



## Optimization of guava edible coating using response surface methodology

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### Abstract

Application of edible coating represents a method that can extend the shelf life of picked guava by minimizing the loss of weight mainly due to natural migration process of moisture and gases. Response surface methodology (RSM) was employed to search for best composition of edible coating which comprised of three variables namely palm stearin, palm olein and beeswax. Based on central composite rotatable designs of RSM and weight loss as response, 15 coating compositions were established involving 8 factorial points, 6 axial points and 1 centre point. From the RSM-generated model, optimum coating composition for minimizing guava weight loss was identified as palm stearin 4.5% (w/v), palm olein 1% (v/v) and beeswax 1% (w/v). Under this optimum composition, the predicted weight loss of coated guava was 7.18%, whereas, the experimental weight loss of coated guava was 7.51% after tenth days of storage period. The RSM-predicted and experimental weight loss were not significantly different from each other. The weight loss of uncoated guava was 3 times higher (25%) after 8 days of storage as compared to coated guava. Thus, the use of optimum composition of edible coating provides acceptable alternative for post harvest control of weight loss of guava during storage.

**Key words:** Response surface methodology (RSM), guava, stearin, olein, beeswax, weight loss

### Introduction

Guava (*Psidium guajava*) is a tropical, climacteric fruit that ripens rapidly and is highly perishable. The shelf-life ranges from 3 to 10 days at room temperature (Campbell, 1994; Vazquez-Ochoa and Colinas-Leon, 1990). If the guavas are kept without any treatment, they may be spoiled mainly due to loss of water from fruit surface, faster respiration rate, attack of microorganisms and development of physiological disorders. The loss of weight in guava is due to migration of moisture, volatile compounds and gases such as oxygen, carbon dioxide and ethylene.

To minimize the post harvest losses of guavas, edible coating can be applied. There are many advantages of fruit coating, among which are retaining freshness, firmness and colour. Lipid-based coatings are generally more effective barriers to moisture while polysaccharide-based coatings are generally good gas barriers (Hagenmaier and Shaw, 1990; Kester and Fennema, 1988). The coating may be applied by dipping or drenching or for experimental purposes by brushing (Smith *et al.*, 1987).

Palm olein and palm stearin are produced by fractionation of palm oil after crystallization at controlled temperature. Palm stearin is a by-product of palm oil, inexpensive and has limited usage. One of the current technological concerns of the edible oil industry is how to expand the multiple usages of palm oil and its by-products. The use of palm stearin and palm olein as part of coating composition can be justified by their constituents of palmitic, oleic and stearic acids. According to Morillon *et al.* (2002), palmitic acid and stearic acid have very low water vapour permeability ( $0.65$  and  $0.22 \times 10^{-12} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ ), respectively. The criterion of water vapour permeability is important because it may reflect the ability of coating to act as a moisture barrier. Beeswax, a product from bee hive, has a lowest water vapour permeability

of  $0.006 \times 10^{-12} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ . With this value, beeswax has many applications in food, cosmetic and pharmaceutical industries.

RSM originally described by Box and Wilson (1951) enables evaluation of the effects of many factors and their interactions on response variables. The advantages of using RSM are reported to be the reduction in the number of experimental runs needed to evaluate multiple variables, and the ability of the statistical tool to identify interactions (Chen *et al.*, 2004; Lee *et al.*, 2000). Therefore, it is less laborious and less time-consuming compared to one-variable at a time. RSM has been widely applied for optimizing conditions and processes in various food studies (Junqueira *et al.*, 2007; Liyana-Pathirana and Shahidi, 2005).

In this study, all lipid-based coating ingredients *i.e.*, palm stearin, palm olein and beeswax were emulsified with water to form coating emulsion and subsequently applied on fruit surface by dipping technique. The central composite rotatable design from RSM was used as a statistical method to optimize edible coating composition comprised of stearin, olein and beeswax in minimizing the weight loss of guava.

### Materials and methods

**Guavas:** Samples of fresh guavas were obtained from a commercial farm, Sui Yuan Fruit Trading, in Bidor, Perak, Malaysia. Guavas were carefully selected (maturity index 2) to obtain uniformity based on size, shape, colour and absence of injuries. The fruits were packed in small boxes and transported to the laboratory in Shah Alam, Selangor, Malaysia. Pre-treatment of guavas were made according to the method applied by Soares *et al.* (2007). Selected guavas were unwrapped, washed and sanitized in potassium sorbate 0.5% for 2-3 minutes and left to dry at ambient conditions (25-27°C, RH 80-90%).

**Coating ingredients:** Refined, bleached and deodorised palm kernel olein and palm stearin with a slip melting point of 49.6°C and iodine value 40.3 were obtained from Cargill Palm Products Pte. Ltd., Port Klang, Malaysia. Beeswax (Sigma-Aldrich, GmbH, Sternheim, Germany) was purchased from a local supplier. All other chemical and ingredients used were either of analytical or food grade.

**Preparation and application of coating emulsion:** Coating emulsion was prepared conceptually similar to the method applied by several researchers (Rojas-Graü *et al.*, 2008; Tapia *et al.*, 2007). Beeswax was heated up to 90°C in distilled water while stirring on hot plate (Fisher Scientific, USA) until the solution became clear. Palm stearin, palm olein and 0.5% (v/v) emulsifier (Tween 40, Sigma-Aldrich, GmbH, Sternheim, Germany) were added immediately followed by high speed mixing using an Ultra Turax T10 (IKA®, Germany). Guavas were labelled and marked before being dipped in coating emulsion at 60°C for 15 seconds and left to dry at ambient conditions. Weight measurement was performed on daily basis until day 10 of storage period.

**Weight loss:** Weight loss was determined by the difference between the initial and final weights of each replicate. Percentage of weight loss was calculated as follows:

$$\text{Weight loss (\%)} = \frac{(\text{Initial weight}) - (\text{Final weight})}{(\text{Initial weight})} \times 100$$

**Experimental design and statistical analysis:** Edible coating composition affecting weight loss of guava was optimized using Design-Expert version 6.0.6 RSM software (Stat-Ease Inc., Minneapolis, USA). Each variable was examined at five different levels (relatively low, low, basal, high, relatively high) coded (--, -, 0, +, ++) as shown in Table 1. The design required 48 runs derived from 15 combinations of the independent variables performed in random order, including replicates of the centre region and factorial points. The obtained responses were subjected to an analysis of variance, R-square and were evaluated for lack of fit (LOF). Accordingly, an equation in terms of coded of second-order polynomial could be calculated of the type:

$$Z = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \quad \text{Equation 1}$$

where, Z was the dependent variable (weight loss); A, B and C were the independent variables for palm stearin, palm olein and beeswax respectively;  $\beta_0$  was the regression coefficient at centre point;  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  were linear coefficients;  $\beta_{12}$ ,  $\beta_{13}$  and  $\beta_{23}$  were second-order interaction coefficients; and  $\beta_{11}$ ,  $\beta_{22}$  and  $\beta_{33}$  were quadratic coefficients.

Optimum composition was obtained using the optimization module of RSM software. The experimental and predicted values were compared in order to determine the validity of the developed model. Verification of model was performed similar to the method applied by Tan *et al.* (2009).

## Results and discussion

**Model fitting and analysis of response:** The response was percentage of weight loss in which a good composition should minimize the weight loss of guava within 10 days of storage period. Details about experimental runs, coating composition and response of weight loss are given in Table 1.

Table 1. Central composite rotatable design for three independent variables: palm stearin (A), palm olein (B) and beeswax (C)

Standard Run	A: palm stearin (g)	B: palmolein (g)	C: Beeswax (g)	Z: Weight loss (%)	
1	22	2 (-)	2 (-)	9.87	
2	12	2 (-)	2 (-)	10.05	
3	18	2 (-)	2 (-)	11.87	
4	15	5 (+)	2 (-)	9.06	
5	26	5 (+)	2 (-)	8.50	
6	3	5 (+)	2 (-)	9.10	
7	19	2 (-)	5 (+)	10.32	
8	38	2 (-)	5 (+)	11.33	
9	31	2 (-)	5 (+)	11.51	
10	5	5 (+)	5 (+)	10.77	
11	20	5 (+)	5 (+)	10.02	
12	7	5 (+)	5 (+)	11.90	
13	17	2 (-)	2 (-)	14.73	
14	11	2 (-)	2 (-)	12.15	
15	6	2 (-)	2 (-)	13.41	
16	25	5 (+)	2 (-)	10.77	
17	23	5 (+)	2 (-)	11.23	
18	13	5 (+)	2 (-)	11.68	
19	32	2 (-)	5 (+)	8.65	
20	9	2 (-)	5 (+)	9.16	
21	43	2 (-)	5 (+)	8.06	
22	40	5 (+)	5 (+)	7.65	
23	35	5 (+)	5 (+)	9.54	
24	21	5 (+)	5 (+)	7.98	
25	36	0.98 (--)	3.5 (0)	12.98	
26	44	0.98 (--)	3.5 (0)	12.53	
27	42	0.98 (--)	3.5 (0)	12.15	
28	39	6.02 (++)	3.5 (0)	10.90	
29	14	6.02 (++)	3.5 (0)	10.76	
30	45	6.02 (++)	3.5 (0)	9.87	
31	46	3.5 (0)	0.98 (--)	12.07	
32	8	3.5 (0)	0.98 (--)	11.65	
33	48	3.5 (0)	0.98 (--)	10.43	
34	33	3.5 (0)	6.02 (++)	7.76	
35	1	3.5 (0)	6.02 (++)	7.53	
36	2	3.5 (0)	6.02 (++)	6.81	
37	27	3.5 (0)	3.5 (0)	0.98 (--)	8.23
38	34	3.5 (0)	3.5 (0)	0.98 (--)	7.76
39	29	3.5 (0)	3.5 (0)	0.98 (--)	9.68
40	30	3.5 (0)	3.5 (0)	6.02 (++)	7.62
41	41	3.5 (0)	3.5 (0)	6.02 (++)	8.47
42	24	3.5 (0)	3.5 (0)	6.02 (++)	9.56
43	37	3.5 (0)	3.5 (0)	3.5 (0)	8.82
44	4	3.5 (0)	3.5 (0)	3.5 (0)	9.09
45	16	3.5 (0)	3.5 (0)	3.5 (0)	9.22
46	10	3.5 (0)	3.5 (0)	3.5 (0)	8.62
47	47	3.5 (0)	3.5 (0)	3.5 (0)	8.66
48	28	3.5 (0)	3.5 (0)	3.5 (0)	8.47

Symbols (--, -, 0, +, ++) indicates relatively low, low, basal, high and relatively high.

Analysis of variance (Table 2) of the model revealed that all selected terms had significant effects on the response. This was indicated by the small value of probability for F value (less than 0.05). However, the term C (or beeswax) did not have significant effect on response. According to Myers and Montgomery (1995), non-significant term can be dropped from a model or fixed at one level. Thus, we decided to fix beeswax at low level (1% w/v) in the coating composition. Furthermore, the interaction between B and C (palm olein and beeswax) showed a significant result on response. Meanwhile, LOF test measured variation of the data around the fitted model. If the model did not fit the data well, LOF

test would be significant. In this case, the selected model showed non-significant results. A model should be rejected if the results showed significance in the LOF test (Myers and Montgomery, 1995).

Table 2. Analysis of variance of the model terms

Source	Sum of square	df	Mean square	F value	Prob > F	Status
Model	125.97	7	18.00	29.27	<0.0001	Significant
A	13.16	1	13.16	21.40	<0.0001	Significant
B	31.27	1	31.27	50.87	<0.0001	Significant
C	0.011	1	0.011	0.018	0.8935	NS
A2	38.54	1	38.54	62.68	<0.0001	Significant
B2	3.16	1	3.16	5.13	0.0290	Significant
AB	4.66	1	4.66	7.57	0.0089	Significant
BC	38.33	1	38.33	62.34	<0.0001	Significant
Residual	24.59	40	0.61			
Lack of fit	5.69	7	0.81	1.42	0.2308	NS
Pure error	18.90	33	0.57			
Cor total	150.57	47				

NS: Non significant

The selected model comprised of several important properties as listed in Table 3. According to Joglekar and May (1987), the coefficient of variance (CV) should be less than the standardized value of 10%. The CV value of the selected model was 7.86% which was below that of the standardized value. The predicted residual sum of square (PRESS) value measures how the model fits each point in the design. The lower the value of PRESS, the better the model fits the point. In our case, when the term AC was added the PRESS value increased to 38.37, reflecting the term AC should be dropped from the model. For the model fitted, the coefficient of determination ( $R^2$ ), which is a measure of degree of fit (Haber and Runyon, 1977), was 0.8367. This implied that 83.7% of the variations could be explained by the fitted model. Joglekar and May (1987) suggested that for a good fit of a model,  $R^2$  should be at least 0.80. Adjusted R-Square was used to measure the amount of variation around the mean which was adjusted for the number of terms in the model. The

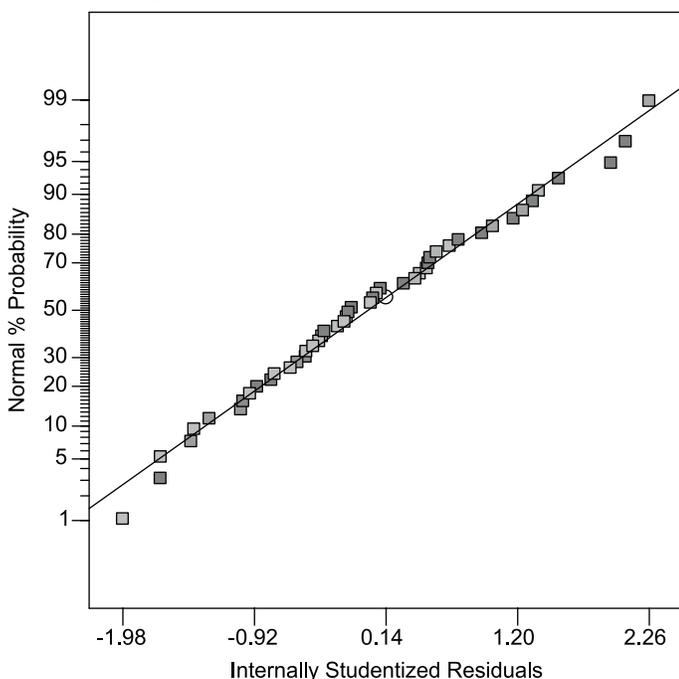


Fig. 1. All points scattered on straight line in normal plot of residuals for the selected model.

Table 3. Collection of summary of statistics for the selected model

Parameters	Value
Standard deviation	0.78
Mean	9.98
C.V	7.86
PRESS	36.27
R-Squared	0.8367
Adjusted R-Squared	0.8081
Predicted R-Squared	0.7591
Adequate Precision	16.895

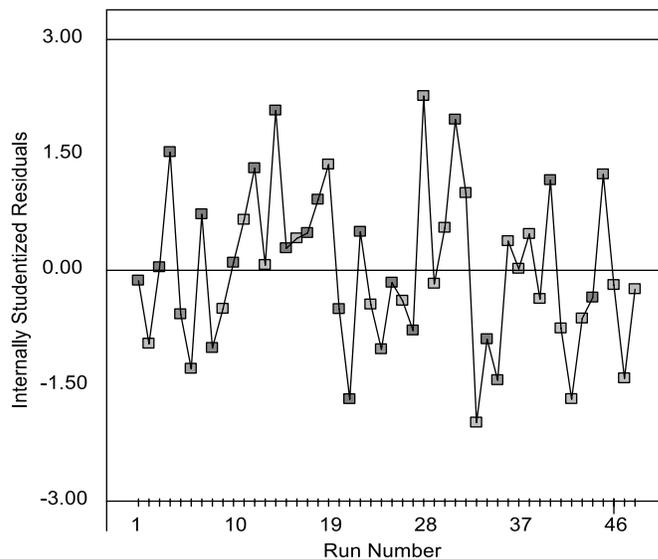


Fig. 2. All points scattered randomly in this plot of residual against experimental run number.

adjusted R-Square decreased as the number of terms in the model increased if those additional terms do not add value to the model. For instance, when the term AC was added, the adjusted R-Square decreased from 0.8081 to 0.8041, which indicated that the term AC did not contribute significantly to the response. The adequate precision value of the selected model was 16.9. Adequate precision measures the signal to noise ratio and a ratio greater than 4 indicates an adequate signal (Stat-Ease Inc., Minneapolis, USA). Thus the model can be used to navigate the design space.

The selected model can also be interpreted into a coded equation as shown in Equation 2. The equation is used to correlate a relationship between the three variables and percentage of weight loss.

$$Z = 8.81 - 0.57A - 0.87B + 0.017C + 1.07A^2 + 0.31B^2 + 0.31B^2 + 0.44AB - 1.26BC \quad \text{Equation 2}$$

**Diagnostic and optimum coating composition:** Normal probability plot indicates whether the residuals follow a normal distribution, in which case the points will follow a straight line. The results showed that the points were scattered along the straight line (Fig. 1) and no bad shape like an “S-shape” was observed. Plot residual against run was used to check lurking variables that may have influenced the response during the experiment. The plot should show a random scatter as in Fig. 2. Similar pattern and shape were observed in the plot outlier against run number (the plot is not shown but is similar to Fig. 2) which indicated no outliers and all points were randomly scattered within plus or minus 3.5 (standard value set by the software).

Fig. 3 expressed the relationship in the form of a three dimensional plot between palm stearin and palm olein when level of beeswax

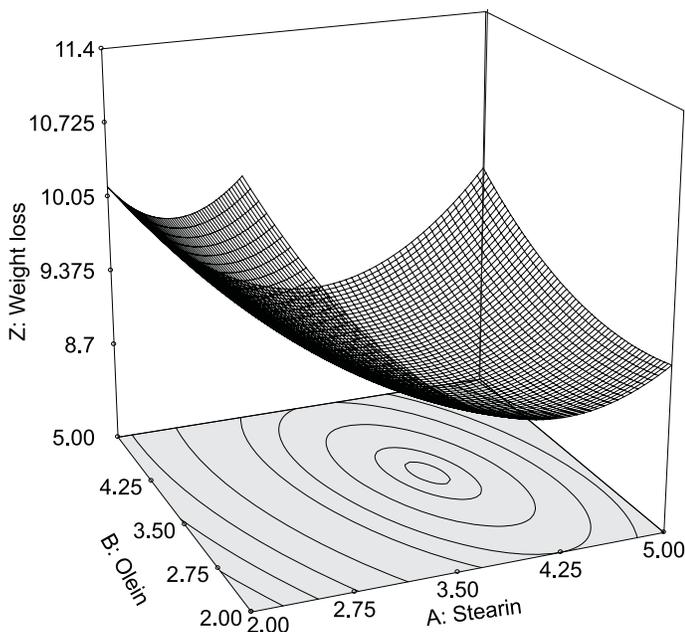


Fig. 3. Three-dimensional plot showing relationship between fruit weight, palm stearin and palm olein when level of beeswax set at 2.61% (w/v).

was set at 2.61% (w/v). It can be observed that the weight loss decreased to a lowest area of about 8% as palm stearin increased from 2 to 4% (w/v). However, the weight loss increased to about 8 to 10% as palm stearin increased from 4.3 to 5% (w/v).

The optimization module was evaluated with the aid of software (Stat-Ease Inc., Minneapolis, USA) by a combination of variables levels that simultaneously satisfy the requirements placed on the response and variables. Based on economic reason, we decided to set the minimum goal for beeswax at 1% (w/v). The goals for both palm stearin and palm olein were set in the range of study. Beside that, the lower limits of variables were extended backward from 2-5 to 1-5% as permitted by the software. Results showed that the optimum composition for minimizing guava weight loss was identified as palm stearin 4.5% (w/v), palm olein 1% (v/v) and beeswax 1% (w/v). Using this optimized coating composition, the predicted response was 7.18% weight loss of guava after 10 days of storage period.

**Model verification:** Model verification was performed by additional four sets of independent trials using the mentioned composition and *t*-test was carried out to determine the validation of the experimental results as compared to the predicted value from RSM. The predicted value suggested by the module and experimental results gave identical values which indicate that the results of validation parameters were reliable and satisfactory. In this study, RSM successfully optimized the coating composition used to minimize weight loss of coated guava compared to uncoated guava. Thus, the use of optimum composition provides acceptable alternative for post harvest control of weight loss of guava during storage.

Optimized coating composition was used to compare the weight loss with uncoated guava (Fig. 4). Both coated and uncoated guava showed a linear increase in weight loss percent against days of storage. However, weight loss for uncoated guava was faster (eight-fold) compared to that of coated guava (five-fold) within 10 storage days. On day 8, the weight loss was about 25% for uncoated guava. Mello Prado *et al.* (2005) reported the weight

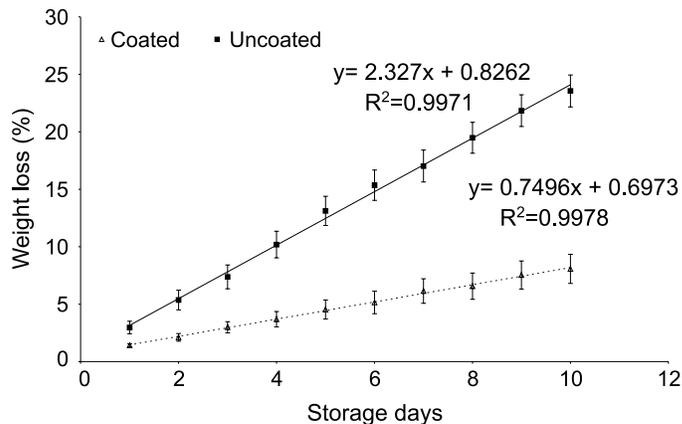


Fig. 4. Fruit weight loss for uncoated and coated guava using optimized composition of stearin 4.5% (w/v), olein 1% (v/v) and beeswax, 1% (w/v).

loss of guava (cv. Paluma) reached 18 and 22% on day 7 and 8, respectively, while Singh and Chauhan (1982) reported an 18% weight loss with guava cv. L-49 after 8 days of storage at room temperature. However, Adsule and Tondon (1983) observed that the weight loss of guava (cv. Allahabad) reached 30%. These differences occurred due to the variation in climatic conditions and the cultivars used by the authors.

The study revealed that weight loss of uncoated guava was 3 times higher after 8 days of storage as compared to coated guava. Thus, the use of optimum composition of edible coating provides acceptable alternative for post harvest control of weight loss of guava during storage.

## Acknowledgements

Thanks are due to University of Kuala Lumpur, Malaysian Institute of Chemical and Bioengineering Technology (UniKL-MICET), Melaka and Science Fund of Malaysian Government (Project number: 03-01 – 01-SF0033) for financial support. Also, thanks to Cargill Palm Products Pte. Ltd., Port Klang, Malaysia, for providing RBD palm stearin and RBD palm olein.

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Received: December, 2009 Revised: April, 2010 Accepted: August, 2010