SUPPLEMENT ISSUE

IIOAB JOURNAL
ISSN: 0976-3104

ARTICLE

RESPONSE SURFACE METHODOLOGY FOR OPTIMISATION OF PARAMETERS FOR EXTRACTION OF *STEVIA REBAUDIANA* USING WATER, H_20

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ABSTRACT

The sweet diterpene glycosides extracted from the leaves of Stevia rebaudiana (Bert) Bertoni are of increasing interest in recent years as possible sugar substitutes. The objectives of this study were to investigate the application of Response Surface Methodology (RSM) in determination of optimum condition of stevia extraction using water, H_2O. Initially, Stevia leaves variety of China was extracted using water. The extraction process treated with water at different immersion temperatures, immersion duration and particle sizes according to the experimental design which was recommended by RSM of MINITAB software version 16. The MINITAB software Version 16 was used to optimise the stevia extraction using water, H_2O. The determination coefficient R2 was 98.72% meaning that the experimental data were acceptable. It was found that the crude extract of Stevia could be optimised 2.1521 % at the optimum condition at immersion temperature of 92 °C, particle size 0.7 mm and immersion duration of 4.7 minutes. 2.1 % was the verification value obtained from Stevia extraction at the feasible optimum condition. Since there is not much difference between the verification and predicted values, therefore, MINITAB software Version 16 could be used for prediction of the optimum condition for the Stevia extraction using water.

KEY WORDS
Stevia, Water, Extraction, Response Surface Methodology (RSM)

Received: 15 Octr 2016
Accepted: 14 Nov 2016
Published: 1 Dec 2016

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INTRODUCTION

Based on the International Diabetes Federation (IDF) report, it was stated that Malaysia is one of the 21 countries and territories of the IDF Western Pacific region. In the year 2015, there were 3.3 million cases of diabetes were recorded in Malaysia from the total population which was 31.2 million. Although the percentage of cases of diabetes is not very high with 10.54 %, however it should be taken seriously so that these percentage could be decreased [1, 2]. Hence, Stevia is a perfect answer to the needs of consumers, combining the qualities of a sweetener, which also constituting a source of many substances with a nutritional effect on the human organism. New types of food products enriched with Stevia bring many benefits [3]. Stevia has been widely used in Japan, China, Russia, USA and UK for food, beverage and tea preparation. These plant which act as a natural sweetener also well known as sugar leaf, candy leaf and sweet leaf due to its sweet taste that estimately 300 times sweeter than cane sugar [4].

In order to minimize the usage of organic solvents, water was selected as the medium for the extraction which offers a feasible green option of biomarker compounds in botanicals [5-7]. It is the conventional extraction method that mostly used [8]. Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques useful for improving, developing, and optimizing processes. RSM it is possible to observe the interaction effect of the independent parameters on the response [9].

MATERIALS AND METHODS

Stevia from variety of China was obtained from Stevia Sugar Coorporation in Malaysia. Filtered distilled water, dH_2O. Refractometer was purchased from Surechem Sdn Bhd (Sri Petaling, Malaysia).

Method

Optimisation of Stevia extraction involved the combination of RSM with central composite design (CCD). Since there are large numbers of variables controlling the yield of Stevia extraction, a few mathematical models are required to represent the process. Nevertheless, rather than including all the parameters, these models have to be developed using only significant parameters that influencing the extraction process. Therefore, in order to achieve this, the data were subjected to analysis of variance (ANOVA). RSM and second-order CCD for three-variables which were immersion temperature X_1, particle size X_2, and immersion duration X_3. Besides, five level combination coded value -2.000, -1, 0, +1, and +2.000 [Table 1] was applied to determine the effects of the independent variables on the Stevia extraction [10].

Table 1 : Design of experiment for coded and uncoded factors

	-2.000 (-α)	-1	0	1	2.000(α)
X_1	0	25	50	75	100
X_2	0.5	1	1.5	2	2.5
X_3	1	2	3	4	5

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Where: X_1 = Immersion temperature (°C), X_2 = Particle size (mm), X_3 = Immersion duration (min)

Table 2: Experimental design suggested by MINITAB software Version 16

No. Of Sample	X_1	X_2	X_3
1	25	1	2
2	75	1	2
3	25	2	2
4	75	2	2
5	25	1	4
6	75	1	4
7	25	2	4
8	75	2	4
9	8.0	1.5	3
10	92	1.5	3
11	50	0.66	3
12	50	2.34	3
13	50	1.5	1.3
14	50	1.5	4.7
15	50	1.5	3
16	50	1.5	3
17	50	1.5	3
18	50	1.5	3
19	50	1.5	3
20	50	1.5	3

Where: X_1 = Immersion temperature (°C), X_2 = Particle size (mm), X_3 = Immersion duration (min)

The Stevia was extracted by following three parameters that has been chosed which were immersion temperature (°C), Particle size (mm), and Immersion duration (min). An amount of 1 g of dried leaves was extracted with 100 mL distilled water using steeping method. Water bath and thermometer are used to control the temperature of extract. The crude extract was then filtered using Whatman filter paper no. 1. Distilled water act as a control in this experiment.

The 3D plot is a graphical representation of the regression equation, and is often plotted to aid the understanding of the interaction of the independent variables and locate the optimal level of each variable for maximum response. 3D response surface plot was plotted by different combinations to two test variables at one time and maintaining the other variable at zero level [11, 12]. Three variables are considered in the Stevia extraction, the value of one variable was fixed to obtain the contours. The non-variant parameters were therefore set at optimum level and a new relationship was developed and plotted between the independent and dependent variables [11].

RESULTS AND DISCUSSION

Result in [Table 3] shows that the predicted responses and highest actual were 1.56061 % and 1.8 % respectively at factors whereby immersion temperature (°C) was 92 °C, particle size (mm) was 1.5 mm and immersion duration (min) was 3 mins. Meanwhile, the predicted responses and lowest actual were 0.42027 % and 0.5 % respectively at factors in which immersion temperature (°C) at 25 °C, particle size (mm) was 2.0 mm and immersion duration (min) was 2 mins.

[Table 4] shows that the linear factors such as immersion temperature (X_1), particle sizes (X_2) and immersion duration (X_3) indicated a positive coefficients. Square factors such as immersion temperature (X_1X_1), particle sizes (X_2X_2) and immersion duration (X_3X_3) indicated a negative coefficients. Quadratic or interaction factors such immersion temperature and particle sizes (X_1X_2), particle sizes and immersion duration (X_1X_3) showed positive coefficient respectively, meanwhile immersion temperature and immersion duration (X_2X_3) showed negative coefficient. Analysis of response surface regression was performed and results of estimated regression coefficients of second-order polynomial model for optimisation of Stevia extraction using water, H_20 are shown in [Table 4]. By referring [Table 4], the second-order polynomial model equation for Stevia extraction using water, H_20 optimisation was given in equation :

$$Y = 2.68391 + 0.03375\, X_1 + 1.77223\, X_2 + 0.90630\, X_3 - 0.00017\, X_1X_1 - 0.42469\, X_2X_2 - 0.10532\, X_3X_3 + 0.05966\, X_1X_2 + 0.00197\, X_1X_3 - 0.24298\, X_2X_3 \quad (1)$$

Where: X_1 = Immersion temperature (°C), X_2 = Particle size (mm), X_3 = Immersion duration (min).

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Table 3: Comparison of factors between predicted (FITS) and actual (Y) responses

No	Test variables X_1	X_2	X_3	Responses FITS	Y
1	25	1	2	1.08933	1.0
2	75	1	2	1.40352	1.2
3	25	2	2	0.42027	0.5
4	75	2	2	0.91507	0.8
5	25	1	4	1.34642	1.2
6	75	1	4	1.47930	1.3
7	25	2	4	0.65798	0.6
8	75	2	4	0.97217	0.8
9	8.0	1.5	3	0.95089	1.0
10	92	1.5	3	1.56061	1.8
11	50	0.66	3	1.40991	1.7
12	50	2.34	3	0.50088	0.5
13	50	1.5	1.3	0.98299	1.1
14	50	1.5	4.7	1.24720	1.5
15	50	1.5	3	1.51057	1.5
16	50	1.5	3	1.51057	1.5
17	50	1.5	3	1.51057	1.5
18	50	1.5	3	1.51057	1.5
19	50	1.5	3	1.51057	1.5
20	50	1.5	3	1.51057	1.5

Where: X_1 = Immersion temperature (°C), X_2 = Particle size (mm), X_3 = Immersion duration (min)

Table 4: Optimisation of Stevia, China extraction using water, H_20 by estimated regression coefficients of second-order polynomial model

Term	Coefficient	SE Coefficient	t	p
Constant	2.68391	0.915387	2.932	0.000
X_1	0.03375	0.011835	2.852	0.000
X_2	1.77223	0.654201	2.709	0.000
X_3	0.90630	0.327100	2.771	0.020
X_1X_1	-0.00017	0.000067	-2.524	0.030
X_2X_2	-0.42469	0.168251	-2.524	0.030
X_3X_3	-0.10532	0.042063	-2.504	0.020
X_1X_2	0.05966	0.004516	1.321	0.000
X_1X_3	0.00197	0.002258	0.874	0.020
X_2X_3	-0.24298	0.112911	-2.152	0.040
R^2 = 98.72 %	R^2 (adj) = 97.85 %			

The significance of each co-efficient was determined by student's t-test and p-values which are listed in [Table 4]. Determination of the significance for estimated coefficient of the regression model equation [eq. 1] was using the student t. Division of each coefficient by its SE results in the student t test value [13]. The larger the magnitude of the t-value and the smaller the P value, the more significant is the corresponding coefficient [14, 15]. Based on the [Table 4], it shows that the large number of t value, meanwhile the p value for each interaction was less than 0.05. Hence, the present study resulted that linear factors (X_1, X_2, X_3,), square factors ($X1^2$,$X2^2$, $X3^2$) and quadratic or interaction factors (X_1X_2,X_1X_3,X_2X_3) terms were highly significant. It suggests that all linear, square and quadratic or interaction factors gave significant ($p<0.05$) effects on Stevia extraction. The p values were act as a tool to assess the significance and contribution of each factor and the statistical polynomial model equation [16].

The coefficient of determination [R^2] and the significance of lack-of-fit indicates the fitness and adequacy of the model. Definition of R^2 was the ratio of the explained variation to the total variation, used as a calculate of the degree of fit [17]. The coefficient of determination R^2 which was calculated to be 98.72 % of variability in the response could be explained by the model. The closer the R^2 value to unity, the better the empirical model fits the actual data [18]. Meanwhile, the variability in the observed response values cannot be explained by the model only 1.28 %. Probably, 1.28 % of the total variations would be due to other factors which were excluded in the model. The adjusted R^2 represents a rectified value for R^2 after the riddance of unnecessary model terms. Moreover, if there were massive influx of non-significant terms in the model, the adjusted R^2 would be outstandingly smaller than the R^2 [19]. In this study, the adjusted R^2 was close to the R^2 value.

Table 5: ANOVA for optimisation of Stevia extraction using water, H_20

Source	DF	Seq SS	Adj SS	Adj MS	f	p
Regression	9	2.82502	2.82502	0.313892	12.31	0.000
Residual Error	10	0.25498	0.25498	0.025498		
Lack of fit	5	0.25498	0.25498	0.050995	1.59	0.501
Pure Error	5	0.16000	0.16000	0.032000		

ISSN: 0976-3104

Evaluation of f and p values using Fischer's and null-hypothesis tests was to determine the significance of regression. The quality prediction of the entire model considering all design factors at a time was through the f value. The p value defined the probability of the factors having insignificant or very small effect on the response. The RSM model will signifies better fit to the experimental data when the f value was large [20]. High significance of the regression model when the f value with low p value [21]. However, the p value should be lower than 0.05 [$p<0.05$] for the model to be statistically significant [22]. Based on above discussion, the large f and low p values with 12.31 and 0.000, respectively indicates that the regression model found in this study was very significant [Table 5].

The test for Lack of fit was also conducted. It explains the variation in the data around the fitted model [23]. Indication of a good model as there might be contributions in the regresses-response relationship that are not accounted for by the model was via the insignificant lack of fit [22]. By dividing the lack of fit mean square by its pure error mean square will results in the f value for the lack of fit . [Table 5] shows the results of the lack of fit and it was found that the f and p values for the lack of fit were 1.59 and 0.501, respectively. Besides, the absence of any lack of fit ($p>0.05$) also strengthened the reliability of the models. Thus, it exhibits that the model was fitted well to the experimental data [24][28].

[Fig. 1 (a)], [Fig. 1 (b)] and [Fig. 1 (c)] shows the results for target, maximum and minimum goals are shown in respectively at optimum condition that performed by response optimiser. Determination of the feasibility of experiment for target, maximum and minimum goals was from the overlaid contour plot which are shown in [Fig. 2 (a)],[Fig. 2 (b)] and [Fig. 2 (c)], respectively. Results of different goals of actual and predicted responses and the feasibility of experiments at the optimum conditions were obtained from response optimiser of MINITAB software Version 16 are shown in [Table 6].

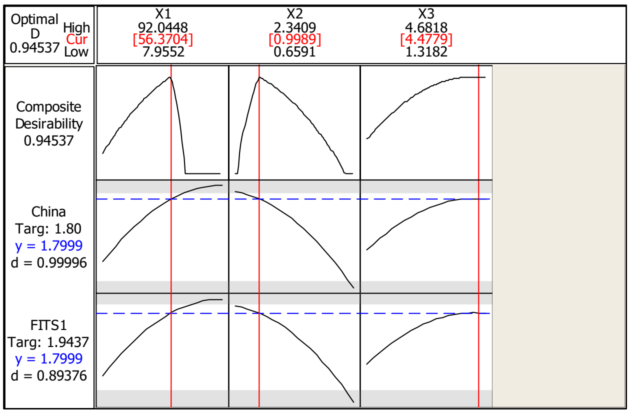

Fig. 1 (a) : Response optimiser for target goal at optimum condition.

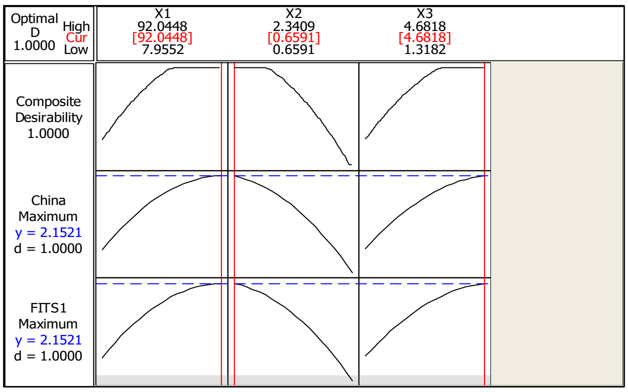

Fig. 1 (b): Response optimiser for target maximum at optimum condition.

IIOAB JOURNAL
ISSN: 0976-3104

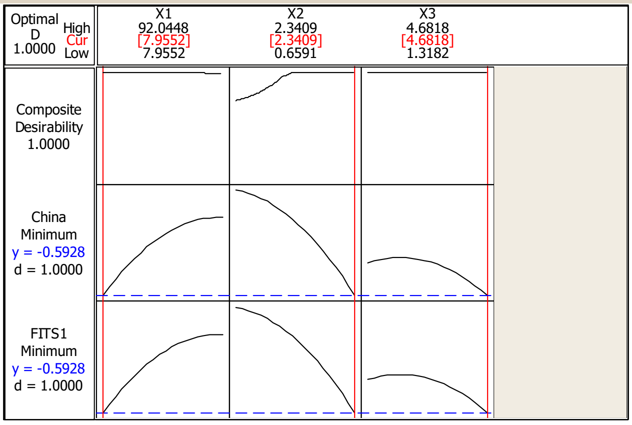

Fig. 1 (c): Response optimiser for minimum goal at optimum condition .

It shows that the target goal for optimum conditions with immersion temperature of 56°C, particle size of 1 mm and immersion duration of 4.5 minutes, and maximum goal with immersion temperature of 92°C, particle size of 0.7 mm and immersion duration of 4.7 minutes were feasible to be carried out. Meanwhile, for minimum goal at optimum condition with immersion temperature of 8 °C, particle size of 2 mm and immersion duration of 4.7 minutes was not feasible to be carried out. According to the overlaid contour plots for target and maximum goals as shown in [Fig. 2 (a)] and [Fig. 2 (b)] respectively, the optimum conditions of target and maximum goals situated at a feasible region which in white. Meanwhile, the overlaid contour plot of minimum goal as shown in [Fig. 2 (c)] situated at a not-feasible region which in grey. However, the optimum condition from maximum goal was selected due to the target and FITS values was much closer.

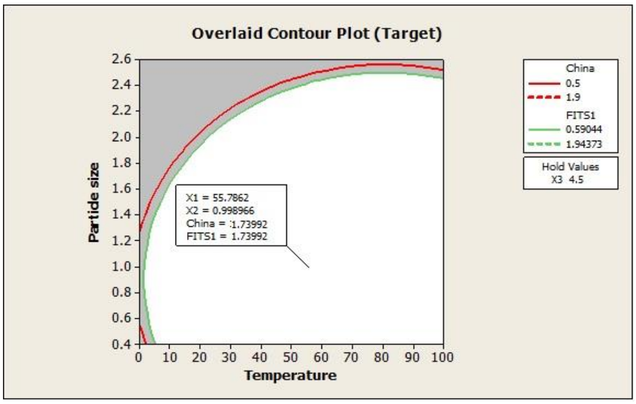

Fig. 2 (a) : Overlaid contour plot for target goal at optimum condition; immersion temperature of 56 °C, particle size of 1.0 mm and immersion duration of 4.5 minutes.

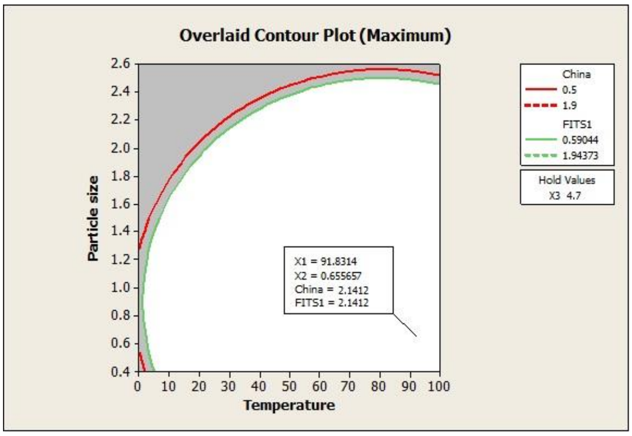

Fig. 2 (b) : Overlaid contour plot for maximum goal at optimum condition; immersion temperature of 92 °C, particle size of 0.7 mm and immersion duration of 4.7 minutes.

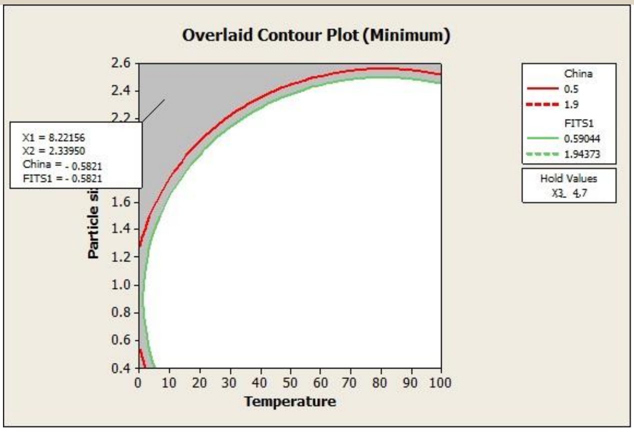

Fig. 2 (c) : Overlaid contour plot for minimum goal at optimum condition; immersion temperature of 8 °C, particle size of 2.0 mm and immersion duration of 4.7 minutes.

Table 6: The values comparison of target and predicted responses at different optimum conditions and feasibilities of experiment

Goal		Lower	Target	Upper
Target	Y	0.5	1.8	1.9
	FITS	0.59044	1.94372	1.94373
Max	Y	0.5	1.9	1.9
	FITS	0.59044	1.94373	1.94373
Min	Y	0.5	0.5	1.9
	FITS	0.59044	0.59044	1.94373

Goal	Optimum Condition			FITS (%)	F/NF
	X1	X2	X3		
Target	56	1	4.5	1.7999	F
Max	92	0.7	4.7	2.1521	F
Min	8	2	4.7	-0.5928	NF

Where: X_1 = Immersion temperature (°C), X_2 = Particle size (mm), X_3 = Immersion duration (min)

The 2D contour plots and 3D surface plots for Stevia, China extraction using water at feasible optimum condition are shown in[Fig. 3] and [Fig. 4], respectively that showed the immersion temperature (°C), particle size (mm) and immersion duration (min). 2D contour and 3D surface plots were defined as the graphical representatives of the regression equation potraying the function of two factors at a time while holding other factors at a fixed level [21][27]. The plots illustrating the values for immersion temperature and particle sizes while holding the value of immersion duration at 4.7 min.

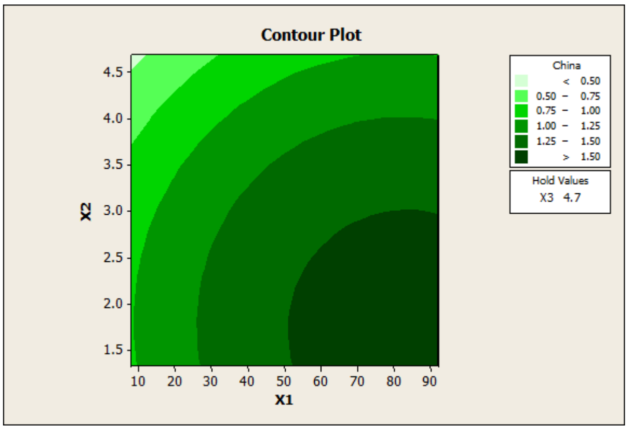

Fig. 3: Contour plot at Stevia, China extraction using water at feasible optimum condition; immersion temperature of 92 °C, particle size of 0.7 mm and immersion duration of 4.7 minutes (holding value: immersion duration 4.7 min).

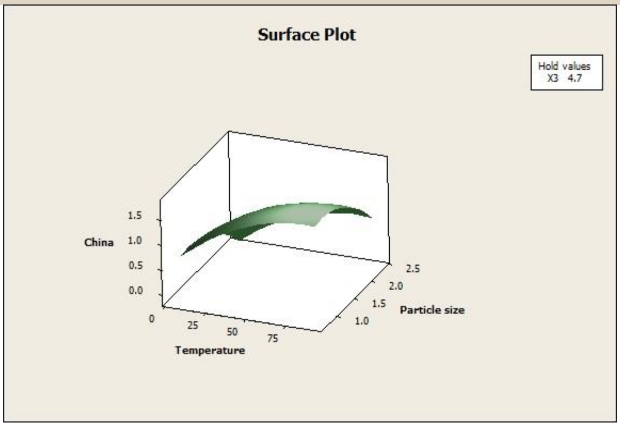

Fig. 4: Surface plot at Stevia, China extraction using water at feasible optimum condition; immersion temperature of 92 °C, and particle size of 0.7 mm and immersion duration of 4.7 minutes (holding value: immersion duration 4.7 min).

Table 7: Comparison of verification and predicted values of Stevia, China extraction using water, H_20 at a feasible optium condition

Optimum condition			V (%)	P (%)
X_1	X_2	X_3		
92	0.7	4.7	2.1	2.1521

Where: X_1 = Immersion temperature (°C), X_2 = Particle size (mm), X_3 = Immersion duration (min)

The interactions between corresponding factors describes by the circular contour plot are negligible, meanwhile elliptical contour plot are significant. Hence, both shapes of contour plot describes whether the reciprocal interactions between the factors are significant or insignificant [14, 25]. Results of the present study showed that the contour plot was elliptical shape. Hence, it indicates significant interaction effect between pH of beef and immersion temperature on beef tenderization [26]. Therefore Figure 3 indicated the significant interaction between immersion temperature and particle size of Stevia, since the contour plot showed the elliptical shape. The surface plot showed that the Stevia extraction escalated at the higher immersion temperature, meanwhile at the higher particle sizes, the extraction of Stevia declined.

Verification of Stevia extraction using water at the feasible optimum condition was performed and the result is shown in [Table 7]. The verification value of Stevia extraction using water at the feasible optimum condition was 2.1 % which was very close to the predicted value with 2.1521 %. Since there is not much difference between the verification and predicted values, hence the feasible optimum condition of the Stevia extraction predicted by MINITAB Software Version 16 was acceptable.

CONCLUSION

As we can see, the experimental results obtained confirming that the RSM could be effectively used to optimize the process parameters in complex process using the statistical design of experiments. The ascertainment coefficient R^2 (98.72 %) was high, thus the experimental data was acceptable. Optimum condition for the extraction of Stevia (China) using water via RSM had been determined. It was found that Stevia extraction could be optimised at the immersion temperature of 92 °C, particle size of 0.7 mm and immersion duration of 4.7 minutes. It was also found that the difference between the verification and predicted values was small , therefore, the optimum condition for the Stevia (China) extraction using water predicted by MINITAB Software Version 16 could be accepted.

CONFLICT OF INTEREST

There is no conflict of interest.

ACKNOWLEDGEMENTS

The author wish to express their gratitude to Faculty of Plantation and Agro technology, UiTM for providing laboratory facilities.

FINANCIAL DISCLOSURE

Financial support from Faculty of Plantation and Agrotechnology for this research.

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