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ORIGINAL ARTICLE

Optimization of Process Parameters for the Edible Coating Application on Guava using Response Surface Methodology

*[1]Ung Hua Ting, [1,2]Mohd Zahid Abidin, [1,2]Mohd Syafiq Abdullah, [1,2]Abdul Fattah Ab Razak, [1,2]Mohammad Hafsanjani Salleh and [1,2]Siti Nadhira Mohamad Basri

[1]Food Technology Department, School of Engineering and Technology, University of Technology Sarawak (UTS), 96000 Sibu, Sarawak, Malaysia
[2]Centre of Research for Innovation and Sustainable Development (CRISD), University of Technology Sarawak (UTS), 96000 Sibu, Sarawak, Malaysia

ABSTRACT - The effects of dipping temperature and duration for the edible coating application on guava fruits were evaluated using response surface methodology (RSM). The optimum coating condition was determined using the coating pickup and weight loss percentage as the responses. From the central composite rotatable design (CCRD) in RSM, a total of 29 experimental runs which based on 9 sets of independent variables were generated randomly with axial distance (α) = 1.4142. The optimized coating condition with the highest desirability was identified as 34.8°C and 12.31 s. Under this optimized coating condition, the predicted mean values of coating pick up and weight loss on Day 6 were 0.1247% and 4.27% respectively for the guava samples which had been subjected to the ambient storage. Meanwhile, the actual mean values for the coating pickup and weight loss were 0.1139% and 4.25% correspondingly. The actual mean values of both responses were within the 95% prediction interval, which 0.1036 – 0.1458% for coating pickup and 4.10 – 4.45% for weight loss. After optimizing the coating condition, the developed edible coating emulsion was able to control the respiration rate of the guava samples by reducing their weight loss through a minimized thickness for the coating layer.

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INTRODUCTION

Guava (*Psidium guajava*) faces commercial challenge as it easily deteriorates due to insufficient postharvest handling technologies and storage information. It has a short shelf-life of about 3 – 4 days under the tropical ambient atmosphere at 26 – 30°C [1]. The limited shelf-life is majorly due to the natural metabolism processes, like respiration and ripening. These processes cause weight loss and obvious changes in terms of colour, taste, odour, reduction in the sugar content and decaying of guava [2]. It causes unsaleable loss to the local sellers and retailers.

Edible coating derived from natural biopolymers is a promising approach to maintain the quality of fruits and extend their postharvest shelf-life. It controls the respiration and ripening processes, which is indicated by the changes of physicochemical properties. For example, there is a reduced rate of weight loss and softening through the application of edible coating [1];[3]. When applying the coating, only a small amount of coating material is required to cover the entire surface of substrate [4];[5]. It has been reported that the duration and temperature of coating application had an impact on the adherence of coating to the surface [5–8]. The general way of applying coating to the fruits are dipping, brushing and spraying. Among all, most researchers utilize the dipping [7];[9],[10] The fruits will be dipped for a while, ranging from seconds to minutes whereas the dipping temperature differing from ambient to increased setting [5];[8];[10].

Response surface methodology (RSM) has been used extensively to optimize the conditions and processes in numerous food studies. As compared to one-variable-at-a-time (OVAT), it allows the analysis

*Corresponding Author: Ting Ung Hua. University of Technology Sarawak (UTS), email: mas22030001@student.uts.edu.my

of the impacts of numerous factors and their interactions on the response, with a reduced cost and time [11].

From the previous study, the edible composite coating emulsion was developed from gum Arabic and some lipid components. It could prolong the postharvest shelf-life of the guava by minimizing the undesirable changes in terms of their physicochemical properties under the ambient storage. Since the time and temperature of coating process have a high influence on the coating effectiveness and sensorial acceptability of the fruits, the developed coating emulsion were tested under different process parameters to further the study. A high coating pickup is unfavourable to the coated fruits since it might block the cellular respiration and give rise to the anaerobic respiration issue. Besides, thick coating affects the acceptability of fruits due to the extreme oily or waxy sensation. However, if the coating pickup is not enough, the layer of coating is not durable as it will gradually delaminate off the surface in the process of handling and transportation.

In this study, gum Arabic, beeswax and coconut oil were used to form the coating emulsion with the aid of tween 80 as the surfactant. The guavas were dipped and subsequently stored under the ambient condition (24 – 26^{o}C, 65 – 75% RH). The central composite rotatable design (CCRD) from RSM was employed as the statistical tool to optimize the coating process parameters. It is expected that there is a reduced coating pickup and weight loss percentage under the optimized coating condition.

MATERIALS AND METHODOLOGY

Materials

The guavas of Cambodia cultivar were procured from a local orchard in Sarikei, Sarawak. Based on the evaluations like skin colour and size, the fruits that had reached physiological maturity (typically 60 – 90 days after flowering) were harvested at one-week intervals, for a period of three months within August and October. They were sorted for uniformity in size, shape and weight (300 – 400 g), free from the microbial infection and physical injury. The sorted guavas were washed and left to dry at the ambient conditions (24 – 26^{o}C, 65 – 75% RH) as such performed by many researchers [5]

Gum Arabic (Evachem Sdn Bhd, Selangor, Malaysia), beeswax (Sigma-Aldrich, USA) and coconut oil (Mama Lim Handmade, Negeri Sembilan, Malaysia) were used as the edible coating components and tween 80 (Sigma–Aldrich, France) was used as surfactant. All materials and chemicals used were food or analytical grade.

Preparation and Application of Coating Emulsion

The gum Arabic powder was dissolved in the distilled water at 40^{o}C by constant stirring [1]. Beeswax was melted in a hot water bath (62 – 64°C) until the solution become clear [12]. The coconut oil (3.6% w/v) was added with the surfactant of tween 80 (3% w/v), followed with the gum Arabic solution (6.6% w/v) and the melted beeswax (5.5% w/v). The mixture was subjected to the high-speed mixing at 12000 rpm in a homogenizer (Omni, USA) for 5 min to produce the coating emulsion.

The coating emulsion was left aside to cool down to the desired temperature as shown in Table 1. Then, the guavas were dipped in the coating emulsion accordingly. The temperature of the coating emulsions was controlled by using water bath (Memmert, Germany). The coated samples were dried in the circulating air of the table fan for 15 min before being subjected to the ambient storage. The percentage of coating pickup and weight loss were calculated for the responses. The uncoated guavas were used as the control.

Coating Pickup Percentage

Before coating, the guavas were labelled and weighed. Their initial weight was denoted as W_i. After being coated and fan-dried, the samples were weighed again which was denoted as W_o. As shown in Equation 1, the coating pickup was calculated and expressed in percentage prior to the ambient storage.

$$P_C = \frac{W_o - W_i}{W_i} x100 \quad (1)$$

Weight Loss Percentage

The initial weight of coated guavas on Day 0 was recorded as W_o while their final weight at the end of ambient storage as W_f. The weight measurement was done repeatedly until Day 6. The weight loss percentage (P_{WL}) was calculated as shown in Equation 2.

$$P_{WL} = \frac{W_o - W_f}{W_o} x100 \quad (2)$$

Experimental Design and Statistical Analysis

The coating process parameters that affected coating pickup and weight loss were optimized using Design-Expert® Version 13 RSM software (Stat-Ease Inc., USA). As depicted in Table 1, each independent variable was tested at five different levels namely lower axial, lower factorial, medium, upper factorial, upper axial which coded as --, -, 0, +, ++, respectively. A total of 29 experimental runs based on 9 sets of independent variables were generated randomly by CCRD with axial distance (α) = 1.4142. It included five replicates of centre points (0) and three replicates of each factorial (-, +) and axial points (--, ++). The data of both responses were subjected to a series of analysis, which were analysis of variance (ANOVA), lack of fit (LOF), R-square (R^2) and predicted error sum of square (PRESS) determinations as well as residuals plotting for fitting the second order polynomial order as shown in Equation 3.

$$Z = \beta_0 + \beta_t T + \beta_i I + \beta_{ti} TI + \beta_{tt} T^2 + \beta_{ii} I^2 \quad (3)$$

Where, Z was the response variable (coating pickup or weight loss percentage); T and I were the independent variables for coating temperature and time respectively; β_o was linear coefficient at centre point of the model; β_t and β_i were linear coefficients; β_{ti} were linear interactive coefficient; β_{tt} and β_{ii} were quadratic interactive coefficient. The model verification was conducted by running the confirmation runs in the end of CCRD. The variation between the predicted and actual responses must be within 95% prediction interval [13];[16].

RESULTS AND DISCUSSION

The information about experimental runs, coating conditions and responses are illustrated in Table 1.

Table 1. Results of Coating Pickup Percentage and Weight Loss Percentage under Different Coating Conditions in the CCRD

Standard	Run	A (°C)	B (s)	P_C (%)	P_{WL} (%)
1	6	30 (-)	10 (-)	0.2724	3.65
2	27	30 (-)	10 (-)	0.2736	3.71
3	3	30 (-)	10 (-)	0.2779	3.82
4	25	40 (+)	10 (-)	0.1552	4.14
5	13	40 (+)	10 (-)	0.1402	4.37
6	20	40 (+)	10 (-)	0.1245	4.58
7	24	30 (-)	20 (+)	0.3185	3.92
8	19	30 (-)	20 (+)	0.3372	3.75
9	17	30 (-)	20 (+)	0.3590	3.59
10	11	40 (+)	20 (+)	0.1632	4.26
11	23	40 (+)	20 (+)	0.2298	4.57
12	4	40 (+)	20 (+)	0.1915	4.38

13	14	27.93 (--)	15 (0)	0.3739	3.55
14	7	27.93 (--)	15 (0)	0.3287	3.77
15	8	27.93 (--)	15 (0)	0.3575	3.81
16	15	42.07 (++)	15 (0)	0.1376	4.75
17	22	42.07 (++)	15 (0)	0.1489	4.63
18	5	42.07 (++)	15 (0)	0.1403	4.46
19	16	35 (0)	7.93 (--)	0.1767	3.92
20	10	35 (0)	7.93 (--)	0.1688	3.84
21	9	35 (0)	7.93 (--)	0.1782	3.63
22	26	35 (0)	22.07 (++)	0.2700	3.82
23	29	35 (0)	22.07 (++)	0.2635	4.03
24	18	35 (0)	22.07 (++)	0.2913	3.95
25	1	35 (0)	15 (0)	0.1603	3.74
26	12	35 (0)	15 (0)	0.1632	3.97
27	28	35 (0)	15 (0)	0.1745	3.76
28	21	35 (0)	15 (0)	0.1663	3.87
29	2	35 (0)	15 (0)	0.1584	4.01

Notes: The signs '--', '-', '0', '+' and '++'show the low axial, low factorial, centre, and high factorial and high axial points respectively

Model Fitting

As to interpret the results in Table 1, the data of both responses were fitted to different models, such as linear, two-factors interaction (2FI), quadratic and cubic to find out the most suitable polynomial model. The model fitting results as summarized in Tables 2 and 3 were first examined.

Table 2. Model Fitting for Coating Pickup Percentage Analysis

Source	Sequential p-value	LOF p-value	Adj. R^2	Pred. R^2	PRESS	Status
Linear	< 0.0001	< 0.0001	0.8266	0.8144	0.0332	
2FI	0.8253	< 0.0001	0.8201	0.8059	0.0347	
Quadratic	< 0.0001	0.7125	0.9615	0.9495	0.0090	Suggested
Cubic	0.4978	0.9250	0.9605	0.9414	0.0105	Aliased

Table 3. Model Fitting for Weight Loss Percentage Analysis

Source	Sequential p-value	LOF p-value	Adj. R^2	Pred. R^2	PRESS	Status
Linear	< 0.0001	0.0241	0.7326	0.6908	1.02	
2FI	0.9498	0.0141	0.7219	0.6694	1.10	
Quadratic	0.0008	0.8134	0.8374	0.7874	0.7045	Suggested
Cubic	0.8656	0.4250	0.8243	0.7450	0.8451	Aliased

It was observed that the quadratic models were the most suitable model in predicting the effects of dipping temperature and time. The data of coating pickup and weight loss percentage fitted well in the quadratic models with a non-significant LOF p-value (> 0.05), which was 0.7125 and 0.8134 respectively. In addition, the lowest PRESS values were depicted by the quadratic models. The PRESS value indicated how well the model fitting the points and was needed to predict the R^2 value. A lower PRESS value reflected a better model fitting. From Tables 2 and 3, the values of R^2 of the selected quadratic models had fulfilled the minimum standardized R^2 value (0.8). The predicted R^2 was also in reasonable agreement with the adjusted R^2 as their difference was less than 0.2 [14–16]. The cubic model was aliased as the experimental runs in RSM were not enough to estimate for the cubic model, thus, it was negligible. These adequacy measures supported the decision to select the quadratic model for continued analysis of ANOVA.

ANOVA and Model Reduction in Coating Pickup Percentage Analysis

When analysing the ANOVA, the p-values for each term were examined first. If the p-value was greater than 0.05, the factor was insignificant and should be dropped for a better model fitting and prediction. This step was known as model reduction. Table 4 depicts the ANOVA results of the reduced model.

Table 4. ANOVA for the Reduced Model of CCRD using Coating Pickup Percentage as the Response

Source	Sum of Squares	df	Mean Square	F-value	p-value	Status
Model	0.1732	4	0.0433	181.73	< 0.0001	significant
T	0.1247	1	0.1247	523.30	< 0.0001	significant
I	0.0254	1	0.0254	106.73	< 0.0001	significant
T^2	0.0215	1	0.0215	90.24	< 0.0001	significant
I^2	0.0113	1	0.0113	47.31	< 0.0001	significant
Residual	0.0057	24	0.0002			
LOF	0.0004	4	0.0001	0.3999	0.8064	not significant
Pure Error	0.0053	20	0.0003			
Cor Total	0.1789	28				

In the initial ANOVA table, the term of TI (interaction between dipping temperature and time) was insignificant with p-value of 0.6343, was dropped from the model. Thus, it was not shown in Table 4. Similarly, the adequacy of the developed second order model in the CCRD was checked by LOF test, the predicted and adjusted R^2, PRESS and residual analysis as in diagnostics step [11];[15]. A non-significant LOF value with p-value greater than 0.05 was crucial to ensure there was an adequate data fitting after the model reduction. In this case, the LOF p-value after the model reduction was 0.8064. This value was higher than the initial LOF p-value before the model reduction (0.7125), indicating an improvement of model fitting after reducing the CCRD model in the coating pickup response. Table 5 shows the other statistical values using the coating pickup as the response.

Table 5. Statistical Summary for the Reduced Model of CCRD using Coating Pickup Percentage as the Response

Statistics	Value
Standard Deviation	0.0154
Mean	0.2242
CV%	6.89
PRESS	0.01
R^2	0.9680
Adjusted R^2	0.9627
Predicted R^2	0.9552
Adequate Precision	34.0274

Overall, the reduced model fulfilled adequacy as the ANOVA results in Table 2 showed p-value < 0.05 and LOF p-value > 0.05 for the selected quadratic model. Besides, from Table 5, for the reduced CCRD model, the difference between the predicted and adjusted R^2 was within 0.2 and the adequate precision was greater than 4 [14–16]. The reduced CCRD model could be expressed into the coded second order mathematical equation as shown in Equation 4 to establish the relationship between the independent variables and the response of coating pickup percentage.

$$P_C = 0.1645 - 0.0721T + 0.0326I + 0.0418T^2 + 0.0303I^2 \quad (4)$$

ANOVA and Model Reduction in Weight Loss Percentage Analysis

From Table 1, the weight loss percentage for the coated samples stored under the ambient condition ranged from 3.55 – 4.75% on Day 6. On the other hand, the weight loss for the control samples had reached greater than 5% on Day 2 under the same storage condition. After being harvested, all types of fruits would experience moisture loss due to the respiration and ripening processes that continually taken place through the surface of the fruits, resulting in the weight loss [17-18]. The weight loss percentage of guava samples that coated under different conditions were lower than the control.

As illustrated in Table 6, the ANOVA results were studied to investigate which coating parameter affected the weight loss percentage significantly.

Table 6. ANOVA for the Reduced Model of CCRD using Weight Loss Percentage as the Response

Source	Sum of Squares	df	Mean Square	F-value	p-value	Status
Model	2.84	2	1.42	78.79	< 0.0001	significant
T	2.47	1	2.47	136.59	< 0.0001	significant
T^2	0.3790	1	0.3790	21.00	0.0001	significant
Residual	0.4693	26	0.0181			
LOF	0.0467	6	0.0078	0.3680	0.8906	not significant
Pure Error	0.4227	20	0.0211			
Cor Total	3.31	28				

The ANOVA results had revealed that the terms of TI (p-value = 0.9344), I (p-value = 0.2630) and I^2 (p-value = 0.8107) were insignificant towards the weight loss percentage and had been removed from the initial quadratic model for a better data fitting. This was indicated by a higher LOF p-value (0.8906) than that in the initial model (0.8134). The adequacy of the reduced CCRD model using weight loss percentage as the response was also confirmed by the further analysis of statistics. Table 7 summarizes the statistics of the reduced CCRD model based on the weight loss response.

Table 7. Statistical Summary for the Reduced Model of CCRD using Weight Loss Percentage as the Response

Statistics	Value
Standard Deviation	0.1344
Mean	4.01
CV%	3.35
PRESS	0.59
R^2	0.8584
Adjusted R^2	0.8475
Predicted R^2	0.8232
Adequate Precision	21.4532

The PRESS value of the reduced CCRD quadratic model (0.59) was lower than the initial model (0.70). A lower PRESS value reflected a better model fitting. The adequate precision also increased after the model reduction (21.4532 versus 15.0743). Since the adequate precision revealed the ratio of signal to noise, the increase in this value indicated an improvement of the signal. Equation 5 established the relationship between the independent variables and weight loss.

$$P_{WL} = 3.88 + 0.3205T + 0.1532T^2 \quad (5)$$

Model Validation

As to further validate the model by using coating pickup and weight loss as the response, the diagnostics step was conducted. The diagnostics plots like the plots of normal probability versus externally studentized and residuals versus run as well as the influence plots like Cook's distance and the leverage versus run were inspected for the model validation [11];[15]. Figures 1 and 2 illustrate the normal probability plots in the analysis of coating pickup and weight loss responses respectively.

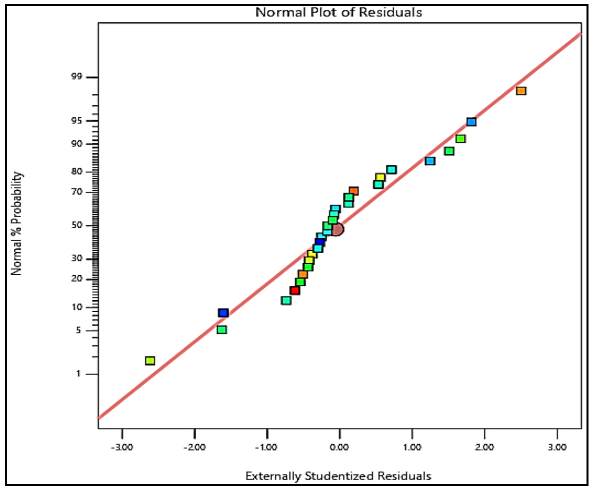

Figure 1. Normal Probability Plot in Coating Pickup Percentage Analysis

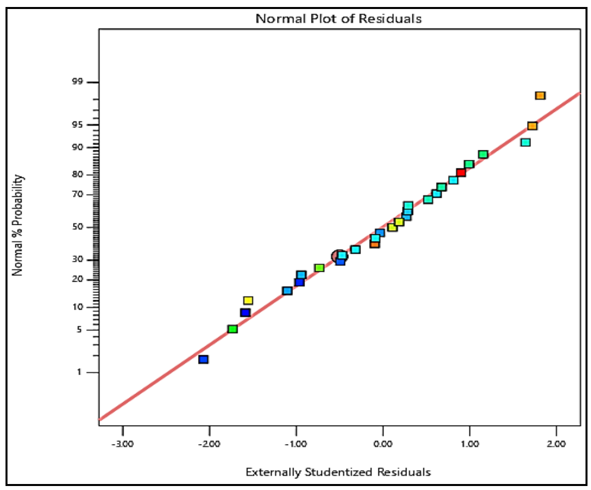

Figure 2. Normal Probability Plot in Weight Loss Percentage Analysis

The normal plot of residuals was checked to identify if there was a need for data transformation. If a S-shaped curve is observed (megaphone pattern for the scattered residuals), there is an issue of abnormality and the data transformation is required. As shown in Figure 1, although there was a slight megaphone pattern (S-shaped) observed from the normality plot in the coating pickup response, the model was still acceptable as there was no data transformation suggested by the analytical tool. Meanwhile, Figure 2 shows that the residuals scattered randomly along the straight line following the normally-distributed pattern.

As illustrated in Figures 3 and 4, the residuals versus run number plots were also diagnosed to check if there was any lurking variable that affects the model validity for both responses. The data in both responses were scattered randomly within the boundaries of ± 3.5 (red lines), which set by the analytical software of Design-Expert. Thus, there was no serious outlier in the responses being observed and no time-related influences lurking behind.

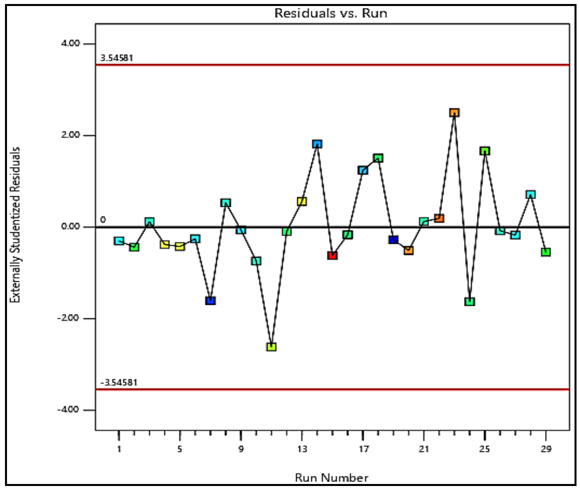

Figure 3. Residuals versus Run Number Plot in Coating Pickup Percentage Analysis

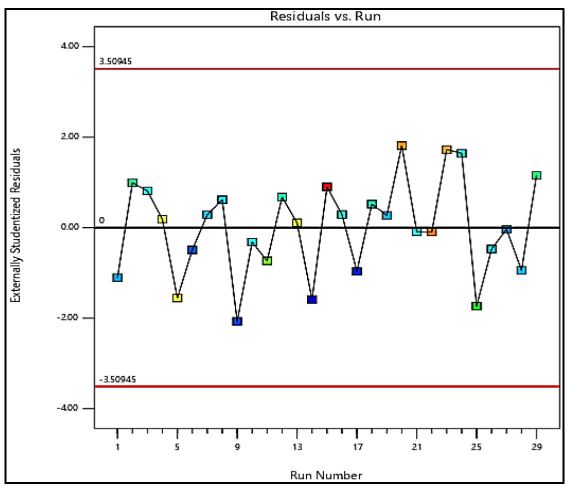

Figure 4. Residuals versus Run Number Plot in Weight Loss Percentage Analysis

The Cook's distance and leverage plots should be viewed to detect the outliers. Cook's distance reveals the sum of differences in every predicted point contributed by omitting one of the points from the model. If there is outlier, the point will locate at a higher region compared to the other points. There are some possible reasons causing outliers to occur, like random error during experiment, inadequate replication and incorrect data collection. The leverage plot is also used to further checking the response validity. It indicates the degree of each point in influencing the model fitting. In case of a high influence, the leverage value will be close to 1 and the model is in the state of 'lack of fit'. The possible way to reduce the influence of outliers is by adding the replicates [16];[19].

As shown in Figures 5 and 6, a few points located slightly higher than the others and had been diagnosed. It might be due to the random error during the experiment but there was no serious outlier as all points were still close to each other for both responses [16];[19].

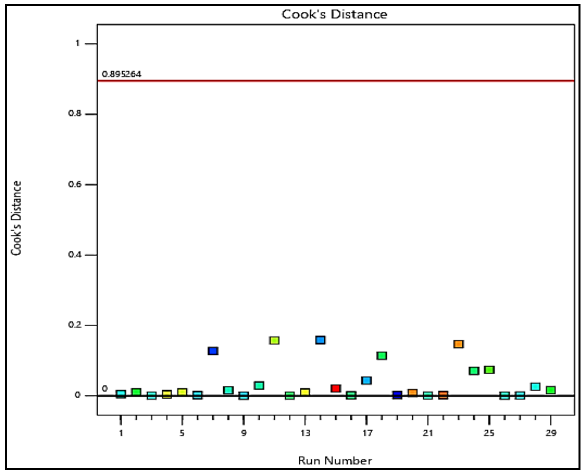

Figure 5. Cook's Distance versus Run Number Plot in Coating Pickup Percentage Analysis

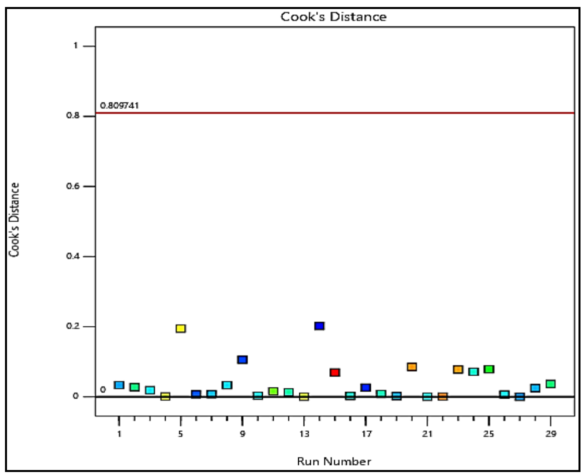

Figure 6. Cook's Distance versus Run Number Plot in Weight Loss Percentage Analysis

Figures 7 and 8 depict the leverage plots for both responses. In both cases, the leverage points were lower than the maximum boundary of 1. It was deduced that adequate replicates had been made. Overall, the reduced models did not need any amendment for both responses.

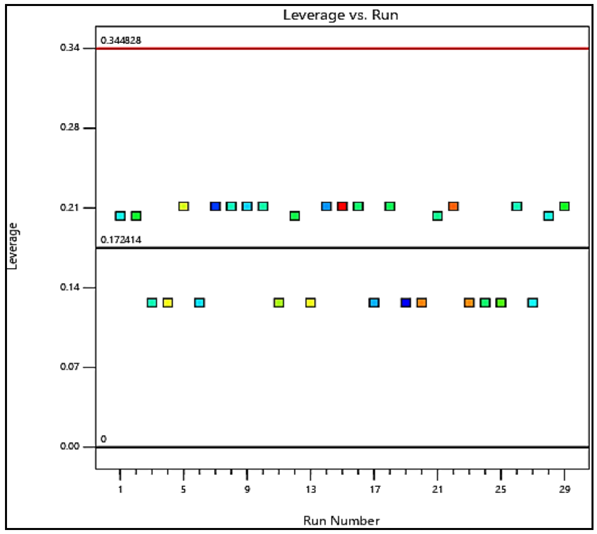

Figure 7. Leverage versus Run Number Plot in Coating Pickup Percentage Analysis

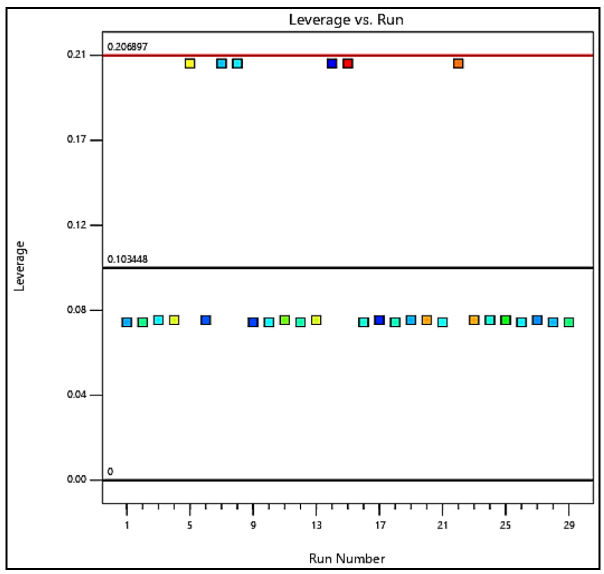

Figure 8. Leverage versus Run Number Plot in Weight Loss Percentage Analysis

Analysis of Model Graphs

In CCRD, the model graph of concern was 3D plot. The 3D plots based on both responses were analysed in order to seek for the optimum solution for the coating condition.

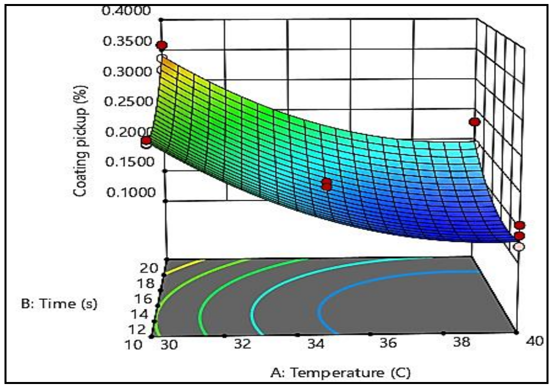

Figure 9. 3D Plot based on the Response of Coating Pickup Percentage

Figure 3 illustrates the 3D plot which uses coating pickup percentage as the response. It demonstrated an obvious curvature contour as the relationship between the variables was expressed in the quadratic equation (as shown in Equation 4). The most desirable response is like the 'peak of a hill' to be attained and the curvature illustrates the most appropriate range of coating temperature and time in order to reach the peak region. In this case, the range of coating temperature and time that could yield the lowest coating pickup percentage was targeted. This was to minimize the coating thickness which may cause the undesirable anaerobic respiration to the guava samples.

As shown in Figure 9, at a constant dipping time, when the dipping temperature had increased, there was a reduced coating pickup percentage. This finding was in conformity with the kinetic theory of the particles. When the temperature increased, the coating emulsion would be less viscous. In this case, the emulsion would tend to flow through the creaks on the guavas surface and dripped off more easily, resulting in a lower coating pickup at a higher range of temperature. On the other hand, at a low temperature, the coating would be more viscous and have a higher tendency to deposit on the surface of guavas. Meanwhile, there was an increment of the coating pickup percentage when the dipping time was extended longer at a particular temperature. Besides, Figure 10 shows that the curvature effect was less obvious when using the weight loss as the response. This was because only temperature (T) affected the weight loss percentage significantly (as shown in Table 6).

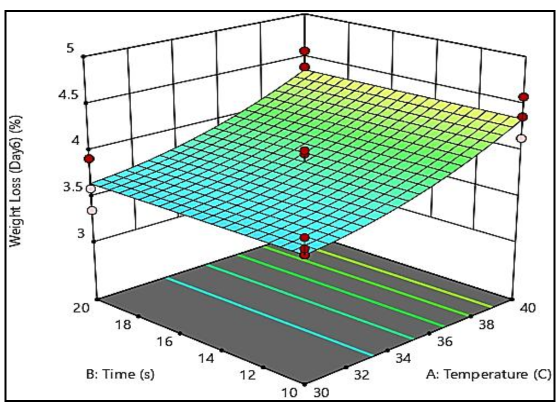

Figure 10. 3D Plot based on the Response of Weight Loss Percentage

The 3D plot using weight loss as the response suggested a low temperature range for minimizing the weight loss. As shown in Figure 10, at a constant time, when the temperature decreased, the weight loss percentage of guava samples declined continually. This could be explained by examining the plot in Figure 9, which showed that there was a higher amount of coating emulsion being adhered to the surface of guavas when the temperature range was reduced to a lower level, possibly < 32°C. However, the amount of coating pickup should be monitored to prevent the coating being applied excessively.

Optimization and Verification

For optimization, the ultimate coating condition that needed was able to minimize both coating pickup and weight loss percentage. The respiration and ripening rates of the guava samples should be slowed down without being blocked completely through a thin layer of coating. If the coating applied excessively, it might block the cellular respiration and give rise to the anaerobic respiration issue. The optimum coating condition with the highest desirability was given as 34.8°C and 12.31 s. The predicted values of coating pickup and weight loss on Day 6 were 0.1247% and 4.27% respectively.

In the end of RSM, the additional runs were conducted for verification and the results had been tabulated as in Table 8.

Table 8. Results of Coating Pickup and Weight Loss Percentage of the Guavas Coated with the Optimized Conditions

Trial	Coating Pickup (%)	Weight Loss (%)
1	0.1203	4.68
2	0.1170	3.76
3	0.1345	4.32

The analytical software would calculate the predicted mean, standard deviation (SD) and actual mean values. Table 9 showed that the actual mean values of both responses were within the 95% prediction interval (PI). Thus, the model had been verified [16].

Table 9. Model Verification of CCRD in Optimizing the Coating Condition

Trial	Predicted Mean	SD	95% PI Low	Data Mean	95% PI High
Coating Pickup	0.1247	0.0154	0.1036	0.1239	0.1458
Weight Loss (Day 6)	4.27	0.13	4.10	4.25	4.45

CONCLUSION

RSM had been effectively adapted to optimize the coating process parameters based on the responses of coating pickup and weight loss. The optimized parameters were identified as 34.8°C for the dipping temperature and 12.31 s for the dipping duration. Under this condition, a thin layer of coating could be adhered on the guava samples. Besides, under this optimum coating process parameter, the weight loss had been reduced effectively by four multiples as compared to the control at the end of ambient storage on Day 6, leading to an overall alleviation of the postharvest quality. Future studies are recommended to explore the effectiveness of the developed coating emulsion when it is applied on the other local fruits and the impact of the coating to the sensorial quality of the fruits.

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