

Optimization of alginate and gellan-based edible coating formulations for fresh-cut pineapples

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Abstract: The effects of alginate-based [sodium alginate, 0-2% (w/v), glycerol, 0-2% (w/v) and sunflower oil 0.025% (w/v)] and gellan-based [gellan, 0-1% (w/v), glycerol, 0-1% (w/v) and sunflower oil 0.025% (w/v)] edible coatings on fresh-cut pineapple were evaluated by response surface methodology (RSM). Weight loss, firmness and respiration rate were considered as response variables. The results showed that for all response variables the RSM models were significantly ($p < 0.05$) fitted. Regression models indicated R^2 (high coefficient of determination) values ranging from 0.931 to 0.955 and 0.949 to 0.968 for alginate and gellan-based coatings, respectively. There was no significant ($p > 0.05$) difference between predicted and experimental values. The overall optimum region predicted by RSM indicated that alginate and gellan-based coatings containing 1.29% (w/v) sodium alginate, 1.16% (w/v) glycerol and 0.56% (w/v) gellan gum, 0.89% (w/v) glycerol were optimized formulations respectively.

Keywords: Fresh-cut pineapple, edible coating, sodium alginate, gellan gum, response surface methodology

Introduction

Pineapple (*Ananas comosus*) is known as one of the most well-liked tropical fruits. Fresh-cut pineapple is nutritious, juicy, aromatic and very tasty (Montero-Calderon *et al.*, 2010). It is more convenient for consumer and has a commercial advantage, because thick peel and inedible crown are removed (Rocculi *et al.*, 2009). Consumer demand for fresh-cut fruits and vegetables is increasing very fast in the world market, and these days fresh-cut pineapple is found in many supermarkets (Marrero and Kader, 2006; Montero-Calderon *et al.*, 2008). However, the process of fruit peeling and cutting increases metabolic activities such as respiration rate and delocalization of enzymes and substrates. This may lead to deterioration such as browning, softening, decay, off-flavour and microbial growth, which in turn make the fresh-cut pineapple has short shelf life (Montero-Calderon *et al.*, 2008; Di Egidio *et al.*, 2009).

Edible coating is defined as edible material (protein, polysaccharide or lipid) that is used as a thin layer on the surface of foods (Gonzalez-Aguilar *et al.*, 2010). It can be applied to providing a selective barrier to oxygen, carbon dioxide and moisture, preserving fresh-cut vegetables and fruits, improving textural and mechanical properties, preventing flavour loss and carrying food additives (Tapia *et al.*, 2008). Several studies have been done to determine the effects of polysaccharide-based edible coatings on fresh-cut fruits such as mango (Chien *et al.*, 2007), papaya

(Tapia *et al.*, 2008), pear (Oms-Oliu *et al.*, 2008) and banana (Bico *et al.*, 2009). However, the information of edible coatings on fresh-cut pineapple is limited. Among polysaccharide-based edible coatings, alginate and gellan have ability to form insoluble polymers or strong gels and very good colloidal properties on reaction with metal cations such as calcium (Rojas-Grau *et al.*, 2008). Alginate is made from marine algae and it is a linear polymer consist of 1,4-linked- β -D-mannuronic and α -L-guluronic acid. Gellan gum is a tetrasaccharide with repeated unit of β -D-glucuronic and α -L-rhamnose in a molar ratio of 2:1:1 and it is obtained from the bacterium *Sphingomonas elodea* (Tapia *et al.*, 2008).

Response surface methodology is a set of statistical techniques for building models, designing experiments, searching the optimum conditions and evaluating the effects of factors (Manivannan and Rajasimman, 2011). Several studies have been done to optimize the edible coating formulations for vegetables and fruits (Avena-Bustillos *et al.*, 1994; Rojas-Grau *et al.*, 2007; Ribeiro *et al.*, 2007; Tapia *et al.*, 2008). However based on our knowledge, no article was published on using RSM for optimization of edible coating for fresh-cut pineapple.

The aim of this study was to optimize the alginate and gellan-based edible coating formulations for fresh-cut pineapple based on weight loss, firmness and respiration rate of the fresh-cut coated pineapple.

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Material and Methods

Materials

Fresh pineapples (*Ananas comosus* cv. Josapine) were purchased from Pasar Borong Selangor, Malaysia. Pineapple fruits of regular shape and uniform size without any defect were selected. The fruits with the maturity stage 5 (about 50% of eyes were orangey yellow, half ripe fruit) were used. The maturity stage was determined based on the Malaysian standard by Federal Agricultural Marketing Authority (FAMA, 2004).

Sodium alginate (Fisher Scientific, UK) and gellan gum (Sigma-Aldrich, USA) were used as polysaccharide-based edible coatings. Glycerol (Sigma-Aldrich, Germany) was applied for plasticizer. Sunflower oil (Sigma-Aldrich, Argentina) was added as emulsifier and lipid source for edible coating formulations. Calcium chloride (Sigma-Aldrich, Germany) was added for gel forming and cross-linking.

Preparation of samples and edible coating solutions

Sodium alginate powder was dissolved in distilled water by heating the mixtures using the stirring hot plate (70°C) until the solutions became clear and then glycerol as plasticizer was added to the solutions (Tapia *et al.*, 2008). Based on previous research by Montero-Calderon *et al.* (2008) 0.025% (w/v) Sunflower oil was used in edible coating formulations. The overall volume for each formulation was 500 ml and this includes different amounts of alginate, glycerol (Tables 1) 0.025%(w/v) sunflower oil and the rest was distilled water. The solutions were mixed with homogenizer (ultra Turrax T25, Germany) for 5 min at 24500 rpm to form emulsions then degassed using vacuum at 80 mbar. For cross-linking of polymers a 2% (w/v) calcium chloride solution was used (Montero-Calderon *et al.*, 2008). A similar procedure was also adopted for preparation of gellan-based formulations. The different concentrations of sodium alginate, gellan gum and glycerol based on the experimental design were shown in Tables 1 and 2.

Before preparation of samples, pineapples, all containers, cutting board, knives and other utensils in contact with pineapple were washed and sanitized with 0.1% sodium hypochlorite solution. After washing, pineapples were peeled manually and cut with a sharp knife into cubes of 2 cm (Rocculi *et al.*, 2009). The pineapple cubes were dipped in the alginate-based edible coating formulation (sodium alginate + glycerol + sunflower oil) for 2 min and then the excess coating materials in pineapple cubes were

Table 1. Experimental design for alginate-based edible coatings

Run	Block	Variables			
		Coded		Uncoded ^a	
		X ₁	X ₂	Alg	Gly
1	1	-1.41421	0.00000	0.00000	1.00000
2	1	1.41421	0.00000	2.00000	1.00000
3	1	0.00000	0.00000	1.00000	1.00000
4	1	0.00000	1.41421	1.00000	2.00000
5	1	0.00000	-1.41421	1.00000	0.00000
6	1	0.00000	0.00000	1.00000	1.00000
7	1	0.00000	0.00000	1.00000	1.00000
8	2	0.00000	0.00000	1.00000	1.00000
9	2	1.00000	-1.00000	1.70711	0.29289
10	2	0.00000	0.00000	1.00000	1.00000
11	2	-1.00000	-1.00000	0.29289	0.29289
12	2	1.00000	1.00000	1.70711	1.70711
13	2	-1.00000	1.00000	0.29289	1.70711
14	2	0.00000	0.00000	1.00000	1.00000

^a Uncoded Variables: Alg: Sodium alginate (% w/v), Gly: Glycerol (% w/v).

Table 2. Experimental design for gellan-based edible coatings

Run	Block	Variables			
		Coded		Uncoded ^a	
		X ₁	X ₂	Gel	Gly
1	1	0.00000	0.00000	0.50000	0.50000
2	1	0.00000	0.00000	0.50000	0.50000
3	1	-1.00000	1.00000	0.14645	0.85355
4	1	-1.00000	-1.00000	0.14645	0.14645
5	1	1.00000	-1.00000	0.85355	0.14645
6	1	1.00000	1.00000	0.85355	0.85355
7	1	0.00000	0.00000	0.50000	0.50000
8	2	0.00000	0.00000	0.50000	0.50000
9	2	0.00000	-1.41421	0.50000	0.00000
10	2	0.00000	0.00000	0.50000	0.50000
11	2	1.41421	0.00000	1.00000	0.50000
12	2	0.00000	0.00000	0.50000	0.50000
13	2	0.00000	1.41421	0.50000	1.00000
14	2	-1.41421	0.00000	0.00000	0.50000

^a Uncoded Variables: Gel: Gellan gum (% w/v), Gly: Glycerol (% w/v).

permitted to drip off. After that, coated pineapple cubes were dipped in the calcium chloride solution 2% (w/v) for 2 min. The polystyrene trays were used for packing the coated pineapple cubes and then trays were wrapped with PVC film. The packed pineapples were stored at 10±1°C; 65% RH for 10 days. Fresh pineapple cubes without any treatments were packed and stored at the same condition, served as control. Similar procedure was also carried out for coating of samples with gellan-based formulations.

Analytical methods

Sample weight loss was determined by comparing the weights of coated pineapple cubes after storage with initial weights and expressing the results as percentage (Chien *et al.*, 2007).

Firmness of pineapple cubes was evaluated with texture analyzer (TAXT2i, UK). Penetration tests were used by a 2 mm-diameter stainless steel cylindrical probe, 5 kg load cell and 0.5 mm s⁻¹ test speed. The maximum peak measured during the test was selected as firmness (Rocculi *et al.*, 2009).

Respiration rate was determined by O₂/CO₂ analyzer (Mocon inc., USA). 10 g of coated or uncoated pineapple cubes were placed in 200 ml glass jars and incubated at 10°C for 1 hour. The glass jar

had air-tight screw caps and rubber septum to allow headspace sampling. The calculation of respiration rate was based on the production of carbon dioxide (ml CO₂/kg.h) (Bhande *et al.*, 2008).

Statistical analysis and experimental design

Two polysaccharide-based edible coating, sodium alginate and gellan gum, were used in these experiments. For each type, response surface method was applied to study the effect of glycerol and polysaccharide (sodium alginate or gellan gum) content as independent variables on weight loss, firmness and respiration rate of coated fresh-cut pineapple. The center composite design (CCD) was used for optimization of edible coating formulations. The type of CCD was axial with 2 blocks and fourteen experimental runs. (Tables 1 and 2). For evaluation the repeatability of methods, the center point was repeated six times (Mirhosseini *et al.*, 2008). The linear, quadratic and interaction terms of independent variables in the response surface models were predicted by multiple regressions. For evaluation the relationship between the response and independent variables the generalized polynomial model was used as below:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j \quad (1)$$

In this model, Y is a calculated response; β_i , β_{ii} and β_{ij} are linear, quadratic and interaction coefficients, respectively and β_0 is a constant. Minitab 14 statistical package was used to perform data analysis, experimental design matrix, and optimization procedure (Minitab Inc., USA).

Verification and optimization procedures

Numerical and graphical optimization procedures were applied to determine the optimum level of two independent variables (x_1 and x_2). To verify the adequacy of the regression models the fitted values predicted by the models were compared with experimental data.

Results and Discussions

Response surface analysis for alginate and gellan-based edible coatings

The results of experimental data obtained by the response variables were shown in Table 3. Response surface methodology has the ability to determine main, quadratic and interaction effects of two edible coating components on each studied response variable. RSM suggested response surface models to show the relationship between independent variables and experimental data.

Effect of alginate, gellan, and glycerol contents on weight loss

Weight loss is almost similar to water loss because other components like gaseous products of respiration, aroma or flavour are practically undetectable in terms of weight (Olivas and Barbosa-Canovas, 2005). Edible coatings have potential to control the water loss of fresh-cut fruits (Gonzalez-Aguilar *et al.*, 2010). As shown in Table 3, weight loss of alginate-based edible coated fresh-cut pineapples varied from 10.50 ± 0.47 to 15.12 ± 0.55%. Uncoated pineapple cubes at the same condition exhibited a weight loss of 16.80±0.55%. The analysis of variance for final reduced models (Table 4) showed that the interaction, quadratic and main effects of alginate and glycerol on weight loss were significant (p<0.05). Table 3 shows weight loss of gellan-based coated fresh-cut pineapples varied from 10.16 ± 0.38 to 15.20 ± 0.75%. The quadratic and main effects of gellan gum and glycerol were significant (p<0.05) on weight loss (Table 4). The results of optimization based on weight loss indicated that the optimized formulations for alginate and gellan were [1% (w/v) sodium alginate, 1.22 % (w/v) glycerol] and [0.81% (w/v) gellan gum, 0.7% (w/v) glycerol] respectively. The results obtained show that increasing the concentration of alginate, gellan and glycerol decreases the weight loss. However, due to hydrophilic nature of alginate, gellan and glycerol, high concentrations led to increase in weight loss. The 3D surface plot of weight loss versus glycerol and alginate-based edible coating were shown in Fig.1. Rojas-Grau *et al.* (2007) reported that in their study of fresh-cut apple, WVR (water vapor resistance) for alginate and gellan-based coating increased when glycerol concentration increased. However, in high concentrations of glycerol, WVR decreased (water loss increased). In the present study, result obtained show that weight loss was significantly (p<0.05) lower in both optimized coating formulations as compared to control. Similar research indicated that gellan and sodium alginate-based coatings used on fresh-cut apples were effective to reduce the water loss when sunflower oil was applied in coating formulation as lipid source (Rojas-Grau *et al.*, 2008).

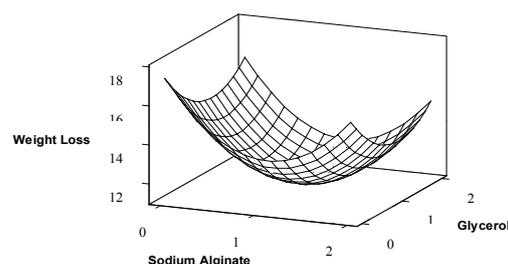


Figure 1. Response surface plot for weight loss as a function of sodium alginate and glycerol content

Table 3. Responses for alginate and gellan-based edible coatings

Edible coating	Run ^a	Responses		
		Weight Loss (%)	Firmness (N)	Respiration rate (ml CO ₂ /kg.h)
Alginate-based	1	15.12 ± 0.55	1.48 ± 0.22	81.81 ± 3.93
	2	14.41 ± 0.23	2.57 ± 0.17	44.80 ± 1.57
	3	10.90 ± 0.41	2.34 ± 0.22	35.06 ± 1.94
	4	12.50 ± 0.48	2.12 ± 0.20	49.00 ± 4.01
	5	13.85 ± 0.30	1.75 ± 0.16	65.90 ± 2.24
	6	11.54 ± 0.30	2.45 ± 0.18	42.17 ± 2.57
	7	11.67 ± 0.42	2.49 ± 0.16	40.83 ± 1.90
	8	11.45 ± 0.54	2.31 ± 0.21	38.45 ± 4.01
	9	12.72 ± 0.41	2.30 ± 0.15	43.77 ± 2.15
	10	11.50 ± 0.14	2.48 ± 0.17	41.50 ± 2.87
	11	15.07 ± 0.62	1.80 ± 0.23	70.90 ± 3.42
	12	12.93 ± 0.46	2.65 ± 0.23	43.37 ± 1.55
	13	12.99 ± 0.22	1.75 ± 0.20	60.10 ± 3.31
	14	10.50 ± 0.47	2.30 ± 0.15	39.80 ± 1.59
Gellan-based	1	12.10 ± 0.40	2.55 ± 0.22	28.40 ± 2.84
	2	11.08 ± 0.42	2.43 ± 0.12	33.50 ± 1.60
	3	13.30 ± 0.56	2.01 ± 0.17	58.20 ± 3.17
	4	14.70 ± 0.48	1.67 ± 0.24	66.00 ± 4.44
	5	11.90 ± 0.18	2.40 ± 0.12	35.00 ± 2.00
	6	10.16 ± 0.38	2.60 ± 0.26	30.20 ± 3.94
	7	10.90 ± 0.73	2.28 ± 0.12	37.10 ± 2.83
	8	11.75 ± 0.39	2.34 ± 0.21	33.00 ± 1.64
	9	14.00 ± 0.58	1.92 ± 0.14	50.50 ± 4.16
	10	11.70 ± 0.23	2.47 ± 0.19	28.24 ± 1.98
	11	11.60 ± 0.73	2.65 ± 0.21	41.80 ± 3.76
	12	11.65 ± 0.57	2.41 ± 0.16	30.90 ± 2.39
	13	12.30 ± 0.44	2.50 ± 0.16	33.16 ± 2.38
	14	15.20 ± 0.75	1.43 ± 0.17	79.80 ± 1.40

^aDetails for each run were shown in Tables 1 and 2

Effect of alginate, gellan and glycerol contents on firmness

Firmness is an important factor that influences the consumer acceptability of fresh-cut fruits and it is related to water content and metabolic changes (Rojas-Grau *et al.*, 2008). Table 3 shows firmness of alginate-based coated fresh-cut pineapples varied from 1.48 ± 0.22 to 2.65 ± 0.23 N. Firmness of uncoated fresh-cut pineapples after 10 days was 1.45 ± 0.18 N. The firmness of gellan-based edible coated fresh-cut pineapples also varied from 1.43 ± 0.17 to 2.65 ± 0.21 N (Table 3). The analysis of variance for final reduced models (Table 4) indicated that the quadratic and main effects of alginate, gellan and glycerol for both edible coating formulations on firmness were significant ($p < 0.05$). The results of optimization based on firmness indicated that the optimized formulations for alginate and gellan were [1.87% (w/v) sodium alginate, 1.66% (w/v) glycerol] and [0.58% (w/v) gellan gum, 1% (w/v) glycerol] respectively. Both alginate and gellan-based coatings were effective in controlling water loss and is a good carrier of calcium chloride as a firming agent. Hence, the optimized formulations were effective in maintaining the firmness of fresh-cut pineapples. A similar trend in the results was also reported by Rojas-Grau *et al.*, (2008) and Oms-Oliu *et al.*, (2008). They indicated that gellan and alginate-based edible coatings incorporated with calcium chloride had beneficial effect in maintaining the firmness of fresh-cut apples and melons.

Effect of alginate, gellan and glycerol contents on respiration rate

As shown in Table 3, respiration rate of alginate-based coated fresh-cut pineapples varied from 35.06 ± 1.94 to 81.81 ± 3.93 ml CO₂/kg.h. Respiration rate of uncoated fresh-cut pineapples at day 10 was 82.11 ± 2.55 ml CO₂/kg.h. The respiration rate for gellan-based coated fresh-cut pineapples varied from 28.24 ± 1.98 to 79.80 ± 1.40 ml CO₂/kg.h respectively (Table 3). The analysis of variance for final reduced models (Table 4) indicated that the quadratic and main effects of alginate, gellan and glycerol for both edible coating formulations on respiration rate were significant ($p < 0.05$). The results of optimization based on respiration rate indicated that the optimized formulations for alginate and gellan were [1.28% (w/v) sodium alginate, 1.63% (w/v) glycerol] and [0.68% (w/v) gellan gum, 1% (w/v) glycerol] respectively. Edible coating has potential to reduce respiration rate of fresh-cut fruits (Olivas and Barbosa-Canovas, 2005). It may be associated with decreasing the metabolism by increasing internal CO₂ concentration and decreasing internal O₂ concentration. This may also be associated with slowing down the rate of interchange of CO₂ and O₂ between the environment and coated fruits (Olivas and Barbosa-Canovas, 2005; Gonzalez-Aguilar *et al.*, 2010). The results of the present study showed that respiration rate was significantly ($p < 0.05$) lower in both optimized coating formulations as compared to control.

Table 4. Analysis of variance for final reduced models (p-value)

Source	Alginate			Source	Gellan		
	Weight loss	Firmness	Respiration		Weight loss	Firmness	Respiration
X ₁ (Alg)	0.027 *	0.000 **	0.000 **	X ₁ (Gel)	0.000 **	0.000 **	0.000 **
X ₂ (Gly)	0.018 *	0.044 *	0.014 *	X ₂ (Gly)	0.001 **	0.001 **	0.007 *
X ₁ ²	0.000 **	0.011 *	0.000 **	X ₁ ²	0.002 *	0.001 **	0.000 **
X ₂ ²	0.001 **	0.003 *	0.001 **	X ₂ ²	0.006 *	0.041 *	0.018 *
X ₁ X ₂	0.033 *	-	-	X ₁ X ₂	-	-	-
Regression	0.000 **	0.000 **	0.000 **	Regression	0.000 **	0.000 **	0.000 **
Lack of fit	0.725	0.185	0.175	Lack of fit	0.624	0.582	0.448
R ²	0.955	0.931	0.952	R ²	0.942	0.954	0.986
R ² (adjust)	0.917	0.887	0.922	R ² (adjust)	0.918	0.924	0.947

*Significant at p≤ 0.05
 **Significant at p≤ 0.001

Table 5. Regression coefficients for final reduced models

Model term	Alginate			Gellan		
	Weight loss	Firmness	Respiration	Weight loss	Firmness	Respiration
X ₀ (Constant)	18.55410	1.04448	98.4883	17.0701	1.10286	93.3005
X ₁ ^a	-8.21603	1.11499	-58.5034	-9.38011	2.48002	-147.703
X ₂ (Glycerol)	-5.10057	0.920533	-35.9924	-6.44016	1.20425	-45.0881
X ₁ ²	3.23375	-0.297500	20.7488	5.48000	-1.40333	107.843
X ₂ ²	1.64375	-0.387500	14.8938	4.48000	-0.723333	31.9633
X ₁ X ₂	1.14500	-	-	-	-	-

^a X₁: Sodium alginate in alginate-based edible coatings or gellan gum in gellan-based edible coatings

Optimization Procedure

For determination of the optimum levels of independent variables, multiple response optimizations were used. A numerical optimizer was applied to determine the exact value of multiple response optimization that led to the overall optimum condition. For better visualizing the variation of physico-chemical properties as function of main edible coating components, the 3D surface plot was used. Results obtained show that, in terms of all physico-chemical properties studied, the overall optimized region was predicted to be 1.29% (w/v) sodium alginate and 1.16% (w/v) glycerol respectively. For optimized condition, weight loss, firmness and respiration rate for sodium alginate-based edible coating were predicted to be 11.35 %, 2.53 N and 35.82 ml CO₂/kg.h respectively (Table 6). The predicted results indicated that the overall optimized region for gellan-based edible coating was achieved by formulation comprising of 0.56% (w/v) gellan gum and 0.89% (w/v) glycerol. Under the optimized condition, weight loss, firmness and respiration rate of gellan-based coated fresh-cut pineapples were predicted to be 11.36%, 2.55 N and 29.63 ml CO₂/kg.h respectively (Table 7).

Table 6. Predicted and experimental data for the responses at optimum point for alginate-based edible coating

Responses	Predicted value	Experimental value ^a	P value (t-test) ^b
Weight loss (%)	11.35	11.27±0.25	0.598
Firmness (N)	2.53	2.49± 0.13	0.377
Respiration rate (mlCO ₂ /Kg.h)	35.82	34.83± 2.61	0.504

^a Mean ± S.D.
^b No significant (p>0.05) difference between experimental and predicted value

Table 7. Predicted and experimental data for the responses at optimum point for gellan-based edible coating

Responses	Predicted value	Experimental value ^a	p-value (t-test) ^b
Weight loss (%)	11.36	11.48±0.33	0.504
Firmness (N)	2.55	2.48± 0.11	0.115
Respiration rate (mlCO ₂ /Kg.h)	29.63	31.17± 2.33	0.278

^a Mean ± S.D.
^b No significant (p>0.05) difference between experimental and predicted value

Verification of the Models

The comparison between fitted values predicted by the response regression models and experimental values indicated the adequacy of the response surface equations. Tables 6 and 7 show the predicted and experimental values. These values did not show any significant (p>0.05) difference. The predicted values were indicated to be in agreement with the experimental response values.

Conclusion

Response surface methodology was applied in this study to optimize the gellan and alginate-based edible coating formulations for fresh-cut pineapples. Second-order polynomial regression models were obtained for predicting the effects of sodium alginate, gellan gum and glycerol contents on weight loss, firmness and respiration rate of coated fresh-cut pineapples. All models were fitted significantly (p < 0.05) with R² > 0.90. The lack of fit for response variables in this study, was not significant (p >0.05). It shows the accuracy of proposed models is sufficient to evaluate the variability of responses. The optimum formulation predicted for alginate-based edible

coating was 1.29% (w/v) sodium alginate, 1.16% (w/v) glycerol and 0.025% (w/v) sunflower oil. On the other hand, for gellan-based edible coating, the predicted optimum formulation was 0.56% (w/v) gellan gum, 0.89% (w/v) glycerol and 0.025% (w/v) sunflower oil. The results obtained in this study indicate that after 10 days storage ($10 \pm 1^\circ\text{C}$, 65% RH), weight loss and respiration rate were significantly ($p < 0.05$) lower and firmness was maintained in both optimized coated samples as compared to control. Therefore, either formulation based on alginate or gellan has the potential in increasing shelf life and maintaining the freshness of fresh-cut pineapples. The optimum formulations obtained in this study can be used to determine the effects of alginate and gellan-based edible coatings on changes in physico-chemical, microbiological and sensory characteristics of fresh-cut pineapple during storage at low temperature.

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