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Optimisation of beef tenderisation treated with bromelain using response surface methodology (RSM)

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ABSTRACT

The purpose of this study is to determine the optimum condition for the tenderization of beef by bromelain using Response Surface Methodology (RSM). Initially, bromelain powder was produced from pineapple crown of variety N36. Production of the bromelain powder involves several process steps such as extraction, purification, desalting and freeze drying. The cube size beef of round part was treated with bromelain at different pHs of beef, immersion temperatures, bromelain solution concentrations, and immersion times according to the experimental design which was recommended by RSM of MINITAB software version 15. Beef tenderness was then measured by Texture Analyser. The MINITAB software Version 15 was used to optimise the tenderisation of beef by bromelain. The determination coefficient R^2 was 99.97% meaning that the experimental data were acceptable. It was found that beef could be optimize tenderised 89.907% at the optimum condition at pH of beef of 5.4, immersion temperature of 60℃, bromelain solution concentration of 0.1682% and immersion time of 10 minutes. The verification value of beef tenderisation at the feasible optimum condition which was determined by experiment was 89.571%. Since the difference between the verification and predicted values was less than 5%, therefore, the optimum condition for the tenderisation of beef predicted by MINITAB software Version 15 could be accepted.

Keywords: Beef; Bromelain; Tenderisation; Response Surface Methodology (RSM)

1. INTRODUCTION

Beef from Brahman breed is known for its toughness [1]. Thus, it is unsatisfactory to the consumer, if the meat is so tough that it is difficult to eat [2]. Therefore, numerous attempts have been made to encounter this problem and tenderising beef using bromelain becomes one of the widely used methods to improve the beef tenderness. This is because bromelain showed hydrolytic activity on the connective tissue leading to the better tenderisation of the tough meat [3].

Bromelain is a proteolytic enzyme found in pineapple plant [4-5] where it is accumulated in the entire part with different extents and properties depending on its source [6]. Production of bromelain powder involves extraction, purification, desalting and drying processes which can be performed using varieties of procedure. In the present study, production of the bromelain powder from pineapple crown of variety N36 was carried out according to the method by [7] with slight modification.

The action of bromelain in tenderising beef is affected by pHs of beef [8-9] immersion temperatures, bromelain solution concentrations and immersion times [10,11]. Research on the optimisation of beef tenderisation treated with bromelain has not been carried. Therefore, this study was conducted to determine the optimum condition for the tenderisation of beef by bromelain using Response Surface Methodology (RSM).

RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors on the response and searching for the optimum conditions [12]. The principal advantage of using RSM is the number of experimental runs required to evaluate multiple factors and their interactions can be reduced [13-16]. Consequently, less time-consuming used to optimise a process compared to other approaches [17].

2. MATERIALS AND METHODS

2.1. Materials

Pineapple crown of variety N36 from maturity index 2 was obtained from Peninsula Plantations Sdn Bhd at

Simpang Renggam, Johor, Malaysia. Beef of round part from three years old bull of Brahman breed was purchased from a butcher at Kota Kemuning, Shah Alam, Selangor, Malaysia. Analytical grade of methanol and acetonitrile and food grade of acetic acid, sodium hydroxide, hydrochloric acid and sodium chloride were purchased from Merck Sdn Bhd (Petaling Jaya, Selangor, Malaysia). All other chemicals of analytical grade including standard bromelain were purchased from Sigma Technologies Sdn Bhd (Petaling Jaya, Selangor, Malaysia). Custom-made cation exchange resin and diafiltrator were purchased from IT Tech Research (M) Sdn Bhd (Subang Jaya, Malaysia) and Isetake Enterprise (Kajang, Selangor, Malaysia), respectively.

2.2. Methods

2.2.1. Production of Bromelain Powder

The pineapple crowns were extracted by crushing their small pieces using fruit juice processor with ratio of pineapple crown to purified water 1:1. The extract was filtered through a muslin cloth. Then, the pineapple crown extract was centrifuged at 360 x g for 10 min at 4℃. The clear supernatant was collected followed by purification process by Preparative High Performance Liquid Chromatography (HPLC) using cation exchange resin column of 21.2 mm internal diameter and 250 mm length. The eluents used were acetate buffer (25 mm, pH 4.0) and 1M sodium chloride (NaCl) solution. Removal of salt in the purified bromelain samples was carried out by continuous diafiltrator with hollow fiber membrane using purified water for exchange. Finally, the desalted bromelain solution sample was converted to powder form using freeze dryer (Christ alpha 1-4LD Plus model).

2.2.2. Optimisation of Beef Tenderisation Treated with Bromelain Using RSM

2.2.2.1. Tenderisation of Beef Using Bromelain

Beef of round part was cut into cube size of approximately 2 cm^3. Then, the beef cube was treated with bromelain at different pHs of beef, immersion temperatures, bromelain solution concentrations, and immersion times according to the experimental design which was recommended by RSM of MINITAB software Version 15 as shown in **Table 2**. Non-immersed or untreated beef cube was served as a control.

2.2.2.2. Texture measurement

Beef tenderness was measured by Texture Analyser TAX-T2*i* (Stable Micro Systems, Ltd., England, UK) using P2N needle probe with a load cell of 10 kg cross head speed equipped with a 0.5 in diameter. The depth of beef was 5 mm.

2.2.2.3. Optimisation using RSM of MINITAB Software Version 15

The range of four selected factors namely pH of beef, immersion temperature, bromelain solution concentration and immersion time is as shown in **Table 1**.

The factors listed in **Table 1** were then applied into MINITAB software Version 15 whereby full factorial Central Composite Design (CCD) is employed to obtain the experimental design as shown in **Table 2**.

Table 1. Coded and uncoded factors for the design of experiment.

	-2.000 (-α)	-1	0	1	2.000 (α)
X_1	5.0	5.2	5.4	5.6	5.8
X_2	55	60	65	70	75
X_3	0.10	0.15	0.20	0.25	0.30
X_4	5	10	15	20	25

Where: X_1 = pH of beef, X_2 = immersion temperature (℃), X_3 = bromelain solution concentration (w/v%), X_4 = immersion time (min).

Table 2. Experimental design recommended by MINITAB software Version 15.

No	X_1	X_2	X_3	X_4
1	5.2	60	0.15	10
2	5.6	60	0.15	10
3	5.2	70	0.15	10
4	5.6	70	0.15	10
5	5.2	60	0.25	10
6	5.6	60	0.25	10
7	5.2	70	0.25	10
8	5.6	70	0.25	10
9	5.2	60	0.15	20
10	5.6	60	0.15	20
11	5.2	70	0.15	20
12	5.6	70	0.15	20
13	5.2	60	0.25	20
14	5.6	60	0.25	20
15	5.2	70	0.25	20
16	5.6	70	0.25	20
17	5.0	65	0.20	15
18	5.8	65	0.20	15
19	5.4	55	0.20	15
20	5.4	75	0.20	15
21	5.4	65	0.10	15
22	5.4	65	0.30	15
23	5.4	65	0.20	5
24	5.4	65	0.20	25
25	5.4	65	0.20	15
26	5.4	65	0.20	15
27	5.4	65	0.20	15
28	5.4	65	0.20	15
29	5.4	65	0.20	15
30	5.4	65	0.20	15
31	5.4	65	0.20	15

Where: X_1 = pH of beef, X_2 = immersion temperature (℃), X_3 = bromelain solution concentration (w/v%), X_4 = immersion time (min).

Response surface regression analysis was performed to obtain a second-order polynomial equation or model. Statistical analysis of the model was represented in the form of Analysis of Variance (ANOVA). The MINITAB software Version 15 was also used to generate response contour and surface plots.

3. RESULTS AND DISCUSSION

Results in **Table 3** show that the highest actual and predicted responses were 89.907% and 89.813%, respectively at factors whereby pH of beef was 5.6, immersion temperature at 60℃, bromelain solution concentration of 0.15% and immersion time of 10 minutes. The lowest actual and predicted responses were 58.267% and 58.608%, respectively at factors whereby pH of beef was 5.4, immersion temperature at 65℃, bromelain solution concentration of 0.20% and immersion time of 15 minutes.

Table 3. Factors and comparison between actual (Y) and predicted (FITS) responses.

No	Test variables				Responses	
	X_1	X_2	X_3	X_4	Y (%)	FITS (%)
1	5.2	60	0.15	10	81.530	81.663
2	5.6	60	0.15	10	89.907	89.813
3	5.2	70	0.15	10	86.442	86.429
4	5.6	70	0.15	10	87.551	87.543
5	5.2	60	0.25	10	85.521	85.314
6	5.6	60	0.25	10	86.931	86.971
7	5.2	70	0.25	10	71.343	71.352
8	5.6	70	0.25	10	66.053	65.974
9	5.2	60	0.15	20	85.458	85.263
10	5.6	60	0.15	20	89.147	89.439
11	5.2	70	0.15	20	85.807	86.068
12	5.6	70	0.15	20	83.273	83.208
13	5.2	60	0.25	20	86.146	86.455
14	5.6	60	0.25	20	84.399	84.139
15	5.2	70	0.25	20	68.713	68.534
16	5.6	70	0.25	20	59.013	59.181
17	5.0	65	0.20	15	64.929	64.810
18	5.8	65	0.20	15	64.205	64.209
19	5.4	55	0.20	15	73.532	73.513
20	5.4	75	0.20	15	63.511	63.417
21	5.4	65	0.10	15	62.901	62.589
22	5.4	65	0.30	15	52.202	52.401
23	5.4	65	0.20	5	67.019	67.237
24	5.4	65	0.20	25	65.972	65.640
25	5.4	65	0.20	15	58.663	58.608
26	5.4	65	0.20	15	58.574	58.608
27	5.4	65	0.20	15	58.309	58.608
28	5.4	65	0.20	15	58.267	58.608
29	5.4	65	0.20	15	58.881	58.608
30	5.4	65	0.20	15	58.894	58.608
31	5.4	65	0.20	15	58.328	58.608

Where: X_1 = pH of beef, X_2 = immersion temperature (℃), X_3 = bromelain solution concentration (w/v%), X_4 = immersion time (min).

Table 4. Estimated regression coefficients of second-order polynomial model for optimisation of beef tenderisation treated with bromelain.

Term	Coefficient	SE Coefficient	t	p
Constant	5013.43	122.781	40.832	0.000
X_1	-1433.30	47.267	-30.324	0.000
X_2	-38.43	0.984	-39.051	0.000
X_3	2206.78	50.616	43.599	0.000
X_4	-1.13	0.473	-2.380	0.030
X_1X_1	147.55	4.353	33.895	0.000
X_2X_2	0.39	0.007	56.609	0.000
X_3X_3	-445.22	69.648	-6.392	0.000
X_4X_4	0.31	0.007	44.973	0.000
X_1X_2	-1.76	0.070	-25.083	0.000
X_1X_3	-162.31	7.012	-23.146	0.000
X_1X_4	-0.99	0.070	-14.168	0.000
X_2X_3	-18.73	0.280	-66.762	0.000
X_2X_4	-0.04	0.003	-14.119	0.000
X_3X_4	-2.46	0.280	-8.764	0.000
R^2 = 99.97 % R^2 (adj) = 99.95 %				

Where: X_1 = pH of beef, X_2 = immersion temperature (℃), X_3 = bromelain solution concentration (w/v%), X_4 = immersion time (min), SE = standard error, t = student test, p = probability, R^2 = R – squared, R^2 (adj) = adjusted R – squared.

Response surface regression analysis was performed and results of estimated regression coefficients of second-order polynomial model for optimisation of beef tenderisation treated with bromelain are as shown in **Table 4**.

Based on **Table 4**, the second-order polynomial model equation for optimisation of beef tenderisation treated with bromelain is as given in equation 2:

$$Y = 5013.43 - 1433.30X_1 - 38.43X_2 + 2206.78X_3 - 1.13X_4 + 147.55X_1^2 + 0.39X_2^2 - 445.22X_3^2 + 0.31X_4^2 - 1.76X_1X_2 - 162.31X_1X_3 - 0.99X_1X_4 - 18.73X_2X_3 - 0.04X_2X_4 - 2.46X_3X_4 \quad (1)$$

Where: X_1 = pH of beef, X_2 = immersion temperature (℃), X_3 = bromelain solution concentration (w/v%), X_4 = immersion time (min)

The significant second-order polynomial model equation at the 5% level for the optimisation of beef tenderisation treated with bromelain is same as in equation (1). By referring to **Table 4**, it was found that linear factors such as pH of beef (X_1), immersion temperature (X_2) and immersion time (X_4) showed negative coefficients, respectively while bromelain solution concentration (X_3)

showed positive coefficient. Square factors such as pH of beef (X_1X_1), immersion temperature (X_2X_2) and immersion time (X_4X_4) showed positive coefficients, respectively while bromelain solution concentration (X_3X_3) showed negative coefficient. Quadratic or interaction factors such as pH of beef and bromelain solution concentration (X_1X_3), pH of beef and immersion time (X_1X_4), immersion temperature and bromelain solution concentration (X_2X_3), immersion temperature and immersion time (X_2X_4) and bromelain solution concentration and immersion time (X_3X_4) showed negative coefficients, respectively.

Student t and p tests were performed and it was found that linear effect factors namely X_1, X_2 and X_4 showed negative t values, respectively while X_3 showed positive t value. p value for X_1, X_2, X_3 and X_4 were 0.000, respectively. Square effect factors namely X_1X_1, X_2X_2 and X_4X_4 showed positive t values, respectively while X_3X_3 showed negative t value. p value for X_1X_1, X_2X_2, X_3X_3 and X_4X_4 were 0.000, respectively. Quadratic or interaction effect factors namely X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4 showed negative t values, respectively and the respective p values were 0.000. It means that all linear, square and quadratic or interaction factors gave significant ($p \leq 0.05$) effect on beef tenderisation.

Student t test was used to determine the significance of the estimated coefficient of the regression model equation (equation 1). The student t test value can be obtained by dividing each coefficient by its SE [18]. p values were used as a tool to evaluate the significance and contribution of each factor and the statistical polynomial model equation [19]. [20,21] reported that the larger the magnitude of the t value and the smaller the p value, the more significant is the corresponding coefficient. Based on those reported by [20,21], results of the present study showed that linear factors (X_1, X_2, X_3, X_4), square factors (X_1^2, X_2^2, X_3^2, X_4^2) and quadratic or interaction factors (X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_3X_4) terms were highly significant.

The goodness of fit of the regression model was determined by determination coefficient R^2 which provides a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions [22]. Results in **Table 4** showed that R^2 value was 99.97% which signified 99.97% of variability in the observed response values could be explained by the model while only 0.03% of variability in the observed response values cannot be explained by the model. The remaining R^2 value of 0.03% of the total variations would be due to other factors which were not included in the model.

The adjusted R^2 was a corrected value for R^2 after the elimination of unnecessary model terms. The adjusted R^2 would be remarkably smaller than the R^2 if there were many non-significant terms have been included in the model [23]. In this study, it was found that the adjusted R^2 was high and very close to the R^2 and the respective values of adjusted R^2 and R^2 were 99.95% and 99.97%. The high adjusted R^2 value was attributed to the absence of non–significant terms in the model. The high adjusted R^2 and R^2 values thus, indicated a high dependence and correlation between the observed and predicted value responses. This is based on [24], who also found the high adjusted R^2 (98.81) and R^2 (97.96) values in their study on the optimisation of enzymatic detection of cadmium in aqueous solution using immobilized urease from vegetable waste.

ANOVA was performed to test for the significance and adequacy of the second-order polynomial model [25]. The results are as summarised in **Table 5**.

The significance of regression was evaluated by f and p values using Fischer's and null-hypothesis tests. The f value predicts the quality of the entire model considering all design factors at a time. The p value is the probability of the factors having very little or insignificant effect on the response. Larger f value signifies better fit of the RSM model to the experimental data [26]. [27] stated that f value with low p value indicates the high significance of the regression model. However, the p value should be lower than 0.05 for the model to be statistically significant [28]. Based on those reported by [26-28], the regression model found in this study was highly significant as denoted by the large f and low p values with 4185.84 and 0.000, respectively. The linear, square and quadratic factors were also highly significant as denoted by the large f values of 1073.06, 10142.46 and 1016.48, respectively and low p values of 0.000, respectively.

Lack of fit test was also performed. It describes the variation in the data around the fitted model [29]. [28] testified that insignificant lack of fit indicates a good model. Insignificant lack of fit is desired as significant lack of fit indicates that there might be contributions in the regresses-response relationship that are not accounted for by the model. The f value for the lack of fit can be

Table 5. ANOVA for optimisation of beef tenderisation treated with bromelain.

Source	DF	Seq SS	Adj SS	Adj MS	f	P
Regression	14	4610.77	4610.77	329.341	4185.84	0.000
Linear	4	938.89	337.71	84.428	1073.06	0.000
Square	4	3192.02	3192.02	798.005	10142.46	0.000
Interaction	6	479.86	479.86	79.976	1016.48	0.000
Residual Error	16	1.26	1.26	0.079		
Lack of fit	10	0.83	0.83	0.083	1.16	0.445
Pure Error	6	0.43	0.43	0.071		
Total	30	4612.03				

Where: DF = degree of freedom, Seq SS = sequential sum of square, Adj SS = adjusted sum of square, Adj MS = adjusted mean square, f = fischer, p = probability.

obtained by dividing the lack of fit mean square by its pure error mean square. Results of the lack of fit are shown in **Table 5** and it was found that the f and p values for the lack of fit were 1.16 and 0.445, respectively. The insignificant p value thus indicates that the model was good and fitted well to the experimental data.

Response optimiser was performed and the results at optimum condition for target, maximum and minimum goals are shown in **Figures 1**, **2** and **3**, respectively.

The feasibility of experiment for target, maximum and minimum goals was determined from the overlaid contour plot and the results are shown in **Figures 4**, **5** and **6**, respectively.

Results of optimum conditions for different goals of actual and predicted responses and the feasibility of experiments obtained from response optimiser of MINITAB software Version 15 are shown in **Table 6**.

Based on **Table 6**, it was found that optimum conditions of target goal with pH of beef of 5.6, immersion temperature of 60℃, bromelain solution concentration of 0.1864% and immersion time of 10 minutes, and maximum goal with pH of beef of 5.6, immersion temperature of 60 ℃, bromelain solution concentration of 0.1682% and

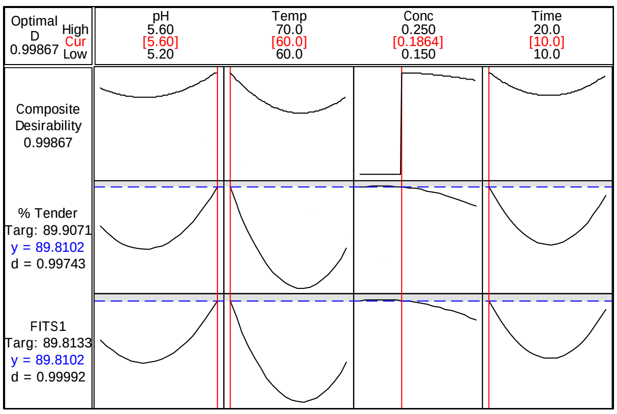

Figure 1. Response optimiser at optimum condition for target goal.

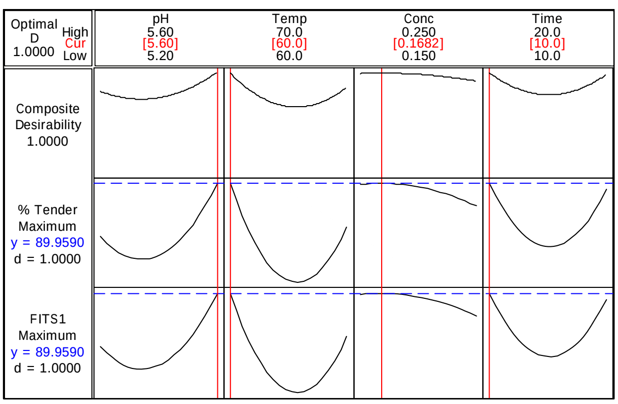

Figure 2. Response optimiser at optimum condition for maximum goal.

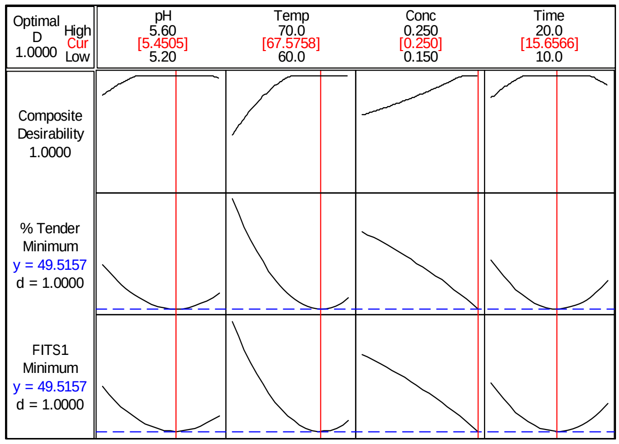

Figure 3. Response optimiser at optimum condition for minimum goal.

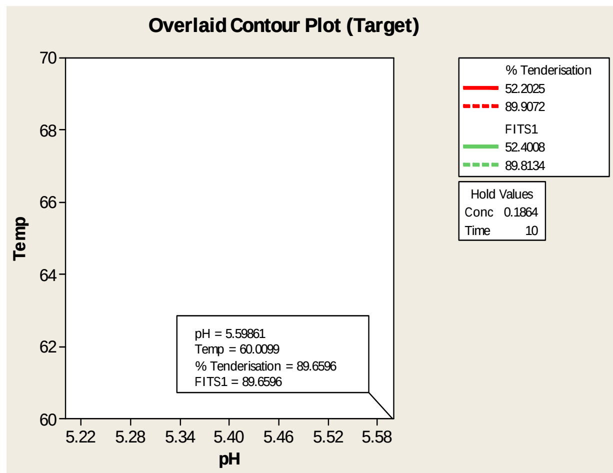

Figure 4. Overlaid contour plot at optimum condition for target goal; pH of beef of 5.6, immersion temperature of 60˚C, bromelain solution concentration of 0.1864% and immersion time of 10 minutes.

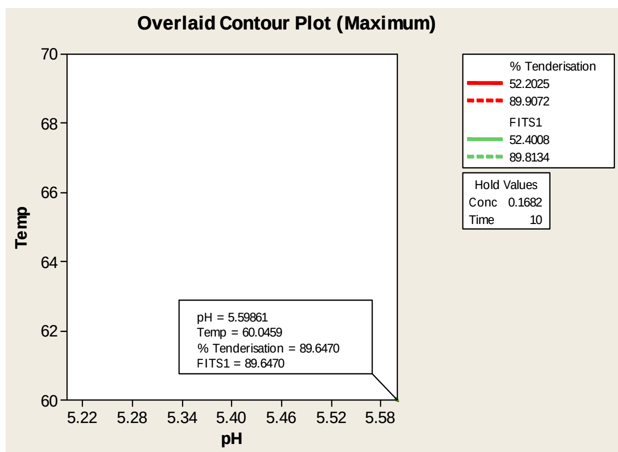

Figure 5. Overlaid contour plot at optimum condition for maximum goal; pH of beef of 5.6, immersion temperature of 60˚C, bromelain solution concentration of 0.1682% and immersion time of 10 minutes.

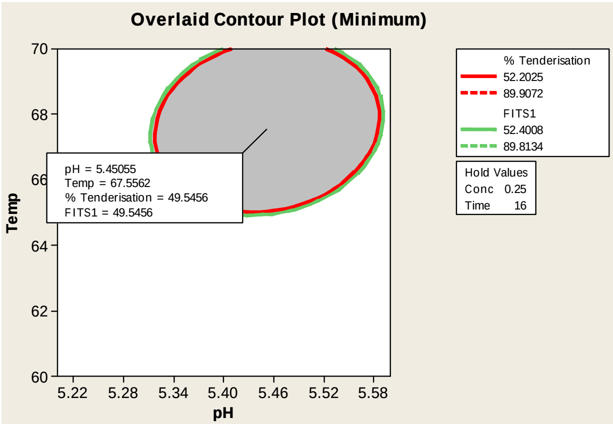

Figure 6. Overlaid contour plot at optimum condition for minimum goal; pH of beef of 5.45, immersion temperature of 68℃, bromelain solution concentration of 0.25% and immersion time of 16 minutes.

Table 6. Comparison values of target and predicted responses for different optimum conditions and feasibilities of experiment.

Goal		Lower	Target	Upper
Target	Y	52.2025	89.9071	89.9072
	FITS	52.4008	89.8133	89.8134
Max	Y	52.2025	89.9072	89.9072
	FITS	52.4008	89.8134	89.8134
Min	Y	52.2025	52.2025	89.9072
	FITS	52.4008	52.4008	89.8134

Goal	Optimum Condition				FITS (%)	F/NF
	X_1	X_2	X_3	X_4		
Target	5.6	60	0.1864	10	89.8102	F
Max	5.6	60	0.1682	10	89.9590	F
Min	5.5	68	0.2500	16	49.5157	NF

Where: X_1 = pH of beef, X_2 = immersion temperature (℃), X_3 = bromelain solution concentration (w/v%), X_4 = immersion time (min), Y = actual response (%), FITS = predicted response (%), F = feasible, NF = not feasible.

immersion time of 10 minutes were feasible to be carried out. Meanwhile, optimum condition for minimum goal with pH of beef of 5.5, immersion temperature of 68℃, bromelain solution concentration of 0.25% and immersion time of 16 minutes was not feasible to be carried out. This is because according to the overlaid contour plots for target and maximum goals as shown in **Figures 4** and **5**, respectively, the optimum conditions of target and maximum goals located in white or feasible region. Meanwhile, the overlaid contour plot of minimum goal as shown in **Figure 6** located in grey or not-feasible region. However, optimum condition from maximum goal was chosen because the target and FITS values was closer.

Contour and surface plots for beef tenderisation treated with bromelain at feasible optimum condition are shown in **Figures 7** and **8**, respectively.

2D contour and 3D surface plots are the graphical representatives of the regression equation illustrating the function of two factors at a time while holding other factors at a fixed level [27]. The 2D contour and 3D surface plots as demonstrated in **Figures 7** and **8**, respectively showed the effect of pH of beef, immersion temperature (℃), bromelain solution concentration (%) and immersion time (min) on beef tenderisation. The plots illustrating the values for pH of beef and immersion temperature while holding the values of bromelain solution concentration and immersion time at 0.1682% and 10 minutes, respectively.

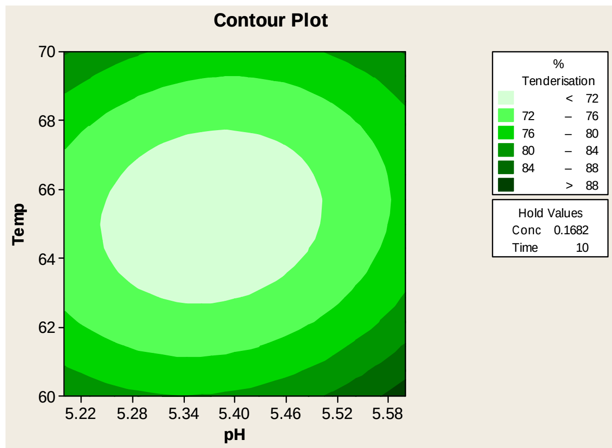

Figure 7. Contour plot of beef tenderisation treated with bromelain at feasible optimum condition; pH 5.6, immersion temperature of 60℃, bromelain solution concentration of 0.1682% and immersion time of 10 minutes (holding value:bromelain solution concentration and immersion time fixed at 0.1682% and 10 minutes, respectively).

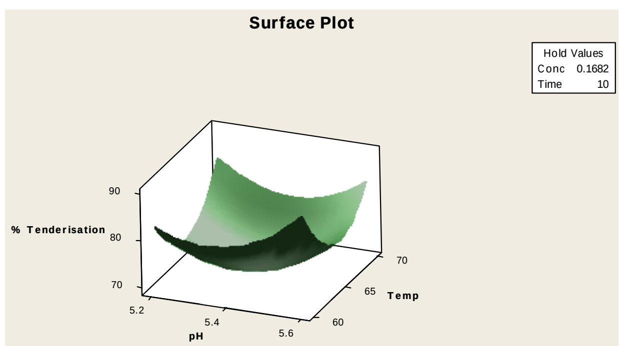

Figure 8. Surface plot of beef tenderisation treated with bromelain at feasible optimum condition; pH 5.6, immersion temperature of 60℃, bromelain solution concentration of 0.1682% and immersion time of 10 minutes (holding value : bromelain solution concentration and immersion time fixed at 0.1682% and 10 minutes, respectively).

Table 7. Comparison of verification and predicted values of beef tenderisation treated with bromelain at feasible optimum condition.

Optimum condition				V (%)	P (%)
X1	X2	X3	X4		
5.6	60	0.1682	10	89.571	89.813

Where: X_1 = pH of beef, X_2 = immersion temperature (℃), X_3 = bromelain solution concentration (w/v%), X_4 = immersion time (min), V = verification value, P = predicted value.

Circular or elliptical shapes of contour plot indicate whether the reciprocal interactions between the factors are significant or not. Circular contour plot indicates the interactions between corresponding factors are negligible, while elliptical contour plot indicates the interactions between corresponding factors are significant [30][20]. Results of the present study showed that the contour plot was elliptical shape thus indicates significant interaction effect between pH of beef and immersion temperature on beef tenderisation.

The surface plot showed that the beef tenderisation increased at the lower and higher levels of pH of beef and immersion temperatures while at the middle level of pH of beef and immersion temperature, the beef tenderisation decreased.

Verification of beef tenderisation treated with bromelain at the feasible optimum condition was performed and the result is shown in **Table 7**.

The verification value of the beef tenderisation treated with bromelain at the feasible optimum condition was 89.571% which was very close to the predicted value with 89.813%. Since the difference between the verification and predicted values was less than 5%, therefore the feasible optimum condition of the beef tenderisation predicted by MINITAB Software Version 15 was acceptable.

4. CONCLUSIONS

The determination coefficient R^2 (99.97%) was high, thus the experimental data was acceptable. Optimum condition for the tenderisation of beef by bromelain using RSM had been determined. It was found that beef could be optimise tenderized 89.907% at the optimum condition pH of beef of 5, immersion temperature of 60℃, bromelain solution concentration of 0.1682% and immersion time of 10 minutes. It aws also found that the difference between the verification and predicted values was less than 5%, therefore, the optimum condition for the beef tenderisation predicted by MINITAB Software Version 15 could be accepted.

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REFERENCES

[1] Maiti, A.K., Ahlawat, S.S., Sharma, D.P. and Khanna, N. (2008) Application of natural tenderisers in meat - A review. *Agricultural Reviews*, **29(3)**, 226-230.

[2] Deatherage, F.E.(1974) New approaches to the marketing of red meats. *New protein food*, Academic Press, New York. doi:10.1016/B978-0-12-054801-9.50012-0

[3] Żochowska-Kujawska, J., Lachowics, K. and Sobczak, M. (2010). The tenderisation of wild boar meat using a calcium chloride, kefir, wine and pineapple marinade. *Electronic Journal of Polish Agricultural Universities*, **13**, 1-8.

[4] Umesh Hebbar, H., Sumana, B. and Raghavarao, K.S.M. S.(2008) Use of reverse micellar systems for the extraction and purification of bromelain from pineapple wastes. *Bioresource Technology*, **99**, 4896-4902. doi: 10.1016/j.biortech.2007.09.038

[5] Hale, L.P., Greer, P.K., Trinh, C.T. and James, C.L. (2005) Proteinase activity and stability of natural brome- lain preparations. *International Immunopharmacology*, **5(4)**, 783-793. doi: 10.1016/j.intimp.2004.12.007

[6] Ngampanya, B. and Phongtongpasuk, S. (2006) Effects of sucrose concentration on crude bromelain production of in vitro culture of pineapple (*Ananas comosus* var. 'Pattavia'). *Kasetsart Journal Natural Science*, **40**, 129 -134.

[7] Devakate, R.V., Patil, V.V., Waje, S.S. and Thorat, B.N. (2009) Purification and drying of bromelain. *Separation and Purification Technology*, **64**, 259-264. doi: 10.1016/j.seppur.2008.09.012

[8] Ke, S., Huang, Y., Decker, E.A. and Hultin, H.O. (2009). Impact of citric acid on the tenderness, microstructure and oxidative stability of beef muscle. *Meat Science*, **82(1)**, 113-118. doi: 10.1016/j.meatsci.2008.12.010

[9] Jerez, N.C., Calkins, C.R. and Velazco, J. (2003) Pre-rigor injection using glycolytic inhibitors in low-quality beef muscles. *Journal of Animal Science*, **81**, 997- 1003.

[10] Ketnawa, S., Sai-Ut, S., Theppakorn, T., Chaiwut, P. and Rawdkuen, S. (2009) Partitioning of bromelain from pineapple peel (*Nang Lae* cultv.) by aqueous two phase system. *Asian Journal of Food and Agro-Industry*, **2**, 457-468.

[11] Ketnawa, S., Chaiwut, P. and Rawdkuen, S. (2011) Aqueous two-phase extraction of bromelain from pineapple peels ('*Phu Lae*' cultv.) and its biochemical properties. *Food Science and Biotechnology*, **20**, 1219-1226.

[12] Tripathi, A. and Srivastava, S.K. (2012) Biodegradation of orange G by a novel isolated bacterial strain *Bacillus megaterium* ITBHU01 using Response Surface Methodology. *African Journal of Biotechnology*, **11**, 1768- 1781.

[13] Jeong, G.T., Yang, H.S. and Park, D.H. (2009) Optimisa-

tion of transesterification of animal fat ester. *Bioresource Technology,* **100(1)**, 25-30.

[14] Sharma, S., Malik, A. and Satya, S. (2009) Application of response surface methodology (RSM) for optimisation of nutrient supplementation for Cr (V1) removal by *As- pergillus lentulus* AML05. *Journal of Hazardous Materi- als*, **164**, 1198-1204.

[15] Cepeda, E.A. and Calvo, B. (2008) Sunflower oil hydrogenation: Study using response surface methodology. *Journal of Food Engineering*, **89(4)**, 370-374. doi: 10.1016/j.jfoodeng.2008.01.012

[16] Siti Mazlina, M.K., Abdul Ghani, L.A., Nur 'Aliaa, A.R., Siti Aslina, H. and Rozita, O. (2008) Comparison on optimisation of star fruit juice using RSM between two Malaysian star fruit varieties (B11 and B10). *Pertanika Journal of Science and Technology*, **16**, 1-13.

[17] Kongkaew, A., Usansa, U. and Wanapu, C. (2012) Optimisation of wort production from rice malt using enzymes and barley malt. *African Journal of Biotechnology*, **11**, 9941-9949.

[18] Mullai, P.N., Syed Ali F. and Eldon R.R. (2010) Statistical analysis of main and interaction effects to optimize xylanase production under submerged cultivation conditions. *Journal of Agricultural Science,* **2**, 144-153.

[19] Thanapimmetha, A., Vuttibunchon, K., Saisriyoot, M. and Srinophakun, P. (2011) Chemical and microbial hydrolysis of sweet sorghum bagasse for ethanol production. *World Renewable Energy Congress-Sweden,* Linköping, Sweden. 8-13 May 2011, **1**, 389-396.

[20] Rengadurai, S., Preetha, B. and Viruthagiri, T. (2012) Response surface technique for optimisation of parameters for decolorization of Reactive red BS using *Trametes hiruta*. *International Journal of ChemTech Research,* **4**, 21-28.

[21] Sudamalla, P., Saravanan, P. and Matheswaran, M. (2012) Optimisation of operating parameters using response surface methodology for adsorption of crystal violet by activated carbon prepared from mango kernel. *Sustainable Environment Research,* **22**, 1-7.

[22] Sudamalla, P., Saravanan, P. and Matheswaran, M. (2012) Optimisation of operating parameters using response surface methodology for adsorption of crystal violet by activated carbon prepared from mango kernel. *Sustainable Environment Research*, **22**, 1-7.

[23] Fang, X.L., Feng, J.T., Zhang, W.G., Wang, Y.H. and Zhang, X. (2010) Optimization of growth medium and fermentation conditions for improved antibiotic activity of *Xenorhabdus nematophila* TB using a statistical approach. *African Journal of Biotechnology*, **9**, 8068-8077. doi: 10.5897/ajb09.1939

[24] Hismath, I., Wan Aida, W.M. and Ho, C.W. (2011) Optimisation of extraction conditions for phenolic compounds from neem (*Azadirachta indica*) leaves. *International Food Research Journal*, **18**, 931-939.

[25] Prakash. O, Talat, M., Hasan, S.H. and Pandey, R.K. (2008) Factorial design for the optimisation of enzymatic detection of cadmium in aqueous solution using immobilised urease from vegetable waste. *Bioresource Technology,* **99**, 7565-7572.

[26] Panwal, J.H., Viruthagiri, T. and Baskar, G. (2011) Statistical modeling and optimization of enzymatic milk fat splitting by soybean lecithin using response surface methodology. *International Journal of Nutrition and Metabolism,* **3**, 50-57.

[27] Datta, D. and Kumar, S. (2012) Modeling and optimisation of recovery process of glycolic acid using reactive extraction. *International Journal of Chemical Engineering and Applications,* **3**, 141-146. doi: 10.7763/ijcea.2012.v3.175

[28] Patel, S., Kothari, D. and Goyal, A. (2011) Enhancement of dextransucrase activity of *Pediococcus pentosaceus* SPAm1 by Response Surface Methodology. *Indian Journal of Biotechnology,* **10**, 346-351.

[29] Noordin, M.Y., Venkatesh, V.C., Sharif, S., Elting, S. and Abdullah, A. (2004) Application of response surface methodology in describing the performance of coated carbide tools when turning AISI 1045 steel. *Journal of Materials Processing Technology,* **145**, 46-58. doi: 10.1016/s0924-0136(03)00861-6

[30] Trinh, T.K. and Kang, L.S. (2010) Application of response surface method as an experimental design to optimise coagulation tests. *Environmental Engineering Research,* **15**, 63-70. doi: 10.4491/eer.2010.15.2.063

[31] Yu, S.-J., Zhang, Y., Li, C.-R., Zhang, Q., Ma, Z.-Y. and Fan, M.-Z. Optimisation of ultrasonic extraction of mycelia polysaccharides from *Paecilomyces hepiali* using response surface methodology and its antioxidant activity. *African Journal of Biotechnology,* **10**, 17241-17250.

