Development of an edible coating based on chitosan-glycerol to delay 'Berangan' banana (*Musa sapientum* cv. Berangan) ripening process

[1]Jafarizadeh Malmiri, H., [1*]Osman, A., [2]Tan, C.P. and [2]Abdul Rahman, R.

[1]*Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*
[2]*Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*

Abstract: Response surface methodology (RSM) was used to optimize the concentrations of chitosan and glycerol for coating Berangan banana (*Musa sapientum* cv. Berangan). The effects of main edible coating components, chitosan (0.5-2.5%, w/w) and glycerol (0-2%, w/w) on weight loss, firmness, total colour difference, total soluble solids content (TSS) and titratable acidity (TA) of coated banana were studied during 10 days of storage at 26±2°C and 40-50% 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 0.745 to 0.930 for all the variables studied. In general, the chitosan concentration appeared to be the most significant ($P < 0.1$) factor influencing all variables except for TSS. The optimum concentration of chitosan and glycerol were predicted to be 2.02% and 0.18%, respectively. Statistical assessment showed insignificant difference between experimental and predicted values.

Keywords: Berangan banana, edible coating, chitosan, glycerol, response surface methodology

Introduction

Banana is one of the most favoured specialty tropical fruits and is very popular worldwide. Banana fruits are climacteric in nature which ripen rapidly and soften after harvest (Smith *et al.*, 1989). Due to its high nutritive value, banana is susceptible to diseases caused by microorganisms. In addition, banana is also sensitive to low temperature storage (Cano *et al.*, 1997). All these mentioned factors limit the handling, storage, distribution and marketing potentials of banana fruit. Banana fruit are usually harvested at mature green stage and stored either at ambient or at low temperature. Some extension of banana's shelf life has been demonstrated using modified atmosphere (MA) storage (relatively high CO_2 and low O_2) and controlled atmosphere (CA) storage (Ben-Yehoshua, 1966; Stewart *et al.*, 2005; Baez-Sanudo *et al.*, 2009). However, CO_2 injury, taste and flavour problems because of anaerobic respiration and ethanol production have been reported for banana fruit (Yousaf *et al.*, 2006; Bhande *et al.*, 2008).

Edible coatings are thin layers of edible component applied to the fruit's surface in addition to or as a replacement for natural protective waxy coatings and act as physical barrier towards carbon dioxide, oxygen and moisture movement for the fruits (Baldwin *et al.*, 1999). Coatings can be formulated from different components such as hydrocolloids (polysaccharides and proteins), lipids (waxes and resins) and synthetic polymers. These edible materials have different barrier properties against gases and physico-chemical and mechanical characteristics. Therefore, most coatings are made of more than one material with the addition of low molecular weight molecules including sorbitol, polyols or glycerol that serves as plasticizers (Risse and Miller, 1983; Olivas and Barbosa-Canovas, 2005). Edible coatings are applied on fruits and vegetables to improve appearance, delay ripening, reduce water loss and decay, and extend shelf life, but may also change flavour (Baldwin *et al.*, 1996; Saucedo-Pompa *et al.*, 2007). In fact, semi-permeable coatings can modify internal atmosphere of fruits by changing the composition or concentration of standard atmosphere gases like CA storage, with less expense incurred (Nisperos-Carriedo *et al.*, 1992). The atmosphere created by coatings is strongly related to permeability of coating and fruit respiration rate, and can alter as function of environmental conditions including temperature and humidity (Baldwin *et al.*, 1996).

Chitosan is a cationic polysaccharide with high molecular weight, obtained by the alkaline deacetylation of chitin (a long-chain polymer of a *N*-acetylglucosamine) and is soluble in dilute organic acids (El Ghaouth *et al.*, 1991; El Ghaouth *et al.*, 1992; Cheah *et al.*, 1997). Sensory evaluation indicated that coated longan fruit with chitosan was better in quality when compared to controls (Jiang and Li, 2001). Results of coating litchi fruit with chitosan-based edible coating, demonstrated that chitosan coating efficiently reduced browning and

*Corresponding author.
Email: *azosman@putra.upm.edu.my, azizahmasahuling@yahoo.com*

weight loss, decreased changes in contents of total phenolics, flavonoids and anthocyanins, reduced activity of polyphenolase and improved storability in coated fruit (Zhang and Quantick, 1997). Salvador *et al.* (1999) applied coating based on chitosan on avocado fruits and increased the avocado storage life to 24 days at 3–10°C and to 6 days at 27–29°C.

Glycerol is a plasticizer and is included in the edible coating formulation with the purpose of modifying the mechanical properties of the base edible components (hydrocolloids and/or lipids), producing more flexible coatings (Chillo *et al.*, 2008; Rivero *et al.*, 2009). In fact, glycerol by combining with the edible components and interspersing between polymer chains, moving the chains apart and improve the mechanical properties of edible films, such as rigidity and flexibility (Olivas and Barbosa-Canovas, 2005). High concentrations of plasticizers unfortunately, increase free volume in the coating matrixes and coating permeability to moisture, oxygen, aroma and oil. Therefore, the objective of this study was to determine the optimum concentrations of chitosan and glycerol (as independent variables) used as edible coating formulation for Berangan banana using RSM. Weight loss, firmness, total colour difference, total soluble solids content (TSS) and titratable acidity (TA) of coated banana were selected as response variables.

Materials and Methods

'Berangan' banana (*Musa sapientum* cv. Berangan) of green mature stage, were purchased from a local wholesale market, Pasar Borong Selangor, Malaysia. The banana hands were carefully selected to be uniform in appearance (weight, shape and colour) from the second hand of each bunch. The individual hands were sectioned into fingers and then the latex allowed to dry for one hour at ambient temperature (26±2°C and 40-50% relative humidity). The fingers were then disinfected by immersing in 0.1% (w/v) sodium hypochlorite solution for 2 min, washed and air-dried. Fourteen banana fingers were used for each treatment and three banana fingers were allocated for control samples. Chitosan (medium molecular weight) as main edible component was purchased from Aldrich (Aldrich Co. Steinheim, Germany) and glycerol (87%) as plasticizer was purchased from Fluka (Fluka Co. Steinheim, Germany). Tween 80 was obtained from Sigma-Aldrich (Sigma-Aldrich Co. Steinheim, Germany).

Coating and storage of bananas
Fourteen edible coating emulsions were prepared by dissolving chitosan (0.5 – 2.5% w/v) in 100 mL of 0.5% glacial acetic acid in distilled water. Then, glycerol (0 – 2% w/v) and Tween 80 (0.1% w/v) were added and the solutions were agitated overnight. The pH of the solutions was adjusted to pH 5.6 with 0.1 M sodium hydroxide. Bananas were dipped into the prepared coating emulsions for 1 min and then drained. Uncoated bananas as control samples were immersed in a 0.5% glacial acetic acid solution at pH 5.6 for the same duration of time. The treated and control banana samples were dried in ambient conditions (26±2°C and 40-50% relative humidity) for 2 hours. After setting a thin layer of edible coating on the surface of treated samples, control and coated banana samples were stored at ambient conditions in the laboratory for 10 days.

Analytical methods
All coated and control banana samples were allowed to ripen at ambient condition for 10 days and at days 0 and 10 of storage the following analysis were carried out to determine the effect of different concentrations of edible coating components on the delaying of banana ripening.

Moisture loss
Moisture loss occurred due to vapour phase diffusion driven by a gradient of water vapour pressure between inside and outside of fruit (Nisperos-Carriedo *et al.*, 1992). Weight loss was determined by weighing the samples on a digital balance (Presica 4000 C, Zurich, Switzerland) and was reported as percentage loss in moisture based on the original mass (day 0).

Firmness
Changes in texture of fruits are related to the degradation of insoluble protopectin to the more soluble pectin and pectic acid (Olivas and Barbosa-Canovas, 2005). In order to measure the changes in firmness of fruit samples, a texture analyzer machine (TA-XT2i, Stable Micro System., Ltd, England) equipped with a 30N load cell was used. Samples were subjected to a puncture test at a constant speed of 2 mm/sec, using a 2 mm diameter round stainless steel probe. Maximum force (N) required to penetrate the sample was recorded and used as the indicator of textural property. At least three measurements were made on each fruit at different locations, apex, middle and peduncle, and the results were averaged.

Colour
Evaluation of the changes in colour of the peel of both coated and control banana samples were

determined using a Minolta Chroma meter (CR-300, Minolta Corp. Tokyo, Japan) coupled with a Minolta Chroma C (VO. 27) software to determine L value (lightness or brightness), a^* value (redness or greenness) and b^* value (yellowness or blueness). The colorimeter was warmed up for 20 min and calibrated with a white standard tile (L=97.67, a^*=0.08 and b^*=1.54). Measurements were taken from at least three points on each fruit at different locations, apex, middle and peduncle, respectively and the average of L, a^* and b^* values were obtained. The total colour difference (ΔE) was calculated as the root mean square of the differences in individual L, a^* and b^* values (Maftoonazad and Ramaswamy, 2005).

$$[\text{i.e., } \Delta E = (\Delta L^2 + \Delta a^{*2} + \Delta b^{*2})^{0.5}] \quad (1)$$

ΔL, Δa^* and Δb^* were obtained as differences in L, a^* and b^* values of coated banana on day 10 and day 0, thus representing the time related changes.

TSS

TSS of fruit includes carbohydrates (sugars), organic acids and amino acids (Cano *et al.*, 1997). In order to determine the TSS of fruit samples, a digital refractometer (Palette PR-32, Atago Co Ltd., Tokyo, Japan) with a scale of 0-32 °Brix was used. Results were expressed as degree Brix (°Brix).

TA

Titratable acidity of samples was determined according to method described by Josylin (1970) based on titrating banana juice with 0.1 N sodium hydroxide. The results were expressed as mg of malic acid per 100 g sample, since malic acid is the prevalent organic acid in banana fruit (Cano *et al.*, 1997).

Microstructure

The surface microstructure of peel from both coated and control samples was analysed using the SEM technique in a Philips XL 300 ESEM microscope, (Philips Ltd, MD Eindhoven, Netherlands). Square pieces (10 mm × 10 mm) of 5 mm thickness of the coated and control sample peel were obtained from the middle portion of the fruit. Samples were vacuum fixed in glutaraldehyde (4%), washed with sodium cacodylated buffer (0.1 M) and post fixed in 1% osmium tetroxide for 2 hours at 4°C. This was followed by dehydration of samples in a series of acetone (35, 50, 75, 95 and 100%). Samples were mounted on the aluminum stubs and dried in a flow of CO_2 in critical point dryer (Bal-Tec CPD 030, Kettleshulme, UK). Finally, samples were coated with Au/Pd nanoparticles using a sputter coater (Bal-Tec Csd 005, Kettleshulme, UK) and viewed under a SEM using an accelerating voltage of 20 kV.

Experimental design and statistical analysis

The effects of two independent variables: chitosan concentration (C_1) and glycerol concentration as a plasticizer (C_2) on the response variables namely, weight loss, peel firmness, total colour difference, TSS and TA of coated banana samples were evaluated using a two-factor central composite design (CCD). The levels of independent variables used in this design are listed in Table 1. One center point with six replications was used in this design (Table 2) to calculate the repeatability of the method (Mirhosseini *et al.*, 2009). A generalized polynomial equation was used to relate the responses, weight loss (Y_1), peel firmness (Y_2), total colour difference (Y_3), TSS (Y_4) and TA (Y_5) of coated samples, to the independent variables as follow:

$$Y_i = a_0 + a_1 X_1 + a_2 X_2 + a_{11} X_1^2 + a_{22} X_2^2 + a_{12} X_1 X_2 \quad (2)$$

Where Yi represents the response variables, a_0 is a constant, a_i, a_{ii} and a_{ij} are the linear, quadratic and interactive coefficients, respectively. The coefficients of the quadratic polynomial equations were calculated using experimental data. Model analysis, lack-of fit test and coefficient of determination (R^2) analysis were performed to determine the adequacy of the model. For any terms of independent variables in the models, a large *F*-value and a small *P*-value would show a more significant effect on the particular response variables (Quanhong and Caili, 2005). The terms statistically found non-significant were dropped from the initial models and experimental data were refitted only to significant ($P < 0.1$) independent variable effects in order to obtain the final reduced model (Mirhosseini *et al.*, 2009). Banana is a perishable agriculture produce with big variations in the quality attributes between one another. Therefore, level of significance was selected at 90% confidence interval. The Minitab v. 14 statistical software (Minitab Inc., PA, USA) was used for design of experiment and statistical analysis.

Table 1. Levels of independent variables used in the center composite design (CCD)

Symbol	Independent variable[a]	levels		
		Low	Center	High
		-1.414 (-α)	0	1.414 (+α)
X_1	C_1 (%w/w)	0.5	1.5	2.5
X_2	C_2 (%w/w)	0	1	2

[a] C_1, concentration of chitosan; C_2, concentration of glycerol.

Table 2. Center composite design (CCD) and experimental data obtained for the response variables studied

Run	Block	Independent variable		Response variable				
		Chitosan (%w/w)	Glycerol (%w/w)	Weight loss (%)	Firmness (N)	Total colour difference	TSS (°Brix)	TA (*[a])
1(C[b])	2	1.5	1	13.81	15.27	14.63	12.9	4.6
2	2	0.5	1	12.99	10.73	26.83	15.6	4.4
3(C[b])	2	1.5	1	14	11.33	23.96	16.5	4.3
4(C[b])	2	1.5	1	12.67	17.22	13.65	10.5	4.1
5	2	1.5	0	11.54	14.38	19.25	13.8	4.5
6	2	2.5	1	15.82	19.35	2.84	5.4	1.5
7	2	1.5	2	14.33	11.09	26.9	16.8	4.3
8	1	0.793	0.293	13.36	9.01	24.98	16.5	4.1
9(C[b])	1	1.5	1	11.79	10.82	25.5	15.9	4.1
10(C[b])	1	1.5	1	12.32	12.98	22.24	15	4.6
11(C[b])	1	1.5	1	12.31	8.74	27.05	17.7	4.1
12	1	2.207	1.707	21.42	6.01	28.98	18.6	2.6
13	1	2.207	0.293	11.35	16.72	6.77	7.2	3
14	1	0.793	1.707	14.18	9.1	27.21	16.5	3.9

[a] *, mg malic acid per 100g sample.
[b] C, center point.

Optimization and validation procedures

Both numerical and graphical optimizations were performed to determine the optimum concentrations of independent variables (chitosan and glycerol). Three dimensional response surface plots were generated from the fitted models for each response to better visualize the interaction effects of chitosan and glycerol concentrations on the responses. A numerical optimization was performed by the response optimizer for determining the exact optimum concentrations of independent variables leading to the overall optimum condition. A comparison Tukey's test was carried out between predicted and experimental responses values of coated banana with optimized concentrations of independent variables, to verify the adequacy of the final response models. The optimization and comparison procedures were carried out using the Minitab v. 14 statistical software (Minitab Inc., PA, USA).

Results and Discussion

Fitting the models

Monitoring of the changes in quality attributes of green mature Berangan banana showed that the control banana samples were ripened at day 10 of storage at ambient conditions. Weight loss, peel firmness, total colour difference, TSS and TA of control banana samples were 16.97 (%), 7.91 (N), 29.51, 17.8 (°Brix) and 4.6 (mg malic acid per 100g sample), respectively, at day 10 of storage. The experimental values of weight loss, peel firmness, total colour difference, TSS and TA of coated banana samples are given in Table 2. The final reduced models were obtained using experimental data. Some variables were kept in the reduced models despite being insignificant. For example, linear terms were also kept in the model if a quadratic or interaction term containing this variable was significant ($P < 0.1$). The individual significance P-value of independent variables and their interactions are shown in Table 3.

Table 4 reveals the predicted regression coefficients for the response variables, along with the corresponding R^2, R^2 (adj), P-value and F-value of lack of fit. The results indicated that the final reduced quadratic polynomial models adequately represented the experimental data with the coefficients of determination (R^2) for the responses of weight loss, peel firmness, total colour difference, TSS and TA values being 0.819, 0.757, 0.745, 0.802 and 0.930, respectively. Results demonstrated that the quadratic polynomial models generated were adequate to explain the effects of the chitosan and glycerol concentrations on the response variables of coated banana samples. Each studied response was evaluated as function of main, quadratic and interaction effects of chitosan and glycerol concentrations. Chitosan concentration and interaction between chitosan and glycerol concentrations had the most significant ($P < 0.1$) effect on the responses as compared to the glycerol concentration. As clearly indicated in Tables 3 and 4, the main linear effects of chitosan and glycerol had no significant ($P > 0.1$) effect on TSS, but they should be kept in the final reduced models since the interaction effect of them showed significant ($P < 0.1$) effect on the reduced model fitted for the TSS. The main linear effects of glycerol had no significant ($P > 0.1$) effect on firmness, total colour difference and TSS of coated samples, but it should also be kept in the final reduced models since the interaction effects of chitosan and glycerol were significant ($P < 0.1$).

The final reduced models to predict weight loss, firmness, total colour difference, TSS and TA of coated bananas as function of chitosan (X_1) and glycerol (X_2) concentrations were;

Table 3. Significance probability (*p*-value) of regression coefficients in the final reduced models

Variables		Main effects		Quadratic effects		Interaction effects
		X_1	X_2	X_1^2	X_2^2	$X_1 X_2$
Weight loss ($Y_{1,\%}$)	*P*-value	0.022	0.083	0.059	-	0.010
Firmness (Y_2, N)	*P*-value	0.009	0.163[b]	-	-	0.040
Total colour difference (Y_3)	*P*-value	0.008	0.297[b]	-	-	0.077
TSS (Y_4, °Brix)	*P*-value	0.880[b]	0.140[b]	0.068	-	0.034
TA (Y_5, *[a])	*P*-value	0.023	0.098	0.034	-	-

[a] *, mg malic acid per 100g sample.
[b] Insignificant at *P*> 0.1.

Table 4. Regression coefficients, R^2, R^2(*adj*), probability values and lack of fit for the final reduced models

Regression coefficients[b]	Weight loss ($Y_{1,\%}$)	Firmness (Y_2, N)	Total colour difference (Y_3)	TSS (Y_4, °Brix)	TA (Y_5, *[a])
a_0	19.951	2.482	42.878	18.926	2.808
Linear					
a_1	-9.679	8.372	-18.894	0.861	3.304
a_2	-4.314	5.400	-8.752	-5.784	-0.156
Quadratic					
a_{11}	2.228	-	-	-3.461	-1.484
a_{22}	-	-	-	-	-
Interaction					
a_{12}	4.625	-5.400	9.990	5.699	-
R^2	0.819	0.757	0.745	0.802	0.930
R^2 (adj)	0.706	0.649	0.632	0.679	0.900
Regression (*P*-value)	0.005 [c]	0.014 [c]	0.008 [c]	0.009 [c]	0.000 [c]
Lack of fit (*F*-value)	11.49	0.55	1.54	0.81	1.16
Lack of fit (*P*-value)	0.018 [c]	0.738 [d]	0.347 [d]	0.578 [d]	0.457 [d]

[a] *, mg malic acid per 100g sample.
[b] a_0 is a constant, a_i, a_{ii} and a_{ij} are the linear, quadratic and interaction coefficients of the quadratic polynomial equation, respectively. 1: Chitosan; 2: Glycerol.
[c] Significant (*P*< 0.1).
[d] Insignificant (*P*> 0.1).

Weight loss = $19.951 - 9.679X_1 - 4.314X_2 + 2.228 X_1^2 + 4.625 X_1 X_2$ (3)

Firmness = $2.482 + 8.372 X_1 + 5.4 X_2 - 5.4 X_1 X$ (4)

Total colour difference = $42.878 - 18.894 X_1 - 8.752 X_2 + 9.99 X_1 X_2$ (5)

TSS = $18.926 + 0.861 X_1 - 5.784 X_2 - 3.461 X_1^2 + 5.699 X_1 X_2$ (6)

TA = $2.808 + 3.304 X_1 - 0.156 X_2 - 1.484 X_1^2$ (7)

Analysis of response surfaces

Weight loss

Water can increase the rate of several reactions in fruits such as browning, vitamin degradation and enzyme activity, enhance the rate of microorganisms growth and cause texture changes (Smith *et al.*, 1989). Fig. 1(A) shows the weight loss of coated banana as function of chitosan and glycerol concentrations. As clearly observed, at low concentrations of glycerol (less than 0.2%), an increase in the concentration of chitosan, significantly ($P < 0.1$) decreased the weight loss. This result can be explained by the fact that attractive forces between chitosan molecules (cohesion) increase by increasing the chitosan concentration in edible coating formulation. As clearly indicated in Figure 1(A), at high concentrations of glycerol, an increase in the concentration of chitosan, significantly ($P < 0.1$) increased the weight loss. Higher concentrations of glycerol as plasticizer seems to reduce intermolecular forces along the chitosan chains, thus increasing free volume, chain movements and finally, increase chitosan based

coating permeability to moisture. Result of present study was in agreement with findings of Cien *et al.* (2007). These authors found that by increasing the concentrations of chitosan in edible coating formulations with low concentration of plasticizer, weight loss of coated fresh cut mango decreased. Olivas and Barbosa-Canovas (2005) reported that by increasing the concentrations of plasticizers such as glycerol in edible coating formulations, permeability of prepared coatings increase towards water vapour.

Firmness

Effect of chitosan and glycerol concentrations on the firmness of coated banana is shown in Figure 1(B). As clearly observed in Figure 1(B), an increase in chitosan concentration at glycerol concentrations less than 1.3% (w/v), there was a beneficial effect on firmness retention. However, at higher glycerol concentrations (more than 1.3%), an increase in chitosan concentration, reduced firmness of coated banana. Generally, chitosan has excellent barrier properties towards O_2 (non-polar molecules) and as a physical barrier decreases respiration rate of fruits (El Ghaouth *et al.*, 1991; Zhang and Quantick, 1998). Breakdown of starch and cell wall to form sugar and soluble pectic substances, respectively, are two enzymatic processes which reduce firmness of fruits (Maftoonazad and Ramaswamy, 2005). It seems that by increasing the chitosan concentration, permeability of edible coating based on chitosan decreased towards

O_2 of storage atmosphere and respiration rate of coated banana decreased. The reduction in respiration rate, reduced the activities of hydrolysis enzymes and retarded the softening of banana. On the other hand, increasing the glycerol concentration, increased free volume in the supporting coating matrix and reduced permeability of edible coating based on chitosan towards O_2. The movement of water from the peel to the pulp during ripening due to the process of osmosis, is another process that decreases the firmness of fruits (Smith *et al*., 1989). Glycerol has hydroxyl groups which form hydrogen bonds with chitosan and high concentrations of glycerol could increase hydrophilic nature of edible coating based on chitosan and increase of water sorption especially in atmosphere with high relative humidity. Result of present study was consistent with observation of Win *et al*. (2007) and Baez-Sanudu *et al*. (2009). They found that high concentrations of chitosan at low concentration of plasticizer, had strong effect on retention of banana firmness.

Total colour difference

As shown in Figure 1(C), the total colour difference (ΔE) of the coated bananas decreased with an increase in the concentration of chitosan and a decrease in the glycerol concentration. This result can be explained by the fact that free volume and edible polymeric chain movements in edible coating formulation decrease by increasing the concentration of edible polymers and decreasing the plasticizers concentrations (Olivas and Barbosa-Canovas, 2005). This reductions enhanced permeability of prepared coating towards O_2 and finally the coating strongly changed internal atmosphere of coated banana. The chlorophyll content of banana peel decreases slowly with ripening of banana as result of chlorophyllase action (Cano *et al*., 1997). Low O_2 and high CO_2 concentrations reduce the activities of chlorophyllase (Stewart *et al*., 2005). Result of present study was in agreement with the findings of Banks (1984), Zhang and Quantick (1997) and Plotto *et al*. (2007). These authors found that edible coatings based on polysaccharides changed internal atmosphere of banana and litchi, and strongly delayed colour changes of the mentioned fruits.

TSS

The effect of chitosan and glycerol concentrations on TSS is shown in Figure 1(D). In general, TSS of banana increased during the ripening process. TSS includes carbohydrates, organic acids and amino acids of fruit. In most ripe fruits, including banana, sugar forms the main component of soluble solids

(Cano *et al*., 1997). As clearly observed in Figure 1(D), the interaction between the concentrations of chitosan and glycerol had significant ($P < 0.1$) effect on TSS of coated banana samples. The coated banana had minimum TSS at high and low concentrations of chitosan and glycerol, respectively. Result can be related to the fact that hydrolysis of banana pulp starch decreases as a result of strong reduction in banana metabolic process. An increase in chitosan concentration at low concentration of glycerol (less than 1%), could increase residues of chitosan in the stomatal apertures of banana peel which impeded the diffusion of gases (O_2 and CO_2) through them and decreased banana respiration rate. It seems that at high concentrations of glycerol, more hydrogen bonds are formed between hydroxyl groups of glycerol and chitosan, and adhesion, attractive forces between edible coating and banana peel, decreases. Results of this study indicated that increasing the concentration of chitosan at low concentrations of plasticizer (glycerol) increased the polysaccharide based edible coating adhesion and reduced respiration rate of banana fruit and this was in line with the findings of Maftoonazad and Ramaswamy (2005). These authors found that low concentration of glycerol (1.9% w/w) conjugated with high concentration of methyl cellulose (3% w/w) strongly decreased the respiration rate of coated avocado as compared to control samples.

TA

Changes in TA of coated banana as function of chitosan and glycerol concentrations are shown in Figure 1(E). Generally, TA in the pulp tissues of banana shows a large increase during ripening (Ben-Yehoshua, 1966). Malic and citric acids are the prevalent organic acids in banana and are used as substrate for the enzymatic reactions of respiration (Banks, 1984; Cano *et al*., 1997). As clearly observed in Figure 1(E), the concentration of chitosan had significant ($P < 0.1$) effect on TA of coated banana. However, glycerol concentration and interaction between glycerol and chitosan concentrations showed no significant effect on the TA of coated banana. Results of this study indicated that by increasing the concentration of chitosan in edible coating formulation, the TA of coated banana decreased. Reduction in respiration rate as a result of filling the cracks and other irregularities on the banana peel may be reflected in lower changes in TA. In fact, following the decrease in the respiration rate, metabolic process and production of substrate for respiration process such as organic acid, decreased. Result of present study revealed that increasing the

chitosan concentration strongly decreased metabolic process of coated banana that can be reflected in delay ripening of fruit, which is in agreement with the findings of Carrillo-Lopez *et al*. (2000). These authors indicated that by increasing the concentration of carboxymethyl cellulose in edible emulsions, rate of ethylene production and metabolic process of coated mangoes decreased.
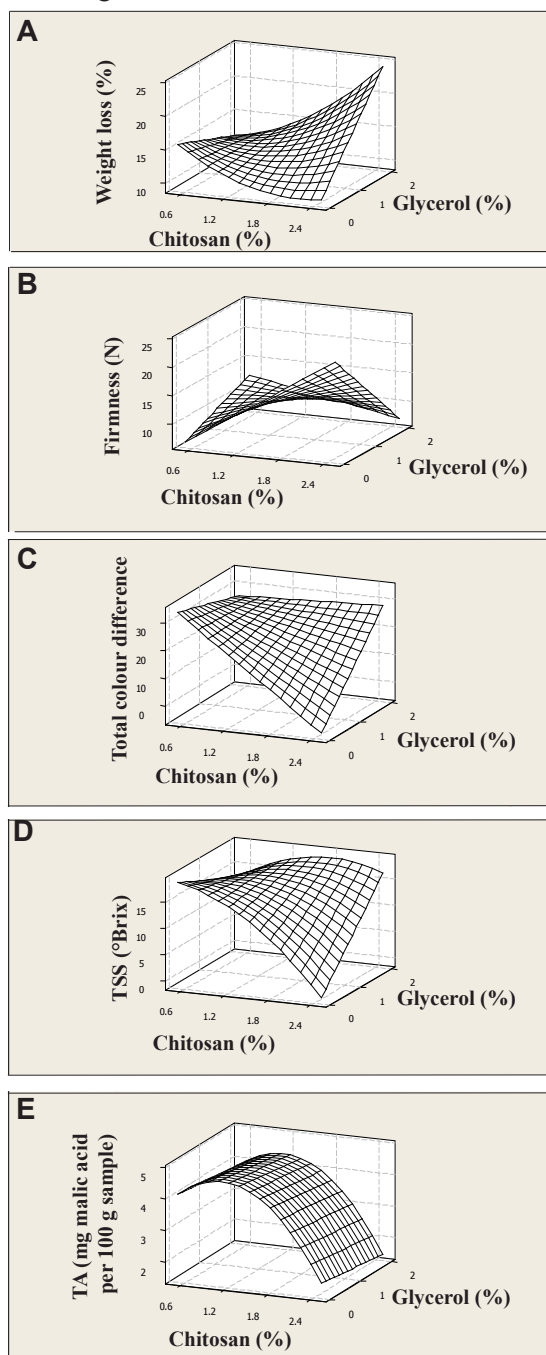
Figure 1 Response surface plot of the weight loss (A), Firmness (B), Total colour difference (C), TSS (D) and TA (E) of the coated samples as a function of the concentrations of chitosan and glycerol

Optimization of chitosan and glycerol concentrations for coating of Berangan banana

During the ripening of banana fruit, weight loss, total colour difference, TSS and TA increase while firmness decreases. Therefore, the optimum concentrations of chitosan and glycerol for coating of Berangan banana would be attained when the coated banana has minimum values for weight loss, total colour difference, TSS and TA and maximum value for firmness. The results indicated that optimum concentrations of chitosan and glycerol were 2.02% and 0.18%, respectively, within experimental ranges. The predicted values of studied responses under optimum condition were observed in Table 5.

Verification experiments were performed at the optimum concentrations of the chitosan and glycerol, and the experimental values of studied responses are given in Table 5. Comparison test demonstrated that there was no significant ($P > 0.1$) difference between predicted and experimental values for all response variables. The closeness between these values of responses verified the adequacy of final reduced models fitted by RSM.

**Table 5.** Predicted and experimental values of responses at optimum concentrations of chitosan and glycerol

Response variable	Predicted value	Experimental value Mean ± SD
Weight loss (%)	10.43[b]	11.03± 0.74 [b]
Firmness (N)	18.36[b]	18.03± 1.82 [b]
Total colour difference	6.84[b]	6.64± 0.70 [b]
TSS (°Brix)	7.61[b]	7.8± 0.3 [b]
TA (*[a])	3.41[b]	3.23± 0.29 [b]

[a] *, mg malic acid per 100g sample.
[b] In significant ($P > 0.1$).

Microstructure

The microstructures of the surface of control and coated banana peel with optimized concentration of edible coating, chitosan (2.02%) – glycerol (0.18%), are shown in Figures 2(A) and 2(B), respectively. The structure of the control banana surface and its stomatal apertures was clear (Figure 2(A)). As clearly observed in Figure 2(B), edible coating based on chitosan mostly filled in the cracks and other irregularities on the banana peel and increased light reflectance. It was also observed that coated banana peel was glossier as compared to that of control sample. It seems that edible coating based on chitosan, by covering the banana peel stomatal apertures, also could reduce respiration rate of fruit. This resulted in less amounts of weight loss, colour changes, TA and TSS, and higer value of firmness of coated banana as compared to control samples.
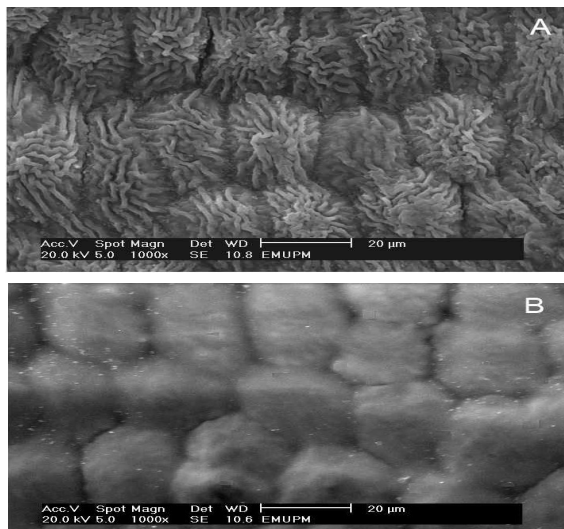
Figure 2. SEM micrographs of the surface of 'Berangan' banana fruit during storage for control (A) and coated banana fruits with chitosan – glycerol (B), at day 10 of storage

Conclusion

Type and concentration of edible components have important effects on the quality characteristics of coated fruits such as weight loss, firmness, colour, TSS and TA. The results of this study showed that it is possible to estimate the optimum concentrations of chitosan, as main edible component, and glycerol, as plasticizer, by response surface methodology in a fixed day of storage. The fact that the coated banana samples had lower weight loss, total colour difference, TA and TSS values and higher firmness values as compared to control samples, implied that chitosan (2.02%) – glycerol (0.18%) edible coating formulation is effective in extending the shelf life and delaying the ripening process of Berangan banana at ambient conditions.

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