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Research Article

Using Response Surface Methodology to Optimize Edible Coating Formulations to Delay Ripening and Preserve Postharvest Quality of Tomatoes

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Tomato is a nutrient-rich but highly perishable fruit. In order to delay the rapid ripening and degradation of fruits and reduce postharvest losses, response surface methodology (RSM) was used as the optimizing method to formulate edible coating based on pineapple peel extract and Arabic gum of twenty concentrations of pineapple (0.5–0.83 kg/l) and 20 concentrations of Arabic gum (5–15%, w/v). Tomatoes were soaked for 10–30 min in any of the coating solution. Five parameters including ripening rate, chlorophyll *a* content, firmness, total flavonoid content, and titratable acidity of tomatoes were evaluated after 8 days of storage at $24 \pm 0.5^{\circ}C$ and $82 \pm 1.5\%$ relative humidity. Results showed that the experimental data could be adequately fitted into a second-order polynomial model with coefficient of determination (R^2) ranging from 0.775 to 0.976 for all the variables studied. The optimum concentrations were predicted as 0.70 kg/l pineapple peel extract and 17.04% with 18.72 min optimum time. Under these conditions, predicted values of response variables are as follows: ripening rate (RR) 40.75, chlorophyll *a* (Chl *a*) 8.11, firmness (Fir) 4.00, total flavonoid content (TFC) 43.51, and titratable acidity (TA) 0.30. It is concluded that RSM can be used to optimize pineapple peel extract and Arabic gum-based edible coating formulation to extend the shelf life or delay the ripening process of tomato fruit at ambient conditions.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most consumed fruits in the world [1]. It is important in human nutrition and health due to high nutrient content and significant amount of bioactive substances such as lycopene, ascorbic acid, tocopherols, folic acid, and flavonoids [2–4]. Due to its high nutritive value and water content, postharvest tomatoes are susceptible to diseases. These fruits are also sensitive to low-temperature storage [5, 6]. This leads to the loss of the quality parameters of the fruits such as color, texture, aroma, and appearance responsible for their commercial interest [4, 7]. Previous studies reported an increase in tomato's shelf life through modified atmosphere storage (relatively high CO_2 and low O_2) and controlled atmosphere storage [8], active packaging of cardboard [9], and genetic engineering [10]. In order to meet the increasing demand and consumption of minimally processed and additive-free foods, different means have been used to extend the shelf life of tomatoes such as edible coatings. Many edible coatings are made from waste agricultural resources through bio-production [11]. Edible coatings are thin layers of the edible component such as hydrocolloids

(polysaccharides and proteins), lipids (waxes and resins), and synthetic polymers, applied to the fruit's surface in addition to or as a replacement for natural protective waxy coatings [12]. They act as a physical barrier towards carbon dioxide, oxygen, and moisture movement for the fruits [13]. The uses of edible films and coatings containing synthetic antimicrobial agents, organic, and vegetable material have been shown to be useful in preserving the quality of tomatoes [13, 14]. Moreover, these materials have been used to incorporate functional ingredients such as antioxidants, antimicrobial agent, plants extract, byproduct extract, and nutraceuticals in fruits [15–19]. Pineapple (*Ananas comosus* L. Merr.) is a fruit rich in several nutrients and bioactive compounds including vitamins C, calcium, and nonvolatile organic acids such as malate and citrate which has been used to preserve the postharvest quality of tomato and strawberry [20–23]. Luo et al. [24] reported that the polysaccharides contained in pineapple peels have some degree of antioxidant activity. Arabic gum (AG) has been used as barrier to CO_2 and O_2 in some edible coating formulations to extend the shelf life of certain fruits such as guava, mango, and tomato [17, 25, 26]. Response surface methodology (RSM) was used to study properties of edible films and the main formulation that have effects on the preservation shelf-life and quality of some fruits [27, 28]. Therefore, the objective of this study was to use the RSM to determine the optimum concentrations of aqueous extract of pineapple peel and Arabic gum as well as the optimal time to coat tomato in order to increase shelf life and reduce postharvest losses.

2. Materials and Methods

2.1. Biological Material. Healthy tomatoes at the mature green stage (Figure 1) were collected from the local farm in Dschang (Cameroon) and kept in the laboratory. The pineapples used as coating material were harvested from farmers field in Melong (Cameroon) at ripening stage four (with low sugar content) [29]. Arabic gum was harvested on *Acacia Senegal* plants in Garoua, northern Cameroon [30].

2.2. Coating Preparation. Pineapples were peeled after washing with water. The peels were dried in the shade, scrambled in the mill, and then grounded to obtain a homogeneous paste. Different quantities of paste were weighted (Table 1) and macerated in a water/ethanol mix (1/1, v/v). 230 μl/l of bleach was added to disinfect the medium. The macerate was transferred on a sieve for filtration. In order to thicken the extract and form an adhesive and transparent film on the surface of the tomatoes, Arabic gum was added to of filtrate as coating matrix according to the quantities shown in Table 1. The mixtures were macerated for 15 hours, allowing pineapple peel extract to adhere to the Arabic gum.

2.3. Coating. Tomatoes were washed and soaked in the different coatings for 10, 20, or 30 minutes depending on the experimental design for coatings T1 to T20 (Table 1). Ten fruits were left without coating as the control. All the fruits were left on the bench at room temperature (24 ± 0.5°C) and 82 ± 1.5% RH.

Figure 1: Mature green tomato fruits.

2.4. Analytical Methods. Two physical parameters and three physiological parameters including ripening rate (RR), firmness, total flavonoid content, chlorophyll *a* content, and titratable acidity (TA) of fruits were evaluated after 8 days of storage.

2.4.1. Ripening Rate. The ripening rate was evaluated by counting the number of red ripped fruits (Figure 2) at the 8th day after treatment according to ripening rate defined by The United Fresh Fruit and Vegetable Association, in cooperation with USDA [31].

2.4.2. Determination of Chlorophyll a Content. Quantitative analysis for chlorophyll *a* content in tomato pulp was performed using a Biochrom Libra S22 spectrophotometer. Chlorophyll *a* content was determined using the method described by Nagata and Yamashita [32]. Six (6) grams of the tomato pulp was crushed and introduced in a test tube, then 10 ml of acetone/hexane (4/6, v/v) was added. The mixture was stored at 4°C for 48 hours. Subsequently, chlorophyll *a* in the hexanolic extracts was detected by spectrophotometry at 663 and 645 nm. The chlorophyll *a* content was calculated using the following equation:

$$\text{Chlorophyll } a\,(\mu\text{g}/100\text{ ml}) = 0.0999\text{A}663 - 0.0989\text{A}645. \quad (1)$$

A663 and A645 are the absorbances at 663 nm and 645 nm, respectively.

2.4.3. Firmness. Firmness is the force (*N*) required to press the fruit against the tip of a penetrometer. Epicarp was removed at equatorial and top region of tomato fruits. The cylindrical tip of the penetrometer was pressed down gradually on tomato notches, and the measurements were read on the board of the penetrometer [23].

2.4.4. Total Flavonoid Content. The concentration of total flavonoids was measured using the aluminum chloride colorimetric method [33] with some modifications. One milliliter (1 ml) of filtered tomato juice was added to a 10 ml Erlenmeyer flask containing 4 ml of distilled water. Then,

Table 1: Center composite design (CCD) and experimental data obtained for the response variables studied.

Run	Independent variables			RR (%)	Chl *a* (*μ*g/g)	Fir (N)	TFC (*μ*g/ml)	TA (%)
	CPE (kg/l)	CGA (%)	Time (min)					
T1	0.83	5	30	46.67	7.02	3.91	59.64	0.275
T2	0.83	15	10	53.33	5.47	4.00	74.58	0.352
T3	0.83	15	30	40.00	7.80	4.00	78.79	0.291
T4	0.51	5	30	46.67	5.57	3.77	86.04	0.293
T5	0.83	5	10	46.67	6.15	3.93	76.47	0.293
T6	0.51	5	10	70.00	6.97	3.98	61.11	0.298
T7	0.51	15	10	66.67	8.37	3.93	37.45	0.283
T8C	0.67	10	20	36.67	8.37	4.00	40.92	0.278
T9	0.51	15	30	36.67	9.84	3.76	51.44	0.259
T10C	0.67	10	20	35.26	8.20	3.99	40.89	0.276
T11C	0.67	10	20	37.36	8.60	3.97	40.29	0.279
T12C	0.67	10	20	35.46	8.82	4.00	40.79	0.272
T13	0.67	0.955	20	36.67	3.65	3.86	49.54	0.299
T14	0.67	10	1.91	63.33	6.55	4.00	64.90	0.345
T15C	0.67	10	20	36.56	8.68	4.00	40.12	0.279
T16	0.381	10	20	50.00	11.98	3.90	57.85	0.26
T17	0.67	19.05	20	40.00	9.38	4.00	39.44	0.305
T18C	0.67	10	20	35.76	8.48	3.99	40.61	0.271
T19	0.67	10	38.09	33.33	8.30	3.75	67.06	0.300
T20	0.959	10	20	46.67	7.63	4.00	72.69	0.292

CPE: concentration of water/ethanol pineapple peel extract, CGA: concentration of gum Arabic, C: center point, RR: ripening rate, Fir: firmness, TFC: total flavonoid content, TA: titratable acidity, Chl *a*: chlorophyll *a*.

Figure 2: Red ripe tomato fruits.

0.3 ml of 5% $NaNO_2$ was added. After 5 min, 0.3 ml of 10% $AlCl_3$ was added. Finally, 2 ml of 1 M NaOH was added after 6 minutes; the volume was completed to 10 ml with distilled water. The solution was mixed thoroughly, and the absorbance was measured at 510 nm using a spectrophotometer. Flavonoid compounds were determined according to a catechin standard curve in *μ*g/ml.

2.4.5. Titratable Acidity. Forty milliliters of distilled water were added to 20 ml of tomato juice. The volume was made up to 40 ml with distilled water. After adjusting the pH to 8.1, the titrate value was measured and was used to calculate the titrable acidity following the method of Gharezi et al. [34].

$$\%\ \text{acid} = \frac{\text{Titrate value} \times \text{Normality} \times \text{M.eq.wt. of acid}}{\text{Volume of sample}} \times 100, \quad (2)$$

$$\text{Milli} - \text{equivalent weight of citric acid} = 0.06404.$$

2.5. Experimental Design and Statistical Analysis. RSM was used to generate the experimental design statistical analysis and regression model with the help of Minitab software. The central composite rotatable design (CCRD) with a quadratic model [35] was employed as Nandane et al. [28]. Each independent variable had three (3) levels: −1.809, 0 and +1.809 (Table 2). Six replicates of the center points were chosen in random order according to a CCRD configuration for three factors divided in two blocks. The p values in the design outside the ranges were selected for rotatability of the design [36]. The center points for these designs were selected with ingredients at levels expected to yield satisfactory experimental results. Twenty (20) edible coating formulations with different concentrations of pineapple peel extracts

Table 2: Level of independent variables used for the center composite design (CCD).

Independent variable	Symbol	Level			
		$-\alpha$ (–1.809)	Low	High	α (1.809)
CPE (kg/l)	X1	0.381	0.5	0.83	0.959
CGA (%)	X2	0.955	5	15	19.045
Time (min)	X3	1.909	10	30	38.091

CPE: concentration of pineapple peel extract in water/ethanol (1/1, v/v) solvent mixture, CGA: concentration of gum arabic.

(0.5–0.83 kg/l), Arabic gum (5–15%), and soaking time 10–30 min were designed. The response functions (y) measured were physiological weight loss, firmness, total soluble solid contents and titratable acidity, total flavonoids, and proteins levels of tomatoes. The response values are related to the coded variables (x_i, $i = 1$, 2 and 3) by a second-degree polynomial equation given as follows:

$$Y_i = a_0 + a_1 X_1 + a_2 X_2 + a_3 X_3 + a_{12} X_1 X_2 + a_{13} X_1 X_3 + a_{23} X_2 X_3 + a_{11} X_1^2 + a_{22} X_2^2 + a_{33} X_3^2. \quad (3)$$

The coefficients of the polynomial equation were represented by a_0 (constant term), a_1, a_2, and a_3 (linear effects), a_{12}, a_{13}, and a_{23} (interaction effects), and a_{11}, a_{22}, and a_{33} (quadratic effects).

The analysis of regression was made and regression tables were generated; the effect and regression coefficients of individual linear, quadratic, and interaction terms were determined. The significance of all terms in the polynomial equation was appreciated statistically by computing the F-value and comparing response variables at standard significance levels of 0.1, 0.05, 0.01, and 0.001. Because tomato fruits are perishables, agricultural products with big variations in the quality attribute between one another [27]. The adequacy of the model was determined using regression coefficient (R^2) analysis. Using Minitab Software, numerical and graphical optimization procedures were applied to determine the optimum level of the independent variables.

3. Results and Discussion

3.1. Effect of Edible Coating on Ripening Rate of Tomato Fruits. As shown in Figure 3, the ripening rate (RR) of the coated tomato decreased with the pineapple peel extract concentration, while the optimum predicted time of treatment was 20 min. The CGA was fixed at 10%. Ali et al. [17] observed that fruit coated with 10% arabic gum delayed the ripening process by slowing down the rate of respiration and ethylene production. The regression coefficient table of RSM analysis with ripening rate as response variable is shown in Table 3. The model F-value of 69.25 obtained for the effect on ripening rate (%) of treated tomato fruit implies that this model was significant. Values of "Prob > F" less than 0.1 indicates significance of the model terms (Table 3). In this case, $a_1 a_3$, a_{13}, a_{11}, a_{22}, and a_{33} are significant model terms. Thus, the ripening rate is affected by linear effect of pineapple peel extract concentrations and time, interactions effects of time of treatment, and concentration of pineapple peel extract and by the quadratic effect of tree factors. The "Lack of Fit p-value" of < 0.001 implies that the Lack of Fit is significantly relative to the pure error. Thus, independent variables had a significant effect on the ripening rate. Observations from RSM analysis suggested that the ripening rate was negatively related to the concentration of the pineapple peel extract used (Figure 3). As the concentration of the pineapple peel extract in the solution increased, there was a relative decrease in ripening rate of the fruit. This shows that the calcium and antioxidants compounds in the pineapple extract may have induced the delay of ripening [37–40]. The final equation in terms of actual factors for ripening rate is as follows:

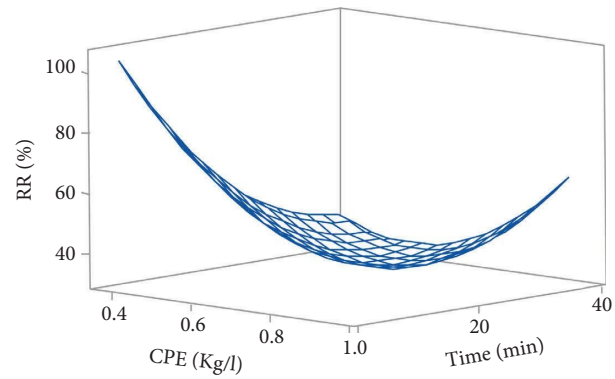

Figure 3: Response surface for ripening rate of coated tomato as function of concentration of pineapple peel extract and time of treatment.

Table 3: Regression coefficients, R^2, R^2 (adj), and probability values for four dependent variables.

Regression coefficients	RR	Chl a	Firmness	TFC	TA
Constant	220.7[c]	8.22[c]	4.043[c]	255.1[b]	0.374[c]
CPE	−349.5[c]	−15.1[c]	0.090[c]	−469.3[b]	−0.0195[c]
CGA	−1.86	1.185[c]	−0.0039[c]	−11.27[b]	−0.0151[a]
Time	−4.329[c]	−0.041[c]	−0.011[c]	−1.00	−0.0025
CPE ∗ CPE	4.76[c]	10.20	−0.562[b]	348.0[c]	−0.0284
CGA ∗ CGA	1.71	−0.0298[c]	−0.0008[c]	0.102	0.00029[c]
Time ∗ time	4.76[c]	−0.0046[b]	−0.0004[c]	0.091[c]	0.00013[c]
CPE ∗ CGA	1.66	−0.872[b]	0.0342[b]	11.80[c]	0.0194[c]
CPE ∗ time	5.00[b]	0.244	0.0282[c]	−4.03[b]	−0.00392[c]
CGA ∗ time	−2.50	0.0108	0.0001	0.025	−0.00015[c]
R^2	0.899	0.881	0.950	0.901	0.976
R^2 adj	0.809	0.775	0.905	0.812	0.955
Model F-value	69.25	28.23	9.41	866.44	3.06
Lack of fit (p value)	<0.001	0.001	0.014	<0.001	0.122

[c]Significant at 0.01 level. [b]Significant at 0.05 level. [a]Significant at 0.1 level. RR: ripening rate; Chl a: chlorophyll a; TFC: total flavonoid content; TA: titratable acidity.

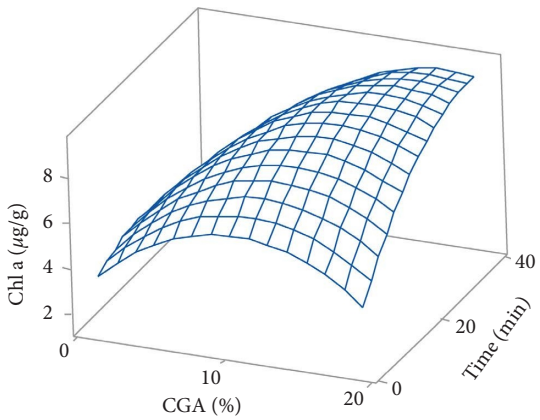

Figure 4: Response surface for chlorophyll *a* of coated tomatoes as function of Arabic gum concentration and time of treatment.

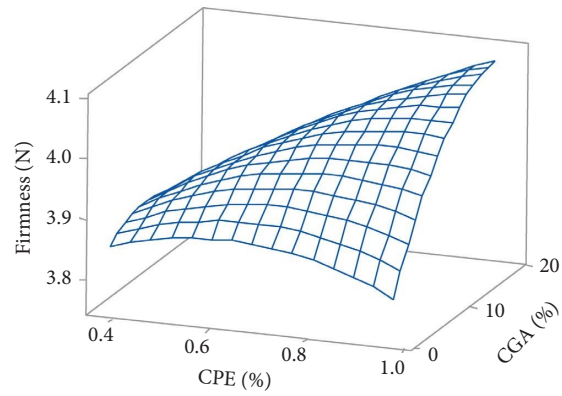

Figure 5: Response surface for firmness of coated tomatoes as function of pineapple peel extract concentration and time of treatment.

$$\begin{aligned}\mathrm{RR}\,(\%) = {} & 220.7 - 349.5\,\mathrm{CPE} - 1.86\,\mathrm{CGA} - 4.329\,\mathrm{Time} + 186.0\,\mathrm{CPE} * \mathrm{CPE} + 0.0683\,\mathrm{CGA} * \mathrm{CGA} \\ & + 0.0476\,\mathrm{Time} * \mathrm{Time} + 2.08\,\mathrm{CPE} * \mathrm{CGA} + 3.13\,\mathrm{CPE} * \mathrm{Time} - 0.0500\,\mathrm{CGA} * \mathrm{Time}.\end{aligned} \tag{4}$$

3.2. Effect of Edible Coating on Chlorophyll a in Tomatoes. Chlorophyll *a* is a more appropriate biomarker for evaluation of the ripening-retarding effects of edible coatings, because it is part of ripening process through the conversion of chlorophyll *a* and chlorophyll *b* into chlorophyllide *a* and then pheophorbide *a* before its complete degradation in nongreen products [11, 37]. As shown in Figure 4, concentration of chlorophyll *a* gradually increased with Arabic gum and with the increase of time of treatment. The regression coefficient table for RSM analysis for chlorophyll *a* as response variable is shown in Table 3. The *F*-value (28.23) of the model implies that this model is significant. In this case, the value of chlorophyll *a* was influenced by concentrations of Arabic gum and time of treatment. But only the coefficient of the linear term effect of time of treatment was significant ($p < 0.1$). The evolution ripening rate was confirmed by alterations level of chlorophyll *a* (Figure 3). The treatment applied induced the inhibition of chlorophyll *a* breakdown at different rate, resulting in the delaying of the ripening process. The final equation in terms of actual factors for chlorophyll *a* is as follows:

$$\begin{aligned}\text{Chlorophyll } a\,(\mu\mathrm{g/g}) = {} & 8.22 - 15.1\,\mathrm{CPE} + 1.185\,\mathrm{CGA} - 0.041\,\mathrm{Time} + 10.20\,\mathrm{CPE} * \mathrm{CPE} - 0.02980\,\mathrm{CGA} * \mathrm{CGA} \\ & - 0.00466\,\mathrm{Time} * \mathrm{Time} - 0.872\,\mathrm{CPE} * \mathrm{CGA} + 0.244\,\mathrm{CPE} * \mathrm{Time} + 0.01085\,\mathrm{CGA} * \mathrm{Time}.\end{aligned} \tag{5}$$

3.3. Effect of Edible Coating on the Firmness of Tomatoes. As shown in Figure 5, the response surface firmness of fruits increased with Arabic gum concentration in the coating solution. Firmness was affected by pineapple peel extract concentration and time of treatment. The regression coefficient table for RSM analysis with firmness as response variable is shown in Table 3. The *F*-value (9.41) obtained on firmness of treated tomato fruit implies that this model is significant. Ali et al. [41] showed that tomato fruit coated with Arabic gum at 10% resulted in a significant delay in change of firmness. Low levels respiration gas (O_2, CO_2) exchanges limit pectin esterase and polygalacturonase activities and allow retention of the firmness. Calcium ion, as a firming agent, in edible coatings could improve the rigidity of the cell wall of coated fruits [37, 39]. The final equation in terms of actual factors for firmness is given as follows:

$$\begin{aligned}\mathrm{Firmness}\,(N) = {} & 4.043 + 0.090\mathrm{CPE} - 0.0039\mathrm{CGA} - 0.01105\mathrm{Time} - 0.562\mathrm{CPE} * \mathrm{CPE} - 0.000849\mathrm{CGA} * \mathrm{CGA} \\ & - 0.000380\mathrm{Time} * \mathrm{Time} + 0.0342\mathrm{CPE} * \mathrm{CGA} + 0.02823\mathrm{CPE} * \mathrm{Time} + 0.000147\mathrm{CGA} * \mathrm{Time}.\end{aligned} \tag{6}$$

3.4. Effect of Edible Coating on Total Flavonoid Content of Tomatoes. Figure 6 shows that the total flavonoid content (TFC) value is increased with pineapple peel extract concentration in the coating solution. This parameter decreased with the time of treatment. The flavonoid content value was affected by interaction effect of pineapple peel extract and Arabic gum, quadratic effect of pineapple peel extract, and quadratic effect of time of treatment in the coating formulation. The regression coefficient table for RSM analysis of flavonoid content as response variable is as shown in Table 3. The *F*-value of 866.44 obtained implies that the model is significant. Flavonoid compounds are secondary

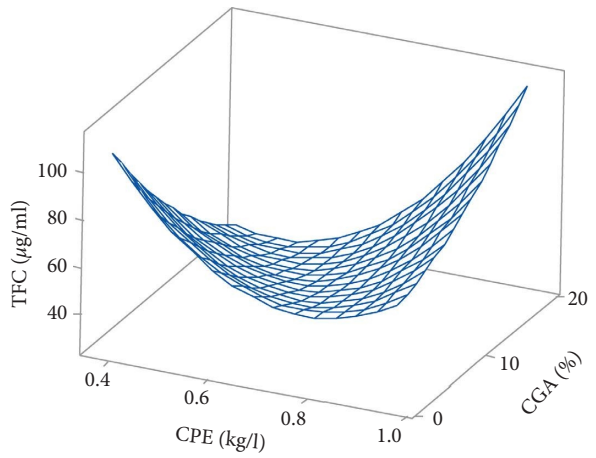

Figure 6: Response surface for total flavonoid content of coated tomatoes as function of pineapple peel extract concentration and Arabic gum concentration.

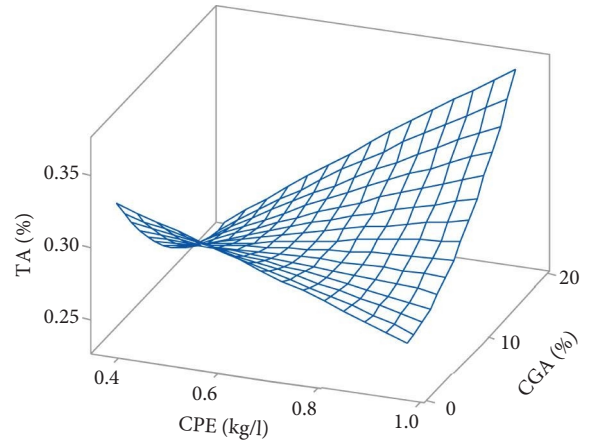

Figure 7: Response surface for titrable acidity of tomatoes as function of pineapple peel extract concentration and Arabic gum concentration.

metabolites in plants with antioxidant capacities that can be produce during abiotic stress by edible coating in tomatoes [13]. The final equation in terms of actual factors for flavonoid content is as follows:

$$\begin{aligned}\text{Total flavonoid content}\,(\mu\text{g/ml}) = {} & 255.1 - 469.3\,\text{CPE} - 11.27\,\text{CGA} - 1.00\,\text{Time} + 348.0\,\text{CPE} * \text{CPE} \\ & + 0.1024\,\text{CGA} * \text{CGA} + 0.0913\,\text{Time} * \text{Time} + 11.80\,\text{CPE} * \text{CGA} \\ & - 4.03\,\text{CPE} * \text{Time} + 0.0252\,\text{CGA} * \text{Time}.\end{aligned} \tag{7}$$

3.5. Effect of Edible Coating on Titrable Acidity of Tomatoes. The titratable acidity (TA) values of coated fruit during storage were maintained with Arabic gum concentration and decreased with concentration peel extract (Figure 7), and the value of linear term was significant ($p < 0.1$). The TA value was positively related to Arabic gum concentration. Regression coefficient table for RSM analysis for titrable acidity as response variable is shown in Table 3. The F-value of 3.06 obtained implies that the model is not significant. The same was observed by Ali et al. [41] who reported that the arabic gum coating delayed ripening of tomato by providing a semipermeable film around the fruit. Since organic acids, such as malic or citric acid, are primary substrates for respiration, a reduction in acidity is expected in highly respiring fruit as reported by El-Anany et al. [42]. The final equation in terms of actual factors for TA is as follows:

$$\begin{aligned}\text{Titrable acidity (\%)} = {} & 0.3740 - 0.0195\,\text{CPE} - 0.01517\,\text{CGA} - 0.002492\,\text{Time} - 0.0284\,\text{CPE} \\ & * \text{CPE} + 0.000289\,\text{CGA} * \text{CGA} + 0.000134\,\text{Time} \\ & * \text{Time} + 0.01940\,\text{CPE} * \text{CGA} - 0.00392\,\text{CPE} * \text{Time} - 0.000155\,\text{CGA} * \text{Time}.\end{aligned} \quad (8)$$

4. Conclusion

Increasing concentration of pineapple peel extract and Arabic gum improved the thickness of edible coating and had important effects on their quality. The ripening rate was correlated with the alterations level of chlorophyll *a* which decreased simultaneously with the ripening of tomato fruits. The thickness of edible coating was confirmed by the correlation between the production of secondary metabolites as flavonoid compounds and the increasing of concentration of pineapple peel extract and Arabic gum. The optimum concentration of CPE, CGA, and time of treatment were predicted to be 0.70 kg/l, 17.04%, and 18.72 min, respectively, with predicted values of response variables denoted as RR 40.75%, chlorophyll *a* 8.106 μg/g, firmness 4.00 N, TFC 43.51 μg/ml, and TA 0.302%. Edible coating formulation with pineapple peel extract and Arabic gum can be used in extending the shelf life and delaying the ripening process of tomatoes at ambient conditions. The RSM method can be effective to study the effect of edible coatings on the ripening of tomato fruits postharvest.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interests.

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