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## Research Article

# **Effect of Beeswax Coating on the Quality of Eggplant Fruit During Cold Storage and Optimization of Coating Conditions**

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Eggplants are highly nutritious and feature prominently in food recipes in the tropics. However, losses in weight, firmness, and nutrients affect the quality of the fruit after harvest. This work investigated the use of beeswax as a potential coating to improve the quality of eggplant fruit. Eggplant fruit were coated with a 3% beeswax solution alone or in combination with a citrate solution and stored at 10°C for 17 days. The coating significantly minimized weight loss, preserved firmness, and delayed colour changes. No changes in total phenols and antioxidant capacity were observed upon coating; however, ascorbate levels decreased. Using a response surface methodology approach the optimum conditions for the application of beeswax coating to improve the quality of eggplant fruit was investigated. The validated optimum coating conditions were 4.6% (w/v) coating concentration, 1% (w/v) citrate concentration, and 3 min coating duration. The validation experiment gave a high correlation coefficient ( $R^2$ ) of 0.93 between the predicted and measured physicochemical properties of the fruit. The optimized conditions can be useful in the preparation of a beeswax-based coating for improving the quality of eggplant fruit during cold storage.

## 1. Introduction

The application of coating agents is a technique that can be employed to extend the storage life and improve the quality of fruit after harvest. Coating agents are applied to surfaces to form thin films that regulate the exchange of water, gases, and volatile substances between fruit and the environment [1, 2]. This helps to reduce moisture and weight loss, improve colour while also helping to preserve firmness. Coatings can also help preserve the biochemical quality and help reduce incidences of fruit decay, especially when applied in conjunction with antimicrobial agents [2].

Coating agents are mainly derived from natural and biodegradable materials as well as food-grade additives [2, 3]. Coating agents can be obtained from carbohydrates, proteins or lipids as well as a combination of these sources (composite coatings). However, coating agents derived from

lipid-based materials offer the most effective barrier against water loss due to their hydrophobic nature [2]. Among the lipid-based coatings, petroleum-based waxes such as paraffin, polyethylene, and mineral oils have been used extensively to coat fruit [4]. The application of petroleum-based waxes is, however, limited by the fact that they can only be applied to fruit where the peel is not ingested [4]. This limitation has raised interest in the use of edible coatings derived from natural lipids and waxes such as beeswax [5].

Several studies have reported the use of beeswax as a coating agent for various fruit. Hassan et al. [6] reported the use of beeswax coating to improve the quality of tangerine during storage. Beeswax has also been used to coat oranges [7], strawberries, apricot [8], and plums [9]. Additionally, the use of beeswax to help improve the quality of lemon [10], mango [11], and lime [12] has been investigated. The findings of these works

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affirm the potential of beeswax as an edible coating agent for fruit. In addition, using beeswax may offer economic benefits, especially in developing countries compared to the high cost of commercial coating agents.

In this work, the possible use of beeswax as a coating agent to improve the quality of eggplant fruit during storage is explored. Eggplant is an important fruit consumed on a daily basis by both rural and urban families in West Africa and other tropical regions [13]. The fruit is used for the preparation of stews and soups, and its nutritional content is comparable to tomato. The cultivation and sale of eggplant fruit also serve as a major source of income for several households [14]. According to Horna et al. [15], eggplant is one of the most important vegetable crops in West Africa and the third most consumed in Ghana. The production of eggplant, however, is constrained by the high loss in quality of the fruit after harvest [13].

The objective of this study was therefore, first, to investigate the potential of beeswax as a coating agent for improving the quality of eggplant fruit during storage. Secondly, based on the results obtained, this study also sought to determine the optimal coating conditions needed for the application of beeswax as a coating agent for eggplant fruit.

#### 2. Materials and Methods

2.1. Plant Material and Reagents. Eggplant fruit (Solanum aethiopicum L.) of known provenance (grown under standard agronomic practices) was harvested from a farm at Mankessim in the Central Region of Ghana. Beeswax was purchased from Honey Centre, a honeybee farm in Saltpond, Ghana, while commercial cooking oil (Frytol®, Unilever Ghana Ltd.) was purchased from a local market. Eggplant fruit were transported on the same day of harvest to the Analytical Laboratory of the School of Agriculture, University of Cape Coast. Sound fruit were washed thoroughly with water and disinfected with 1% sodium hypochlorite solution, rinsed with sterile water, and air-dried at room temperature.

### 2.2. Effect of Beeswax Coatings on the Quality of Eggplant Fruit

2.2.1. Preparation of Beeswax Coating. The beeswax coating (3% w/v) was prepared by melting an appropriate amount of the wax in a commercial oil (Frytol®) at low heat. This concentration of beeswax was selected based on works reported for other fruits [7–9]. The melted beeswax solution was cooled to room temperature prior to coating the fruit.

2.2.2. Coating of Eggplant Fruit. The coating was carried out by dipping the fruit in the beeswax solution for 5 min, followed by air drying for 1 h. Similar to our previous study [16, 17], the effect of pretreating eggplants with an antimicrobial agent (1% citrate treatment for 5 min) prior to the beeswax coating was investigated. All fruit was stored in an incubator at  $10^{\circ}$ C (RH  $88 \pm 5\%$ ). Sampling was carried out periodically to determine the changes in the physicochemical properties of the fruit. The

physicochemical properties analyzed included weight changes, firmness, and colour as well as total phenols, ascorbates, and antioxidant capacity. Uncoated fruit without citrate pretreatment was used as a control. Using a randomized complete block design (RCBD), the treatments were designated as control, beeswax-coated, and citrate-treated beeswax-coated fruit. Each treatment consisted of four replicates with 60 fruits in each replicate.

2.2.3. Physicochemical Analysis. Physicochemical analyses were carried out as described previously [16]. Briefly, changes in weight were measured with an analytical balance while changes in colour and firmness were measured with a colour reader (CS-10, CHN Spec, China) and a digital fruit penetrometer (GY-4, Tsingtao Tokyo Instruments Co., Ltd, China), respectively. The changes in colour of the fruit ( $\Delta E$ ) were estimated as described by Ampofo-Asiama et al. [18].

Total phenolic content and total antioxidant capacity were analyzed based on the Folin-Ciocalteau and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagent-based assays, respectively. In the determination of total antioxidant capacity, 100  $\mu$ L fruit extract (methanolic extract) was mixed with Tris-HCl buffer (900  $\mu$ L of 50 mM solution at pH 7.4) and incubated with 2 mL DPPH solution (0.1 mM in methanol) in the dark for 30 min. A control without DPPH was prepared, and the absorbance of all samples was measured against a methanol blank at 517 nm (Jenway 6400, Bibby Scientific Ltd). Standard calibration curves were prepared using gallic acid and the total antioxidant capacity was expressed as milligrams of gallic acid equivalents per kg of fresh fruit [16, 17].

For the determination of total phenolic content, homogenized fruit (1 g) in 10 mL methanol was incubated for 2 h, and centrifuged for 20 min at 1,790 ×g. Using  $100 \,\mu\text{L}$  of a pooled supernatant (after two rounds of extraction), Folin Ciocalteau reagent (750  $\mu\text{L}$  of 10% (v/v)) and later sodium bicarbonate solution (750  $\mu\text{L}$  of 6%) were added. After 90 min of incubation, the absorbance was measured at 725 nm (Jenway 6400, Bibby Scientific Ltd.). Similar to the total antioxidant capacity, the total phenolic content was expressed as milligrams of gallic acid equivalent per kg of fresh fruit.

Ascorbic acid was measured based on the 2,4-dinitrophenolhydrazine assay [16]. Homogenized fruit samples (1 g) were mixed with a metaphosphoric-acetic solution (3% metaphosphoric acid in 8% acetic acid) and centrifuged for 15 min at 1,790 ×g. The supernatant (1.54 mL) was mixed with bromine water (90  $\mu$ L), thiourea (50  $\mu$ L of 10%), and 2,4-dinitrophenolhydrazine solution (390  $\mu$ L of 2%). After incubation and the addition of chilled  $H_2SO_4$  (2% in 4.5 M), the absorbance was measured at 521. Ascorbic acid was used in the preparation of standard curves.

## 2.3. Optimization of Beeswax Coating Conditions

2.3.1. Experimental Design. The optimal conditions for the application of beeswax as a coating agent for eggplant fruit were carried out based on the beeswax concentration  $[X_1]$  (%

w/v)], citrate concentration  $[X_2 \text{ (% w/v)}]$ , and coating duration  $[X_3 \text{ (min)}]$ . Using a Box-Behnken Design Response Surface Methodology (RSM), optimization experiments were carried out based on Table 1. The physicochemical properties of the fruit were measured at the end of the storage period (day 17) as outlined above. The obtained physicochemical data were modelled with second-order polynomials of the form shown in the following equation [17]:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i \le i \le j}^{k-1} \beta_{ij} x_i x_j, \tag{1}$$

where Y,  $\beta_0$ , and  $\beta_{i-\beta ij}$  represents the predicted response, model constant, and the regression coefficients, respectively.

2.4. Prediction and Validation of Optimized Coating Conditions. To obtain the ideal process conditions and their responses, the numerical optimization approach was used. The overall desirability index (DI) (ranging from 0 to 1, with 0 being the least desirable) was also predicted using the same approach. The goal was to maximize the DI value. As such, goals for independent variables (coating concentration, citrate concentration, and coating duration) were set at any level within the range of the design values. On the other hand, minimization of weight changes,  $a^*$  and  $b^*$  values and maximization of  $L^*$  value, firmness, total phenolic content, total antioxidant capacity, and ascorbic acid levels were set for the response variables. The predicted models were validated using the optimized conditions of the independent variables in a new experiment.

2.5. Statistical Analysis. Data analysis on the effect of beeswax coating on the quality of eggplant fruit was carried out using analysis of variance (ANOVA) in SPSS (IBM, SPSS Statistics 20). Where a significant effect of coating was observed, post hoc analysis was carried out using the Tukey test. Additionally, Pearson correlation coefficients were estimated, and principal component analysis (PCA) performed using Unscrambler (version 10.4; CAMO A/S, Trondheim, Norway) as described previously [19]. Optimization studies were carried out using Design-Expert® 11 Software (Stat-Ease, Inc.). All model parameters were tested at a significant level of 0.05.

#### 3. Results and Discussion

3.1. Effect of Beeswax Coating on the Quality of Eggplant Fruit during Storage

3.1.1. Changes in Weight, Firmness, and Colour. The effect of coating on the changes in weight, firmness, and colour of the fruit during storage is shown in Figure 1. The initial average weight of the fruit was 48.85 g. Losses in weight were observed for all fruit during storage (Figure 1(a)), as reported in other studies [20, 21], albeit at different rates with the highest weight loss observed towards the end of storage. The rate of weight loss in the control fruit was higher compared

to the coated fruit, implying that beeswax coating minimized weight loss of eggplant fruit during storage. Similarly, beeswax coating was effective in reducing the weight loss of oranges [22], strawberries and apricots [8], plums [9], mango [11], and cucumber [23].

The reduced weight loss of the beeswax-coated fruit could be due to the fact that lipid-based coatings are good barriers to water vapour [24, 25]. Bourtoom [26] reported that the primary function of lipid coating is to block the transport of moisture due to their relative low polarity. This could be achieved by interfering with the function of lenticels as well as impeding metabolic processes, such as respiration, which leads to water loss [27]. Prevention of weight loss by beeswax coating is a breakthrough for eggplant fruit since weight loss is one of the primary factors responsible for the loss of quality of eggplant fruit during storage.

Firmness of the fruit generally decreased with increased duration of storage, although higher losses were observed in the control compared to the coated fruit (Figure 1(b)). With an average initial firmness of 34.42 N, more than 25% loss of firmness was observed at the end of the storage period in the control fruit compared to 18 and 10%, respectively, for the beeswax-coated and the citrate-treated beeswax-coated fruit. This observation is similar to that made by Shahid et al. [7], who reported that beeswax coating preserved the firmness of sweet oranges compared to uncoated fruit.

The high loss of firmness in the control fruit may be due to the relatively higher weight losses observed in these fruit. Also, enhanced ripening leading to fruit softening may have resulted in firmness loss in the control fruit. Softening of fruit during ripening is associated with changes (solubilization and depolymerization) in cell wall polysaccharides, which are driven by enzymes involved in cell wall metabolism such as pectate lyases, pectinesterases, and polygalacturonases [28, 29]. Indeed, the essential role of pectin, a cell wall polysaccharide, in helping maintain fruit firmness has been confirmed through several studies [28, 29], as well as using metabolomics and transcriptomics [30]. The delayed firmness loss in the coated fruit could be due to changes in the activities of enzymes involved in cell wall metabolism due to modifications in the internal gas composition of these fruits [31].

Colour is one of the most important quality attributes of fruit [32]. In this study, whitish-cream eggplant fruits which changes colour to yellowish-red with time were used. Figure 1(c) shows the effect of the different treatments on colour loss. The initial  $L^*a^*b^*$  values of the fruit were 80.91, -2.36, and 19.67, respectively. Colour loss was observed in all fruit during storage; however, at the end of the storage period, the highest colour loss was observed in the control fruit. This shows that beeswax coating can help reduce colour changes in eggplant fruit during storage. This observation is very important as colour is one of the most important factors affecting the price of eggplant fruit. In Ghana for instance, the yellowish-red eggplant fruit are usually sold at half the price of the white fruit [15].

TABLE 1: Response surface methodology Box-Behnken	design of experiment for process	s factors $(X)$ and their responses $(Y)$ for beeswax
coating.		

Runs		Factors*		Response (Y)**							
Kuiis	$X_1$	$X_2$	$X_3$	WL	$L^*$	$a^*$	$b^*$	F	TPC	AA	AOA
1	5	3	10	31.68	78.69	-1.7	25.3	32.86	133.53	98.34	131.55
2	5	1	6.5	26.96	80.84	-2.13	23.63	33.14	137.45	113.74	135.70
3	3	5	10	36.13	79.35	-2.05	28.32	33.45	146.60	143.21	136.37
4	3	3	6.5	35.95	81	-2.16	23.63	31.58	132.22	110.27	133.96
5	1	1	6.5	52.97	81.16	0.45	30.31	32.34	154.12	89.24	131.26
6	3	3	6.5	31.47	81.29	-2.32	23.21	31.5	132.88	95.09	133.39
7	1	3	3	52.90	81.19	-1.72	22.83	32.07	131.57	63.58	139.36
8	5	3	3	31.54	80.04	-1.98	25.12	32.57	161.63	89.07	129.05
9	3	3	6.5	31.54	81.77	-2.03	23.16	31.28	137.45	98.01	130.69
10	3	5	3	38.78	77.98	-1.72	28.45	33.49	_	66.06	131.26
11	3	1	3	41.37	80.69	-1.47	22.76	33.13	161.96	130.13	150.74
12	3	3	6.5	31.40	81.4	-2.04	23.87	31.21	137.45	110.27	134.54
13	1	3	10	46.63	80.54	-2.19	26.68	33.14	151.18	69.21	117.86
14	3	1	10	31.22	80.56	-1.76	26.04	33.67	104.12	98.51	121.53
15	1	5	6.5	39.08	80.38	-1.98	25	33.27	128.95	80.85	123.94
16	5	5	6.5	31.35	78.61	0.74	32.99	33.28	130.59	82.45	121.53
17	3	3	6.5	35.95	81.57	-2.03	23.16	31.55	132.22	115.89	134.73

<sup>\*</sup>Factor:  $X_1$  (coating concentration, %w/v),  $X_2$  (citric acid concentration, %w/v), and  $X_3$  (coating duration). \*\*Response (Y): weight loss, WL (g/kg); firmness, F (N); colour ( $L^*a^*b^*$  values); total phenolic content, TPC (mg/kg); ascorbic acid, AA (mg/kg); and total antioxidant capacity, TOA (mg/kg).

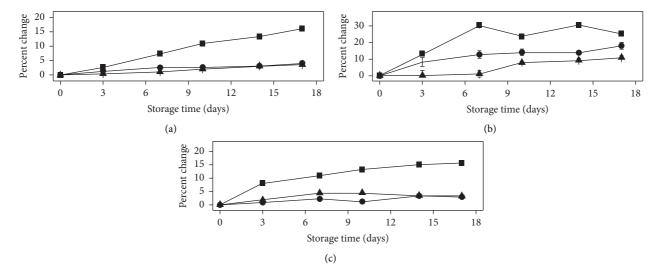


FIGURE 1: Changes in weight (a), firmness (b), and colour (c) of eggplant fruit during cold storage (control fruit (■); beeswax-coated fruit (●); citrate-treated beeswax-coated-fruit (▲)).

Colour changes in fruits during storage are related to the synthesis of pigments such as carotenoids. It was possible that the delayed colour changes in the coated fruits could be related to a reduction in the activity of enzymes involved in the synthesis of carotenoids due to modification in the internal gas composition of the fruit as a result of the beeswax coating affecting the diffusion of gases in the fruit. Thus, the ability of the beeswax coating to delay the synthesis of carotenoids [33] may have accounted for the reduced colour loss in the coated fruit. Mladenoska [8] and Nasrin et al. [10], similarly, observed that beeswax coatings containing coconut oil improved the appearance of strawberry and lemon fruits, respectively. It is possible that the barrier

created by the beeswax coating hinders oxygen and carbon dioxide diffusion resulting in a reduced respiration and other metabolic processes which might result in colour change.

3.1.2. Changes in Total Phenols, Ascorbate, and Antioxidant Capacity. The effect of beeswax coating on ascorbate, total phenols, and antioxidant capacity is shown in Figure 2. The initial levels of ascorbate, total phenols and antioxidant capacity were 230.72, 127.88, and 122.07 mg/kg, respectively. The results in Figure 2 are expressed relative to these initial levels. Generally, no significant changes in total phenols (Figure 2(a)) were observed in the control fruit until at the

end of the storage period. However, significant reductions in total phenols were observed in the citrate-treated beeswax-coated fruit after 10 days of storage. There was no effect of coating on the antioxidant capacity of the fruit (Figure 2(c)). This observation agrees with previous reports that showed that antioxidant capacity of fruit remained steady or increased during storage [16, 20, 34, 35].

Ascorbate levels increased for the control fruit but decreased for beeswax-coated fruit (Figure 2(b)). The beeswax coating promoted ascorbate loss due to a possible modification of the internal gas atmosphere of the fruit, leading to the accumulation of carbon dioxide. Similarly, Wang [36] determined that high levels of carbon dioxide (resulting from lipid-based coatings) inhibited ascorbate synthesis in peppers stored at 13°C. Shiri et al. [22] also reported a decreasing pattern for ascorbate levels in fruit coated with beeswax. The higher ascorbate levels recorded for the control fruit are similar to the report in Ball [24], where uncoated bell peppers recorded higher ascorbate levels than lipid-coated fruit.

3.1.3. Principal Component and Correlation Analysis. A biplot of the first (PC1) against the second (PC2) principal components shows that the distinct effect of coating on the physicochemical properties of eggplant fruits can be observed by the clustering among the coated fruit away from the control (Figure 3).

Among the physicochemical properties,  $a^*$ -and  $b^*$ -values were closely associated with the control fruit, which is similar to the observations of Dadzie et al. [19] who studied the effect of Aloe vera coating on eggplant fruit quality. In addition, weight loss was also closely associated with the control fruit. This shows that weight loss as well as  $a^*$ -and  $b^*$ -values were highly affected in the control compared to the coated fruit.

A strong positive correlation coefficient was observed between weight loss and the  $a^*$ -and  $b^*$ -values. Also, similar to the observations of Dadzie et al. [19], a strong negative correlation was observed between weight loss and firmness,

thus confirming the observation that an increase in weight loss is associated with firmness reduction.

## 3.2. Optimization of Coating Conditions

3.2.1. Fitting and Validation of Models. Table 1 shows the experimental data used in the model fitting. The diagnostic test for total phenolic content revealed run 10 (Table 1) as an outlier and was, therefore, eliminated from the analysis. All responses were fitted with quadratic models, although reduced quadratic models were employed to improve the adequacy of some models. The models had p values less than 0.05 (i.e., significant) and the lack of fit test showed p values greater than 0.05 (i.e., not significant) (Table 2). Additionally, the models had high adequate precisions (greater than 4), indicating they were good models (Table 2). The correlation between the predicted and the actual response for the different physicochemical properties are shown in Figure 4. The predicted and adjusted  $R^2$  for all models were in a reasonable agreement having a difference of less than 0.2, except the predicted and adjusted  $R^2$  values for  $L^*$  value (Table 2) [17].

The measured and predicted physicochemical properties are shown in Table 3. The optimized coating conditions were beeswax and citrate concentrations of 4.62 and 1%, respectively, with a coating duration of 3 min. These optimized coating conditions resulted in an overall desirability of 0.883. The difference between the measured and predicted weight change, firmness,  $L^*$  and  $b^*$  values as well as antioxidant capacity were all less than 3%. The predicted  $a^*$  value, total phenols, and ascorbate levels changed by about 10, 26, and 20%, respectively, compared to the measured value. An overall  $R^2$  of 0.93 was observed between the measured and predicted physicochemical properties (Table 3) [17].

3.3. Models for Changes in Weight and Firmness. The models to explain the changes in weight and firmness are shown in equations (2) and (3), respectively.

Weight change 
$$\left(\frac{g}{kg}\right) = 33.36 - 8.76X_1 - 0.91X_2 - 2.38X_3 + 4.55X_1X_2 + 4.09X_1^2 + 3.37X_3^2$$
, (2)

$$\text{Firmness}\left(N\right) = 31.42 + 0.1288X_1 + 0.1513X_2 + 0.2325X_3 - 0.1975X_1X_2 + 0.4042X_1^2 + 1.18X_2^2 + 0.8318X_3^2. \tag{3}$$

Equation (2) shows that high coating  $(X_1)$  and citrate concentrations  $(X_2)$  along with longer coating durations  $(X_3)$  could decrease weight change of eggplant fruit, although the effect of citrate concentration was not significant (Supplementary Table 1). The relative impact of these factors on

weight change can be compared based on their regression coefficients. In this regard, coating concentration  $(X_1)$  had the highest regression coefficient (8.76), implying that this factor had the highest effect on weight change. All three factors, however, had an opposite effect on fruit firmness

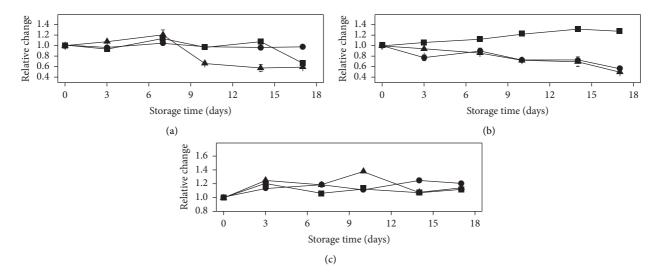


FIGURE 2: Changes in total phenols (a), ascorbate levels (b), and antioxidant capacity (c) of eggplant fruit during cold storage (control fruit (■); beeswax-coated fruit (●); citrate-treated-beeswax-coated-fruit (▲)).

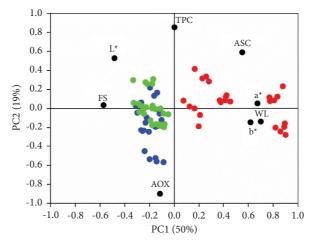


FIGURE 3: PCA bi-plot (PC1 is plotted against PC2) showing eggplant fruit at the different coating conditions. The samples scores (control fruit-red; beeswax-coated fruit-green; citrate-treated-beeswax-coated fruit-blue) is superimposed on the loadings (AOX-total antioxidant capacity; ASC-ascorbate; FS-firmness; TPC-total phenolic content; WL-weight loss;  $L^*$  value;  $a^*$  value;  $b^*$  value).

(Supplementary Table 2) although the interaction between coating and citrate concentration resulted in a slight decrease in firmness (Figure 4(a)).

3.4. Models for Fruit Colour. The colour  $(L^*a^*b^*)$  of the fruit were predicted using reduced quadratic models expressed in equations (4)–(7).

$$L^* \text{value} = 81.26 - 0.6363X_1 - 0.8663X_2 - 0.0950X_3 - 0.8324X_1^2 - 0.9649X_3^2, \tag{4}$$

$$a^* \text{ value} = -2.12 + 0.0463X_1 - 0.0125X_2 - 0.1012X_3 + 1.33X_1X_2 + 0.6193X_1^2 + 0.7667X_2^2 - 0.4008X_3^2,$$
 (5)

$$b^* \text{ value} = 23.40 + 0.2775X_1 + 1.50X_2 + 0.8975X_3 + 3.67X_1X_2 - 0.9175X_1X_3 - 0.8525X_2X_3 + 1.58X_1^2 + 2.99X_2^2. \tag{6}$$

Equation (4) shows that the main effects of coating concentration  $(X_1)$ , citrate concentration  $(X_2)$ , and coating duration  $(X_3)$  had negative regression coefficients, implying that increasing these factors could decrease  $L^*$  values, although the effect of coating duration was not significant

(Supplementary Table 3). However, equations (5) and (6) show that at high coating concentrations  $(X_1)$ ,  $a^*$  and  $b^*$  values could increase (Figure 4(b), Supplementary Tables 4 and 5). This shows that although waxing of fruit can delay respiration, ripening, and colour changes [7], high

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Table 2: Response	surface methodolog	v Box-Behnken	design	model fittir	ig statistics.

Model	F-value	p value	Lack of fit	R-squared	Adj. R-squared	Pred. R-squared	Adequate precision
Weight loss	20.02	<0.0001*	0.39**	0.92	0.88	0.73	15.54
Firmness	24.42	< 0.0001*	0.13**	0.95	0.91	0.75	13.6
$L^*$ value	10.22	0.0008*	0.06**	0.82	0.74	0.48	10.14
<i>a</i> * value	43.72	< 0.0001*	0.12**	0.97	0.95	0.84	20.31
<i>b</i> * value	55.21	< 0.0001*	0.08**	0.98	0.96	0.85	26.09
Total phenolic content	15.13	0.0005*	0.05**	0.93	0.87	0.55	15.99
Ascorbic acid	14.42	0.0002*	0.44**	0.87	0.81	0.66	12.12
Total antioxidant capacity	20.9	< 0.0001*	0.11**	0.93	0.88	0.72	20.36

<sup>\*</sup>Significant \*\*not significant.

Table 3: Predicted and measured physicochemical responses of the optimized coating conditions.

Responses	Measured	Predicted	
Weight loss (mg/kg)	$31.94 \pm 2.15$	31.93	
Firmness (N)	$33.71 \pm 1.26$	33.58	
$L^*$ value	$82.48 \pm 0.77$	80.20	
a* value	$-2.06 \pm 0.28$	-2.27	
$b^*$ value	$22.47 \pm 1.82$	22.18	
Total phenolic content (mg/kg)	$243.90 \pm 10.35$	179.78	
Ascorbic acid (mg/kg)	$107.31 \pm 14.34$	128.51	
Total antioxidant capacity (mg/kg)	$141.36 \pm 5.93$	143.57	

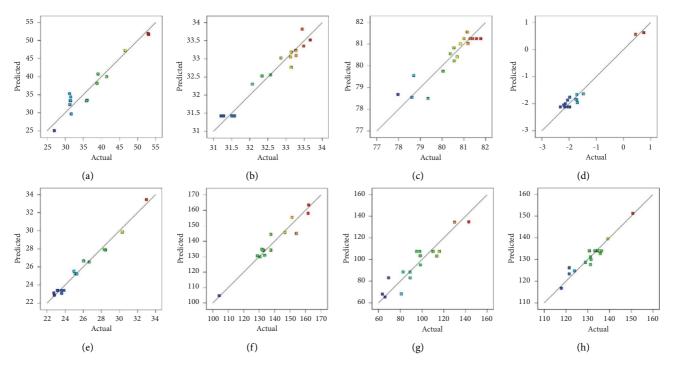


Figure 4: Correlation between predicted and actual values for (a) weight loss (g/kg); (b) firmness (N); (c) $L^*$  value; (d) $a^*$  value; (e) $b^*$  value; (f) total phenolic content (mg/kg); (g) ascorbic acid (mg/kg); and (h) total antioxidant capacity (mg/kg) of beeswax coatings.

concentrations of wax can lead to a modification of the internal gas atmosphere, resulting in undesirable characteristics associated with anaerobic metabolism such as changes in colour [37].

3.5. Models for Total Phenols, Ascorbate Levels, and Antioxidant Capacity. The models used to predict total phenols, ascorbate levels, and antioxidant capacity are shown in equations (7)–(9), respectively. The negative regression

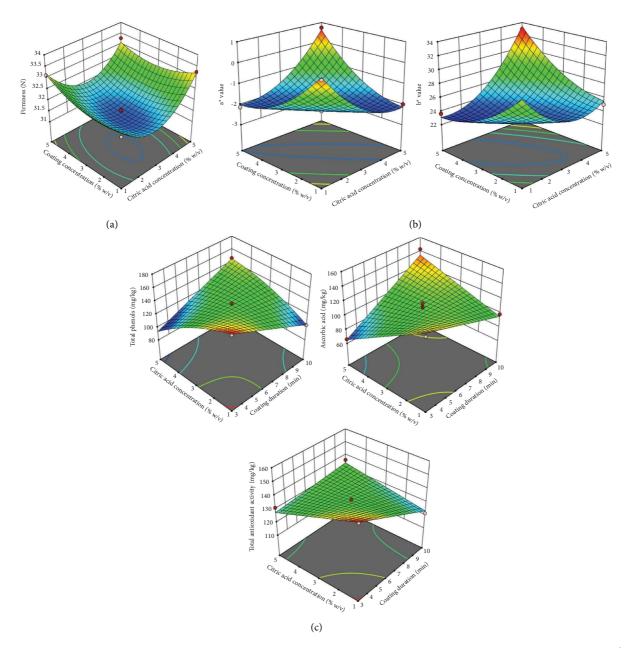


FIGURE 5: (a) Effect of coating concentration and citric acid concentration on firmness of eggplant fruit after 17 d storage period at  $10^{\circ}$ C. (b) Effect of coating concentration and citric acid concentration on  $a^*$  and  $b^*$  values of eggplant fruit after 17 d storage period at  $10^{\circ}$ C. (c) Effect of citric acid concentration and coating duration on total phenols, ascorbate levels antioxidant capacity of eggplant fruit after 17 d storage period at  $10^{\circ}$ C.

coefficients observed for coating concentration  $(X_1)$ , citrate concentration  $(X_2)$ , and coating duration  $(X_3)$  in equation (7) imply that increasing these factors will negatively affect total

phenols (Figure 5). However, the interaction between citrate concentration and coating duration can result in a significant increase in total phenols (Supplementary Table 6).

Total phenols 
$$\left(\frac{mg}{kg}\right) = 134.20 - 0.3273X_1 - 7.24X_2 - 1.66X_3$$

$$-11.93X_1X_3 + 27.71X_2X_3 + 10.59X_1^2 - 7.32X_2^2,$$
(7)

Ascorbic acid 
$$\left(\frac{mg}{kg}\right) = 107.49 + 10.09X_1 - 7.38X_2 + 7.55X_3 + 27.19X_2X_3 - 21.68X_1^2,$$
 (8)

Total antioxidant activity 
$$\left(\frac{mg}{kg}\right) = 134.13 + 0.6748X_1 - 3.27X_2 - 5.39X_3 + 6.00X_1X_3 + 8.58X_2X_3 - 5.35X_1^2.$$
 (9)

Ascorbate levels increased significantly when coating concentration  $(X_1)$  and coating duration  $(X_3)$  were increased. Also, the interaction between citrate concentration and coating duration had a significant increasing effect on ascorbate levels (Supplementary Table 7). Citrate concentration and coating duration had significant negative effect on antioxidant capacity, while the interaction effect between coating and citrate concentrations and citrate concentration and coating duration had a significant positive effect on antioxidant capacity (Figure 4(c), Supplementary Table 8).

#### 4. Conclusion

The coating of eggplant fruit with beeswax minimized weight and firmness changes and delayed colour changes during cold storage. Beeswax coating, however, did not have an effect on total phenols or antioxidant capacity. The results of optimization studies further confirmed that beeswax coating can significantly reduce weight and firmness changes in eggplants fruit, although undesirable colour changes may occur at high concentrations.

#### **Data Availability**

The data used to support the findings of this study are included within the article and the supplementary information files.

## **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

### **Supplementary Materials**

Supplementary Table 1: ANOVA table for reduced quadratic weight loss model of beeswax coating. Supplementary Table 2: ANOVA for reduced quadratic firmness model of beeswax coating. Supplementary Table 3: ANOVA for reduced quadratic  $L^*$  value model of beeswax coating. Supplementary Table 4: ANOVA for  $a^*$  value reduced quadratic model for beeswax coating. Supplementary Table 5: ANOVA for reduced quadratic model of  $b^*$  value for beeswax coating. Supplementary Table 6: ANOVA for reduced quadratic model of total phenolic content for beeswax coating.

Supplementary Table 7: ANOVA for response surface reduced quadratic model of the ascorbic acid levels of beeswax coating. Supplementary Table 8: ANOVA for response surface reduced quadratic model of the total antioxidant capacity of beeswax coating. (Supplementary Materials)

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