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Formulation of Chitosan-Oleic Acid Coating for Kiew Wan Tangerine by Response Surface Methodology

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ABSTRACT

The coating formulation is an essential factor in maintaining the postharvest quality of tangerines. A common problem found in coated tangerines is off-flavor due to improper coating. The aim of this study was to use chitosan and oleic acid to formulate the most suitable coating for tangerines displayed at room temperature (30±2°C). In order to optimize the formulation, response surface methodology (RSM) with a central composite design was applied. The response effect of chitosan (1-2%) and oleic acid (1-4%) on weight loss, ethanol in juice, and carbon dioxide and oxygen in fruit were investigated. A second order model was used to explain the effect of the dependent variables (weight loss, O_2, CO_2, ethanol), which were highly significant and produced R^2 values in the range from 77 to 92%. The optimum coating formulation for tangerine was found to be 1% chitosan (v/v) and 2.5% oleic acid (v/v), which was based on a composite desirability value of 0.80.

Keywords: chitosan, oleic acid, coating, response surface methodology

INTRODUCTION

It is a common practice in many pack houses in Thailand to coat tangerines before delivery to markets. Many types of commercial coatings have been developed recently. The objective of coating is to provide glossiness to the fruit and prevent weight loss. Generally, when fruits have a weight loss of 5-10% they are considered unsaleable (Davies and Albrigo, 1998). Coatings can slow the exchange of gases in the fruit, which can lead to the accumulation of CO_2 and depletion of O_2 inside the fruit. The off-flavors in fruit are mostly the result of anaerobic respiration and therefore, the selection of suitable coating material is essential to pack houses. Several studies (Mannheim and Soffer, 1996; Hagenmaier, 2002; Porat *et al.,* 2005) have proposed coating formulations for mandarins and tangerines based on the gas permeability of the type of wax or coating material; for instance, polyethylene-based coating produced higher gas permeability than shellac- and wood resin-based coatings. Hagenmaier and Shaw (2002) suggested that high gloss shellac and resin-based coatings were not suitable for tangerines, because tangerines were more sensitive to changes during storage than other citrus varieties when coating was applied and thus off-flavors were likely to occur.

Chitosan is a biodegradable film or coating, which has been widely used in the food and cosmetic industries. Some positive advantages of chitosan are its antifungal activity against

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several species (Devlighere *et al*., 2004, Galed *et al*., 2004) and a decrease in transpiration losses in fruit (Jiang and Li, 2001). Chitosan coating has been used to prolong the shelf life of and control decay in many fruits including longan, mango, murcott tangor, fortune mandarins and valencia oranges (Jiang and Li, 2001; Galed *et al*., 2004; Chien *et al*., 2007a; Chien *et al*., 2007b). In a previous study, the formulation of chitosan coating for mandarin was in the range of 1 to 2% (Nilprapruck, 2002). The limitation of using chitosan in the coating was due to its high water vapour permeability (Wong *et al*., 1992; Caner *et al*., 1998), which can result in high weight losses in fruit. Lipid materials and hydrocolloids may be added to improve the moisture barrier property of a coating or film (Amarante and Banks, 2001). Many lipid materials have been used to improve the water vapour permeability of films, including fatty acid, natural waxes and resin (Vargas *et al*., 2009b). Lai and Padua (1998) reported that commonly used plasticizers were liquid compounds, such as polyols, mono-, di- or oligo-saccharides, lipids and lipid derivatives. Oleic acid was used as the plasticizer in zein films. Morillon *et al*. (2002) reported fatty acids were used as an emulsifier and as surface-active agents in films and coatings. Oleic acid is one of the fatty acids that has been used in coating formulations for oranges and mandarin, with a range of 0.5 to 3.8% (Chen and Nussinovitch, 2000a; Chen and Nussinovitch, 2000b; Hagenmaier, 2002). Vargas *et al*. (2006) found that the addition of oleic acid (1-4%) to the chitosan formulation could increase the water vapour resistance in coated strawberries. Vargas *et al*. (2009a) reported that the addition of oleic acid into the chitosan matrix decreased the water vapour permeability and increased the gloss of the film. To date, no studies have been reported using a chitosan-oleic acid coating for Thai tangerines during storage at room temperature. The objective of this study was to formulate the optimum chitosan and oleic acid conditions to coat tangerines displayed at 30°C. To optimize the formulation, RSM was used. RSM can be used to determine the optimization of the process conditions, formulation, ingredient levels (Gan *et al.,* 2007) and coating (Luangwilai *et al.,* 2007) in areas of food technology. It is an effective tool to minimize the numbers of trials. In this study, the effects of chitosan and oleic acid on quality parameters (weight loss, O_2, CO_2 and ethanol concentration) of Thai tangerines during storage at room temperature for 3 wk were investigated. The desirability function approach developed by Derringer and Suich (1980) was used to obtain optimum responses, based on the fitted quadratic response surface model constructed by RSM.

MATERIALS AND METHODS

Raw materials

Kiew wan tangerines (*Citrus reticulate* Blanco cv. kiew wan) that had been harvested at the fully mature stage were purchased from an orchard in Kamphaeng Phet province, Thailand. The fruit size was 5.6-6.0 cm in diameter. Shrimp chitosan (MW, 248 kDa), with a deacetylation degree of 96.5% was purchased from Muew Biosafe Co., Ltd., Phathum Thani, Thailand. Acetic acid (98%), sodium hydroxide, oleic acid and Tween 80 were purchased from Sigma-Aldrich, USA.

Preparation of coating solution

The chitosan-oleic acid coating was prepared according to a method described by Vargas *et al*. (2006). Chitosan (1-2%) was dispersed in an aqueous solution of glacial acetic acid (1%, v/v) at 40°C. Tween 80 at 0.1% (v/v) was added to improve wettability. After 8 h of stirring, oleic acid (1-4%) was added to the chitosan solution. The concentration of chitosan and oleic acid in the coating solution was varied according to the conditions defined in the experimental design (Table 1). Each mixture was

Table 1 Central composite design and response values.

Run	Coded		Uncoded		Response			
	X_1	X_2	Chitosan (%)	Oleic acid (%)	Weight loss (%)	O_2 (%)	CO_2 (%)	Ethanol (ppm)
control	-	-	-	-	15.15	9.61	3.97	1011.74
1	-1	-1	1.15	1.44	13.53	9.48	4.35	1849.43
2	-1	1	1.15	3.56	12.91	7.54	4.67	2086.33
3	1	1	1.85	3.56	12.04	6.36	5.89	2222.61
4	1	-1	1.85	1.44	13.29	6.37	5.17	2038.80
5	0	0	1.50	2.50	12.64	6.92	4.26	2064.99
6	0	0	1.50	2.50	12.42	7.13	4.66	2162.18
7	0	0	1.50	2.50	12.53	7.73	5.62	2268.19
8	0	0	1.50	2.50	12.99	7.91	4.58	2044.10
9	0	0	1.50	2.50	12.88	7.81	4.64	2038.50
10	1.41	0	2.00	2.50	12.06	5.83	6.87	2245.19
11	-1.41	0	1.00	2.50	12.72	8.05	4.04	1885.80
12	0	1.41	1.50	4.00	12.57	6.60	5.93	2350.56
13	0	-1.41	1.50	1.00	13.94	8.30	4.43	1973.45

adjusted to pH 5 using 1 N NaOH (Chein *et al.*, 2007b). All mixtures were emulsified using an Ultra Turrax T25 (IKA®, Germany) at 13,500 rpm for 4 min and the solution was filtered through a filter paper (Whatman No. 1) to remove undissolved material before use.

Coating application

The tangerines were coated using a self-made coating apparatus. The speed of the brush rollers was controlled at 160 rpm and the coating solution was sprayed at 10 mL/min. Each piece of fruit was weighed about 10 s before and again 10 s after application, to determine the wet weight of the coating applied (Hagenmaier, 2005). The mean wet weight of the coating was about 0.20g per fruit. Coated fruits were then dried with an electric fan at room temperature (30±2°C) for 30 min. Fifty coated fruits were packed in a cardboard box (30×40×20 cm) and stored in the laboratory at 30±2°C (to simulate Thai marketing conditions) and 79±5% RH for 3 wk.

Determination of weight loss

Weight loss of the fruit was calculated as a percentage on a weekly basis. Every week ten fruit were weighed using a top load balance.

Determination of internal CO_2 and O_2

Ten fruit from each treatment were sampled. A gas-tight syringe was pierced into the core of the fruit submerged in water and then 1 mL of gas was taken out. The internal concentration of gases was analyzed by a gas chromatograph (GC-8A Shimadzu, Japan) equipped with two columns; a Porapak Q (2 m, 4 mm o.d., GL science Inc, Japan) for CO_2, a molecular sieve (1 m, 4 mm o.d., GL Science Inc, Japan) for O_2; and a thermal conductivity detector (at 90mA). Helium was the carrier gas at a flow rate 50 mL/min, and the column temperature was set at 60°C.

Determination of ethanol

Juice samples from 30 fruit were extracted by a juice extractor and then centrifuged at 10,000 rpm for 20 min. The supernatant was filtered before injection onto the column. The ethanol concentration was analyzed with a gas chromatograph (GC-8A Shimadzu, Japan) equipped with a BX-10 column (3 m, 4 mm o.d., GL Science Inc, Japan) and a flame ionization detector. The operating conditions were: helium as carrier gas at 50 mL/min, column temperature at 85°C and injector and detector temperature at 100°C.

Experimental design and statistical analysis

Response surface methodology (RSM) using a central composite design (CCD) was applied as the experimental design and to optimize the combination of the chitosan (1-2%) and oleic acid (1-4%) concentration in the coating formulation. The five coded levels were incorporated into the design for chitosan: 1.41 (1%), -1 (1.15), 0 (1%), 1 (1.85%) and 1.41 (2%) and for oleic acid: 1.41 (1%), -1 (1.44), 0 (2.5%), 1 (3.56%) and 1.41 (4%). The central point of the design (0,0) was repeated five times to calculate the reproducibility of the method. The effect of the two independent variables on the coating properties was modeled using a second-order model. The second-order model equation used to fit responses followed Lazic (2004) and is shown in Equation 1:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X^2{}_1 + \beta_{22} X^2{}_2 + \beta_{12} X_1 X_2 \quad (1)$$

Where Y is the predicted response value (weight loss; ethanol, CO_2 and O_2), X_1 is chitosan level and X_2 is oleic acid level, β_0 is a constant value, β_1 and β_2 are linear terms, $\beta_{11,}$ β_{22} are quadratic terms, and β_{12} is an interaction term.

Design-Expert 7.6.1 trial version (Stat-Ease, Inc., MN, 2007) was used to fit the model, draw contour plots and determine desirability.

RESULTS AND DISCUSSION

The response surface methodology involving a central composite design, with 13 experiments, was performed for the two factors of chitosan and oleic acid. The combination of chitosan and oleic acid in each formulation and the response values based on weight loss, ethanol, CO_2 and O_2 are presented in Table 1. The levels of chitosan and oleic acid used in the RSM were based on previous studies (Nilprapruck, 2002; Vargas *et al.*, 2006). The prediction of the optimum concentration of chitosan and oleic acid was carried out by Design-Expert 7.6.1 software and the results are shown in Tables 2 and 3. The optimal level of the response variables was determined by desirability with the scale from 0–1.0. The desirability function was based on transforming the measured property of each response to a dimensionless desirability scale and the overall desirability was calculated as the geometrical average of the partial desirability functions. Different desirability functions can be used, depending on the partial response that is required to be maximized, minimized or assigned a target value (Derringer and Suich, 1980).

Table 3 shows the quadratic models of weight loss, oxygen, carbon dioxide and ethanol. The models were found to be adequate, as there was no significant lack of fit in any of the response variables (Table 2). The coefficient values (R^2) of all models ranged between 77 and 93%, indicating that the models could be used to predict the effect of chitosan and oleic acid in the formulation of the parameters associated with the quality of coated tangerines. Chitosan showed no quadratic effect on weight loss, $O_{2,}$ CO_2 and ethanol, but oleic acid had an effect (Table 2).

Weight loss

As the concentrations of chitosan and oleic acid increased, the weight loss decreased (Figure 1a, Table 3, Eq. 1). Only oleic acid showed a quadratic effect on the weight loss (Table 2, Figure 1a) as its concentration increased. Oleic acid appeared to have a greater effect on weight loss than chitosan. The addition of oleic acid into the chitosan film could enhance film hydrophobicity by increasing the surface solid density, which consequently increased the water vapour resistance of the film (Vargas *et al*., 2006). Gontard *et al*. (1994) also reported that oleic acid had a certain degree of mobility due to its double bond, which resulted in a reduction in the moisture barrier properties of the film. Vargas *et al*. (2009a) reported that the water vapor permeability of the chitosan-oleic film was lower than for a pure chitosan film. The current experiment confirmed that oleic acid can reduce the water permeability of the film.

Internal gases

The results obtained showed that the internal gases (O_2 and CO_2) of coated tangerines were affected by the combination of chitosan and oleic acid in the formulation (Table 2). An increase in the concentration of chitosan and oleic acid significantly decreased the internal oxygen and increased the carbon dioxide (Figures 1b and 1c), which showed that coating the tangerine fruit could restrict the exchange of gases between the fruit and the surrounding atmosphere. The results of the current study corresponded with Wu *et al*. (2002). The increased concentration of chitosan lowered the internal O_2 and elevated the CO_2 and the effect of chitosan on gas permeability was directly associated with its concentration. Therefore, inappropriate coatings may result in an off-flavor, as a consequence of insufficient levels of oxygen and excess carbon dioxide levels.

Table 2 Analysis of variance of independent variables on the response variables for chitosan-oleic coating.

Source	*F*-value df	Weight loss	O_2	CO_2	Ethanol
Model	5	17.71***	13.46***	4.89*	5.65*
X_1 (Chitosan)	1	14.03**	43.94***	17.26**	11.45**
X_2 (Oleic acid)	1	48.72***	15.09**	7.04*	14.99**
$X_1 X_2$	1	2.67	5.93*	0.15	0.09
X^2_1	1	2.74	2.13	2.02	1.55
X^2_2	1	18.15***	0.06	0.51	0.05
Residual	7				
Lack of Fit	3	0.20	0.52	1.03	0.48
Pure Error	4				
R^2	-	0.9267	0.9058	0.7774	0.8013

* Significant at $p \leq 0.05$.
** Significant at $p \leq 0.01$.
*** Significant at $p \leq 0.001$.

Table 3 Predictive models from the response surface methodology.

Dependent values	Models	
Weight loss	$12.88+3.23*X_1-1.20*X_2-0.42*X_1X_2-0.97*X^2_1+0.28*X^2_2$	(1)
O_2	$13.80-0.58*X_1-2.60*X_2+1.29*X_1X_2-1.75*X^2_1+0.03*X^2_2$	(2)
CO_2	$7.38-5.18*X_1-0.65*X_2+0.27*X_1X_2+2.22*X^2_1+0.12*X^2_2$	(3)
Ethanol	$558.00+1371.23*X_1+133.99*X_2-35.39*X_1X_2-32930*X^2_1+6.30*X^2_2$	(4)

X_1=chitosan (%)
X_2=oleic acid (%)

An interaction effect of chitosan and oleic acid was found only with the internal O_2 and not with the CO_2 (Table 2). Up to a certain limit, an increase in the concentration of oleic acid reduced the internal O_2 level (Figure 1b). This might have been due to the effect of oleic acid on the structure and formation of the film. Gas permeabilities of edible films and coatings depend on several factors, such as the integrity of the film, the ratio between crystalline and amorphous zones, the hydrophilic-hydrophobic ratio and the polymeric chain mobility. The interaction between the film-forming polymer and the presence of a plasticizer or other additives are also important factors in film permeability as reported by Garcia *et al*. (2000), who found that starch-based film had lower O_2 permeabilities than CO_2. These effects can be explained by the higher solubility of CO_2 in the starch films. Permeability strongly depends on the interaction between the polymer matrix and the permeating gas and environmental conditions, such as temperature and relative humidity.

Using RSM, combinations of chitosan and oleic acid were designed and these are presented in Table 1. The range of O_2 was from 5.83 to 9.48% and for CO_2 was from 4.26 to 6.87%. In general, the respiration of fruit could be shifted to anaerobic if the O_2 and CO_2 levels

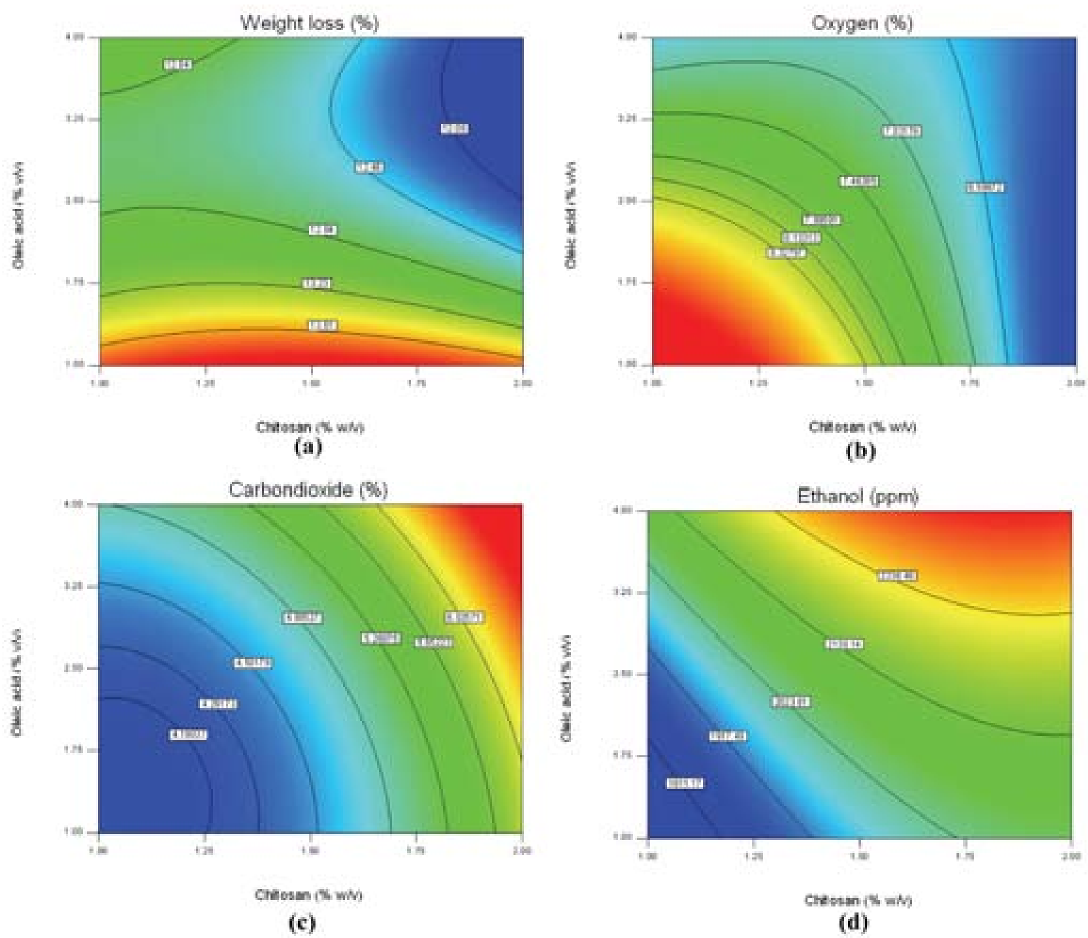

Figure 1 Contour plots for the effect of chitosan (X_1), and oleic acid (X_2) on: (a) weight loss; (b) oxygen; (c) carbon dioxide; and (d) ethanol of coated tangerine.

were less than 1-3% and greater than 10%, respectively (Kader, 1986). Hagenmaier (2002) proposed the extinction point of internal O_2 was 4% and of CO_2 was 5% for a mandarin hybrid at 21°C. Thus, the range of O_2 and CO_2 generated from the current experimental combinations of chitosan and oleic acid coating could be used for tangerines when stored at high temperature. However, the optimal combination must be determined.

Ethanol

Ethanol can be present in many citrus fruits and contribute to the formation of an off-flavor, affecting consumer acceptability (Ahmed and Khan, 1987). Table 2 shows that ethanol concentrations were affected by the concentrations of chitosan and oleic acid in the coating formula. The ethanol content was lower in the uncoated tangerine than in the coated ones. The ethanol level increased with an increased amount of chitosan and oleic acid (Figure 1d). Sinclair (1984) reported that the ethanol concentration in orange juice was affected by waxing. Tangerines kept at 21°C for 5 d were rated as less fresh when the ethanol content was greater than 1500 ppm (Hagenmaier, 2002). Alonso *et al*. (2005) found that the ethanol concentration in mandarins ranged from 1524.3 to 4009.1 ppm, but these levels did not affect consumer acceptance. From the current experiment, the range of ethanol was 1,849 to 2,350 ppm, which corresponded to Alonso *et al.* (2005). In the other part of the current study, the tangerine coated with chitosan-oleic acid and stored at 30°C for 3 wk was accepted by the test panel (unpublished data). This work confirmed the relationship between concentrations of ethanol and the coating formulations.

Optimization of chitosan-oleic acid coating formula

Optimization was based on the generation of results that minimized the response for weight loss, CO_2 and ethanol, and maximized the response for O_2. The desirability function value varied from 0 (undesirable response), to 1 (desirable response). The optimum desirability range proposed by Lazic (2004) was 0.80-0.63. The desirability values for weight loss, O_2, CO_2 and ethanol ranged from 0.66 to 0.95, while the composite desiribility was 0.80. The optimum chitosan-oleic acid concentration for coating was found to be 1% chitosan and 2.50% oleic acid (Table 4). Table 4 shows that this optimum formula gave predicted values of weight loss (12.81%), internal oxygen (8.38%), carbon dioxide (4.24)%, and ethanol (1885.71 mg/L). In contrast, the uncoated tangerine showed a higher weight loss (15.15%), but lower levels of carbon dioxide (3.97%), and ethanol (1011.74 mg/L). The great advantage of the chitosan-oleic acid coating was the reduction in weight loss, which can be beneficial to the fruit grower and retains the fresh look to consumers.

Table 4 Optimization of chitosan coating in terms of composite properties.

Response variable	Optimization of chitosan-oleic coating						
	Goal	Lower	Target	Upper	Weight	Predicted responses	Desirability
Weight loss (%)	Minimize	0	0	13	0.1	12.81	0.66
O_2 (%)	Maximize	4	20	20	0.1	8.38	0.88
CO_2 (%)	Minimize	0	0	15	0.1	4.24	0.95
Ethanol (ppm)	Minimize	0	0	2000	0.1	1885.71	0.75

Local solution :Chitosan=1.0%, oleic acid=2.50%
Composite desirability = 0.80

CONCLUSION

RSM was a useful tool to generate the combination formula for coating mixtures on tangerines. It generated the models that could explain the effect of independent variables on the response variables. The concentrations of chitosan and oleic acid in the coating formulation affected the internal gases and ethanol. Both chitosan and oleic acid had a linear effect on weight loss, ethanol, carbon dioxide and oxygen. Only oleic acid showed a quadratic effect on the weight loss. Thus, the optimum formulation for coating tangerines was identified as 1% (v/v) chitosan plus 2.5% oleic acid (v/v), which was suitable for storage at room temperature (30±2°C) for 3 wk. From these results, this formulation is suggested for short-term storage at room temperature.

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