tabulated in Tables 3 and 4, respectively. Generally, values of "Prob > F" less than 0.05 indicate model terms are significant. Values greater than 0.10 indicate the model terms are insignificant. Model reduction may improve the model if there are many insignificant model terms (not counting those required to support hierarchy). In present study, both quadratic models of TPC and DPPH responses were highly significant where the value of "Prob > F value" is < 0.0001. In addition, both responses are showing insignificant of "lack-of-fit" where F-values at 4.96 and 8.67, respectively. There is only 10.71 % (for TPC) and 5.07% (for DPPH) chance for the model that a "lack-of-fit" F-value of this large could occur due to noise. In other words, the non-significant lack-of fit was good and implied that all data in these experiment were sufficient as well as good predictability of both model [11].

Based on Tables 3 and 4, results show that time factor is the only significant parameter for both responses where the values of both are <0.0001. Meanwhile, for TPC response, other significant terms may include square effects of time (Prob>F = < 0.0001), square effects of initial moisture content (Prob>F = 0.0019) and square effects of inoculum level (Prob>F = < 0.0142). In addition, the significant terms for DPPH response are square effects of time (Prob>F = < 0.0001); square effects of initial moisture content (Prob>F = 0.0104); and all six interactive effects: time*temp (Prob>F = < 0.0001), time*moisture (Prob>F = 0.0007) and time* inoculum (Prob>F = 0.061), temp*moisture (Prob>F = 0.0462), temp*inoculum (Prob>F = 0.0015) and moisture *inoculum (Prob>F = 0.0222), respectively.

Table 3: Analysis of Variance (ANOVA) for TPC response

Source	Sum of square	DF	Mean Square	F value	Prob>F	
Block	55.48	2	27.74			
Model	103.53	14	7.39	36.79	<0.0001	Significant
A	11.21	1	11.21	55.80	<0.0001	
B	0.50	1	0.50	2.50	0.1381	
C	0.17	1	0.17	0.83	0.3785	
D	0.24	1	0.24	1.20	0.2935	
A^2	40.62	1	40.62	202.12	<0.0001	
B^2	0.020	1	0.020	0.098	0.7589	
C^2	3.03	1	3.03	15.05	0.0019	
D^2	1.61	1	1.61	8.01	0.0142	
AB	0.49	1	0.49	2.42	0.1437	
AC	0.29	1	0.29	1.42	0.2549	
AD	0.22	1	0.22	1.09	0.3165	
BC	0.38	1	0.38	1.89	0.1923	
BD	0.070	1	0.070	0.35	0.5653	
CD	0.48	1	0.48	2.40	0.1451	
Residual	2.61	13	0.20			
Lack of fit	2.46	10	0.25	4.96	0.1071	Not significant
Pure error	0.15	3	0.050			
Cor total	161.62	29				
R^2	0.9754					
Adjusted R^2	0.9489					

Note: A: Time; B: Temperature; C: Initial moisture content; and D: inoculum level

On the other hand, for TPC response, those insignificant factors are main effects: temperature (Prob>F = 0.1381), initial moisture content (Prob>F = 0.3785) and inoculum level (Prob>F = 0.2935); square effects of temperature (Prob>F = 0.7589); and all six interactive effects: time*temp (Prob>F = 0.1437), time*moisture (Prob>F = 0.2549) and time* inoculum (Prob>F = 0.3165), temp*moisture (Prob>F = 0.1923), temp*inoculum (Prob>F = 0.5653) and moisture *inoculum (Prob>F = 0.1451), respectively. Also, those insignificant factors in DPPH response are the main effects: temperature (Prob>F = 0.1718), initial moisture content (Prob>F = 0.8989) and inoculum level (Prob>F = 0.5823); square effects of temperature (Prob>F = 0.4819) and square effects of inoculum level (Prob>F = 0.2433), respectively.

Table 4: Analysis of Variance (ANOVA) for DPPH response

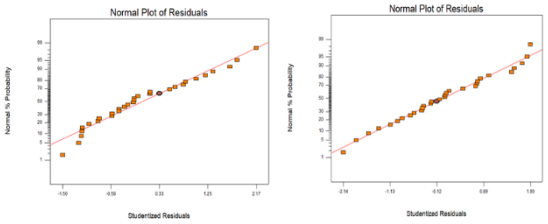
Source	Sum of square	DF	Mean Square	F value	Prob>F	
Block	6909.81	2	3454.90			
Model	15477.28	14	1105.52	161.42	<0.0001	Significant
A	3027.59	1	3027.59	442.06	<0.0001	
B	14.32	1	14.32	2.09	0.1718	
C	0.12	1	0.12	0.017	0.8989	
D	2.18	1	2.18	0.32	0.5823	
A^2	5648.39	1	5648.39	824.73	<0.0001	
B^2	3.59	1	3.59	0.52	0.4819	
C^2	61.35	1	61.35	8.96	0.0104	
D^2	10.23	1	10.23	1.49	0.2433	
AB	210.29	1	210.29	30.70	<0.0001	
AC	134.61	1	134.61	19.65	0.0007	

AD	28.81	1	28.81	4.21	0.0610	
BC	33.24	1	33.24	4.85	0.0462	
BD	110.72	1	110.72	16.17	0.0015	
CD	46.14	1	46.14	6.74	0.0222	
Residual	89.03	13	6.85			
Lack of fit	86.06	10	8.61	8.67	0.0509	Not significant
Pure error	2.98	3	0.99			
Cor total	22476.12	29				
R^2	0.9943					
Adjusted R^2	0.9881					

Note: A: Time; B: Temperature; C: Initial moisture content; and D: inoculum level

3.2 Normality test and predicted versus actual analysis

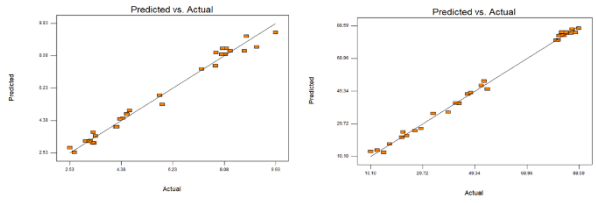
A graphical technique uses to regulate the relevance of assumption on normality is by plotting the data points on a normal probability paper. If a straight line can be constructed through the plotted points, the assumption of normality is reflected to be rational. Figure 1(a) and 1(b) show that the obtained data were fall on the straight line.



(a) (b)

Fig. 1: (a) Normality plot of residuals for TPC response (b) DPPH response

On the other hand, the data in Table 2 shows that the predicted values were in good agreement with the experimental values for TPC and DPPH responses. The results could be further corroborated as illustrated in Figure 2(a) and 2(b) which graphically depicts the correlation between the actual and predicted responses. All obtained data point was closed to the straight line. This shows that no significant violations of the model were found. Moreover, the model should adequate to predict the TPC and DPPH during solid state fermentation within the range in present study as it was highly significant, where the Prob > F value is <0.0001 for both TPC and DPPH responses. In fact, the coefficient of determination, R^2 = 0.9754 for TPC and R^2 = 0.9943 for DPPH, considered sufficient to identify the correlation between the actual and the predicted values.



(a) (b)

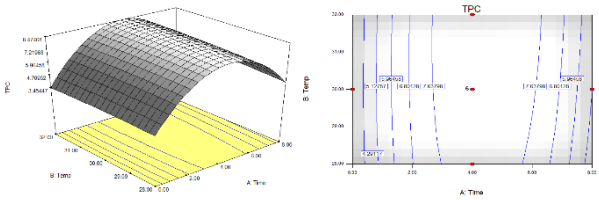
Fig. 2 : Actual and predicted plot for (a) TPC response and (b) DPPH response

3.3 Three-dimensional (3D) response surface plots

Three-dimensional surface plots were illustrated by presenting the response in function of factors and keeping the other constant at its middle level. As shown in Table 3 and Table 4, the main factor: time was statistically significant for both TPC and DPPH responses. Therefore, the interaction effects which involve time*temperature, time*initial moisture content and time*inoculum level should be remained. Further analysis on the interaction effects were carried out.

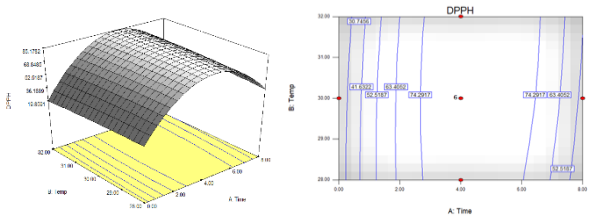
3.3.1 Interaction effects of time versus temperature

Figure 3 and 4 demonstrates the 3D response surface plot and counter plot for the interaction effects of time versus temperature for TPC and DPPH responses respectively. Both of the interaction effects were time and temperature (varying from 0– 8 days and 28 – 32°C, respectively) on TPC, while holding the initial moisture content and inoculum level at center points 70% and 20% (v/v) respectively.



(a) (b)

Fig. 3: (a) 3D response surface plot and (b) counter plot for interaction effects of time versus temperature of TPC response



(a) (b)

Fig. 4: (a) 3D response surface plot and (b) counter plot for interaction effects of time versus temperature of DPPH response

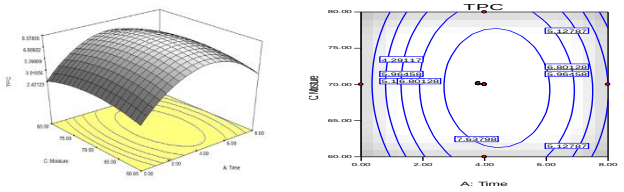
It was noticed that the highest TPC (9.932 mg GAE/g) and DPPH (88.586 % of scavenging activity) could be attained on 4 days fermentation at 30°C. Both TPC and DPPH were increased from 0 to 4 days and subsequently decreased when fermentation was extended to 8 days. This finding suggested that the optimum growth of *A. oryzae* was at day 4. Bae and Kim [3] reported that the highest total phenolic content and antioxidant activity of sea tangle extract fermented with *A. oryzae* was found at day 4 at 30°C. The mycelium growth of fungi was also related to the increasing of enzyme production. *A. oryzae* could able to produce high amount of enzymes at day 4 which will breakdown the cell wall of seaweed to release the bound phenolic compounds present in the seaweed [19].

As a result, more phenolic compound released when the temperature was raised from low to mid-point in present study. However, further increase in number of days led to decrease in TPC and % of radical scavenging activity. Polyphenolic compounds are known to possess antioxidant activity [20]. Thus, there is a strong correlation between TPC and DPPH. Referring to Table 2, the highest TPC obtained was on 4th day when the temperature and inoculum was fixed at 30°C and 10% (v/v) and the % of radical scavenging activity also was higher for the same parameters. Studies show that phenolic play an important role in antioxidant activity, and higher phenolic contents lead to stronger antioxidant activity [21].

3.3.2 Interaction effects of time versus initial moisture content

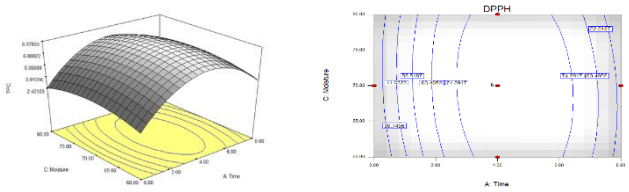
Figure 5 and Figure 6 reveals the interaction between time and initial moisture content on TPC and DPPH respectively. The TPC and DPPH increased from 0 to 4 days. At 0 day low TPC and DPPH were observed, 4.291 mg GAE/g and 30.746 % of radical scavenging activity respectively. When the incubation day extended to day 4, the highest TPC (8.34 mg GAE/g) and DPPH (83.92 %

of scavenging activity) were obtained while the temperature and inoculum level was fixed at 30^{0}C and 20 % (v/v) respectively.
In general, fermentation process will enhance the content of phenolic compound in seaweeds due to the release of bound phenolic compounds through the enzymes produced by the microorganism during fermentation. When the samples were harvested on 0 day, no fermentation was occurred [22]. At the start of fermentation, there is no growth of microbes were observed and this phase is known as lag phase, which is the phase for microbial adaptation with the environment. Besides that, Eom et al., [23] have reported that in comparison of fermented and non-fermented samples, fermented brown algae has produced higher total phenolic content and antioxidant activities. Thus, the production of phenolic compound and antioxidant activity was lower on 0 day and increased to 8.34 mg GAE/g and 83.92 % of scavenging activity on the 4th day of fermentation. The result was supported by a study conducted by Adom et al., [24]. His findings stated that the maximum amount of total phenolic content was higher on 4th day of incubation. Similar trends were observed for DPPH scavenging activity.



(a) (b)

Fig. 5 : (a) 3D response surface plot and (b) contour plot for interaction effects of time versus initial moisture content of TPC response

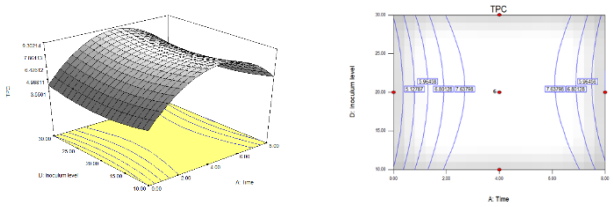


(a) (b)

Fig. 6 : (a) 3D response surface plot and (b) contour plot for interaction effects of time versus initial moisture content of DPPH response

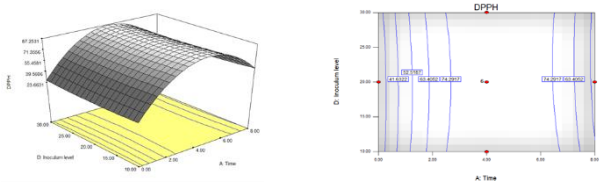
3.3.3 Interaction effects of time versus inoculum level

Figure 7 and 8 demonstrates the 3D response surface plot for the interaction effects of time versus inoculum level. Both of the interaction effects were time and inoculum level (varying from 0– 8 days and 10 – 30 %, respectively) on TPC and DPPH, while holding the temperature and initial moisture content at center points 30°C and 70% respectively. At 0 day, TPC 5.128 mg GAE/g and then increased up to 8.34 mg GAE/g at day 4 and declined to 5.96 mg GAE/g on the 8th day. Few researchers suggested that, low phenolic compounds were observed during the initial stage of the fermentation. This could be explained by the fact that the phenolic compounds exist in the membrane bound form and only low amounts is in the free soluble form [25]. After day 4, the growth of *A. oryzae* may become ceased and enzyme production might also decreased, thus the production of phenolic compound also declined. Usually, enzyme production steadily increases during exponential phase and maximized at the end of this phase or during the stationary phase. After that, the enzyme production slows down because during secondary phase, feedback repression by glucose or interactions with other detrimental components in the medium might occur [26; 27]. This explains why lower TPC and DPPH obtained on 8th day.



(a) (b)

Fig. 7: (a) 3D response surface plot and (b) contour plot for interaction effects of time versus inoculum level of TPC response



(a) (b)

Fig. 8: (a) 3D response surface plot and (b) contour plot interaction effects of time versus inoculum level for DPPH response

3.4 Prediction and verification of optimization conditions

Response optimizer function has provided a set of solutions that are used to predict the optimum conditions of TPC and DPPH from solid state fermentation of seaweed. The criteria of the variables must set accordingly as to maximize both of the responses. The high desirability value, i.e. 0.979 is proposed by RSM software; representing that one would able to attain TPC of 9.251 mg GAE/g and DPPH of 86.278 % radical scavenging activity for at least 97 out of 100 times trial when the experiment conditions are conducted based on criteria as shown in Table 5. Therefore in order to ascertain this, three replicates validation run were performed as to verify the RSM prediction. Table 5 tabulates both of the RSM prediction and experimental values for TPC and DPPH responses are in close agreement to each other with error in the range of 0.99 – 2.10 %, respectively.

Table 5: Optimum conditions suggested by the RSM for TPC response

A	B	C	D	TPC mg GAE/g		Error %
				RSM prediction	Exp	
4	30	70	10	9.251	9.449 ± 0.198	2.10
				DPPH % of scavenging activity		**Error %**
				RSM prediction	Exp	
4	30	70	10	86.278	87.135 ± 0.857	0.99

Note: A: Time; B: Temperature; C: Initial moisture content; and D: inoculum level

In conclusion, the RSM model has successfully modeled the TPC and DPPH radical scavenging activity obtained from solid state fermentation of seaweed in this investigation.

4. Conclusion

The attained data could fit the second order equation well with an R^2 up to 97.54 % and 99.43% for TPC and DPPH, respectively. The RSM optimization of TPC and DPPH responses, 9.449 mg GAE/g and 87.135% of scavenging activity, respectively was achieved when the experiment was performed according to the software optimizing settings. The model suggested the best conditions when time, temperature, initial moisture content and inocu-

lum level were set at 4 days, 30 °C, 70% and 10% (v/v), respectively.

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