

# Effect of Coating Formulations and Packaging Perforation on Biochemical Properties of Litchi under Ambient Storage Conditions

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## Abstract

The quality of litchi affected during storage due to deterioration and change in chemical properties. The total soluble solids of the coated litchi fruits showed slight changes under ambient conditions, each in the range of 17.78 to 20.87 (° C Brix). The acidity of the coated packaged litchi fruit was significantly reduced in ambient storage conditions, ranging from 0.12 to 0.27%. The synergistic effect of coating and packaging could be seen under ambient storage.

**Keywords:** Litchi,  $\alpha$ -Tocopherol, Salicylic acid, Perforation, Biochemical.

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## Introduction

Litchi (*Litchi chinensis* Sonn.) is a subtropical fruit with white, translucent aril and attractive red colour. Litchi is highly perishable and shelf life of litchi under ambient conditions does not more than 24-78 hours. Postharvest decay and browning along with major changes in TSS, acidity and sugar content were identified as major problems restricting expansion of the industry in litchi-exporting countries as well as in the domestic market. Long storage conditions can put the fruits under tremendous physiological stress, that can cause major chemical changes. Postharvest losses of litchi are anticipated to be 20–30% of the harvested fruit and might also be as excessive as 50% previous to consumption[1].

Minimally processed fruits are one of the major growing segments in food retail market. The aim of this work was to study the effect of coatings formulations to maintain chemical properties under ambient conditions to enlarge litchi market.

## Material and Methods

### Materials

The Rose Scented of litchi fruits were harvested at 90 – 100% of the peel exhibiting red colour. Immediately after harvesting, grading was done. The  $\alpha$ -Tocopherol, chitosan and salicylic acid, Tween-20 and other chemicals required for experiment were purchased from Sigma Aldrich Chemicals Pvt. Ltd. Mumbai (Maharashtra).

Fruits were treated with aqueous solutions of according to the treatment details. The aqueous solutions of  $\alpha$ -Tocopherol, chitosan and salicylic acid, in combination according to the treatment details.

Treatments were performed by dipping the fruits in 5 litre treatment solutions containing Tween-20 (2 g L<sup>-1</sup>) as surfactant, at 25°C for 5 min. Whereas, control fruits were dipped in distilled water. Following treatment application, fruits were air-dried at room temperature, then packaged in perforated polyethylene bags and stored at room temperature (25°C) in plastic basket. After 3, 6, 9, 12 and 15 days of storage, fruits from each treatment were sampled at random for analysis.

### Experimental plan

The variables that have greatest influence on the response could be selected as operating parameters of final experiments [2] (Table 1).

### Perforation Percentage

The polypropylene packages with 1.57 mm<sup>2</sup> perforation surfaces showed lowest fungal infestation and reduced deterioration [3].

**Table 1** Selected independent, dependent and constant parameters and their values

Sr. No	Parameters	Levels	Range	Responses
<b>Independent variables</b>				
1	$\alpha$ -Tocopherol (%)	5	0.1,0.2,0.3,0.4,0.5	Total soluble solids
2	Chitosan (%)	5	0.5,1.0,1.5,2.0,2.5	Titrateable acidity
3	Salicylic acid (mM)	5	0.5,1.0,1.5,2.0,2.5	
4	Perforation (%)	5	0.1,0.2,0.3,0.4,0.5	

**Dependent variables***Total soluble solids (TSS)*

The extracted juice of fruit was homogenized and then reading recorded for sample by refractometer. The results were expressed as °Brix.

*Titrateable acidity (TA)*

The 5 g of fruit sample was crushed with 100 ml water. The filtered sample was titrated against 0.1 N sodium hydroxide solution using 3-4 drops of 1% phenolphthalein solution as indicator. The sample was observed for 3 times to get equal titre value. The titre value was used for calculating the values as malic acid by using the following formula.

$$\text{Titrateable acidity (\%)} = \frac{\text{Titre value} \times \text{Normality of alkali} \times \text{Vol. made up} \times \text{Eq. wt. of acid} \times 100}{\text{Volume of sample taken for estimation} \times \text{Wt. or Vol. of sample taken} \times 1000}$$

**Statistical analysis**

Experiments were designed using Central Composite Rotatable design (CCRD) method for satisfying the principle of randomization of Response Surface Methodology (RSM). For vanishing the effect of residuals during Response surface analysis, levels of the independent variables have been expressed in coded form. **Table 2** shows the actual and coded values of different levels of each independent variable considered during study.

**Table 2** Experimental variables (independent) for enhancement of shelf life of Litchi (coded and actual values)

Independent variables Name	Coded Levels Code	Actual Levels				
		-2	-1	0	1	2
$\alpha$ -Tocopherol (%)	A	0.1	0.2	0.3	0.4	0.5
Chitosan (%)	B	0.5	1.0	1.5	2.0	2.5
Salicylic acid (mM)	C	0.5	1.0	1.5	2.0	2.5
Perforation (%)	D	0.1	0.2	0.3	0.4	0.5

**Fitting of Second Order Model**

To determine the relationship between the dependent and independent variables, the obtained experimental data were subjected to the multiple regression analysis. Regression analysis was done by using Design Expert 8.0.6 software. Each response was represented by a second order polynomial equation. A second order response function for four independent variables had the following general form:

$$Y = \beta_0 + \sum_{i=1}^4 \beta_i A_i + \sum_{i=1}^2 \sum_{j=i+1}^4 \beta_{ij} A_i B_j + \sum_{i=1}^4 \beta_{ii} A_i^2 \quad (1)$$

Where,  $\beta_0$ ,  $\beta_i$ ,  $\beta_{ii}$ ,  $\beta_{ij}$  are constants, A, B are variables (coded)

The adequately qualified model with respect to known criteria (i.e., High  $R^2$ ,  $F_{cal} > F_{tab}$  and non-significant lack of fit) was selected to describe the behaviour of response, otherwise, transformation of the dependent variable was performed [2].

### Statistical Analysis and Optimization of Variables

Multivariate optimization and data analysis (ANOVA) of independent and dependent variables were carried out through Response Surface methodology (RSM) using Design Expert 8.0.6. 3-D surface plot were drawn using Sigma plot-10 software.

## Results and Discussions

### Effect of independent variables on total soluble solids (TSS)

Total soluble solids of coated litchi fruits stored at ambient storage condition varied in the range of 17.78 to 20.87%. The maximum value obtained at experiment number 30 with experimental conditions of  $\alpha$ -Tocopherol 0.2% (A=-1), 2% chitosan (B= 1), salicylic acid 2 mM (C=1) and perforation 0.4% (D=1), while minimum (17.78) with experimental conditions of  $\alpha$ -Tocopherol 0.4% (A=1), 2% chitosan (B=1), salicylic acid 2 mM (C=1) and perforation percentage of 0.4% (D=1) at experiment no.15 (**Table 3**). The full second-order mathematical model equation (1) was used to fit TSS data and the result of regression analysis for total soluble solids were imitated by equation (2). The coefficient of determination ( $R^2$ ) for the regression model for this parameter was 74.21% and adj  $R^2$  was 50.14%, which implies that the model could account for 74.21% data. The model was found to be significant at 5% level of significance with non-significant lack of fit. Therefore, the second-order model was adequate for describing the change in total soluble solids with the specified values of independent parameters.

**Table 3** Experimental data on optimization of coating and packaging perforation of litchi for ambient storage condition (15<sup>th</sup> Day).

Treatments	$\alpha$ - Tocopherol (%)	Chitosan (%)	Salicylic acid (mM)	Perforation (%)	TSS (°Brix)	Acidity (%)
1	0.2	2	1	0.2	19.53	0.13
2	0.5	1.5	1.5	0.3	18.38	0.25
3	0.3	1.5	1.5	0.3	18.23	0.19
4	0.1	1	2	0.4	19.33	0.17
5	0.4	2	1	0.2	18.21	0.15
6	0.4	1	1	0.2	18.64	0.14
7	0.4	2	2	0.2	18.32	0.17
8	0.3	1.5	1.5	0.3	18.12	0.19
9	0.3	1.5	0.5	0.3	20.17	0.2
10	0.3	1.5	1.5	0.3	18.18	0.19
11	0.4	2	1	0.4	19.62	0.18
12	0.3	1.5	1.5	0.3	18.21	0.19
13	0.2	1	1	0.2	20.43	0.12
14	0.2	1	1	0.4	19.45	0.12
15	0.4	2	2	0.4	17.78	0.27
16	0.2	2.5	1	0.4	20.53	0.14
17	0.3	2.5	1.5	0.3	18.76	0.24
18	0.3	1.5	1.5	0.3	18.18	0.18
19	0.4	1	2	0.2	18.78	0.16
20	0.3	0.5	1.5	0.3	20.26	0.2
21	0.4	1	1	0.4	19.21	0.14
22	0.2	2	2	0.3	18.51	0.15
23	0.5	1.5	1.5	0.3	20.53	0.19
24	0.3	1.5	1.5	0.5	18.24	0.23
25	0.3	1.5	1.5	0.3	18.16	0.18
26	0.3	1.5	1.5	0.3	18.78	0.23
27	0.3	1.5	1.5	0.1	20.08	0.21
28	0.2	0.5	1.5	0.2	19.96	0.13
29	0.2	0.5	1.5	0.4	20.31	0.15
30	0.2	2	1.5	0.4	20.87	0.16

$$\text{TSS} = 18.18 - 0.58A - 0.23B - 0.18C + 0.04D - 0.08AB - 0.07AC - 0.05AD - 0.19BC + 0.23BD + 0.04CD + 0.30A^2 + 0.32B^2 + 0.31C^2 + 0.23D^2 \quad (2)$$

Where, A is  $\alpha$ -Tocopherol, Bis chitosan, Csalicylic acid and D is perforation percentage (all in coded form).

It is revealed (**Table 4**) that at linear levels, only Tocopherol effected significantly with 1% of level of significance while at quadratic levels Tocopherol, chitosan, and salicylic acid had a significant effect on the TSS at 5% level of significance. The chitosan and salicylic acid at particular concentration might develop suitable microclimate around litchi fruit which might help to maintain total soluble solids, whereas the higher concentration might be beyond optimum range which might lead to negative effect. There was no any significant effect in interactive terms of independent variables. Therefore, Eq.2 was modified as:

$$\text{TSS} = 18.18 - 0.58A + 0.30A^2 + 0.32B^2 + 0.31C^2 \quad (3)$$

Interpretation of the interaction effect between coating and packaging treatments and storage days in ambient condition affirmed that there was a continuous increase in TSS of fruits up to 12 days, after that it decreases upto the 15<sup>th</sup> day of storage. This initial increase is due to the disruption of starch and polysaccharides into monomers of sugars and organic acids, but after 12 days the decline in TSS might be due to their utilization in evapotranspiration and other biochemical activities. The reason was also supported by [4].

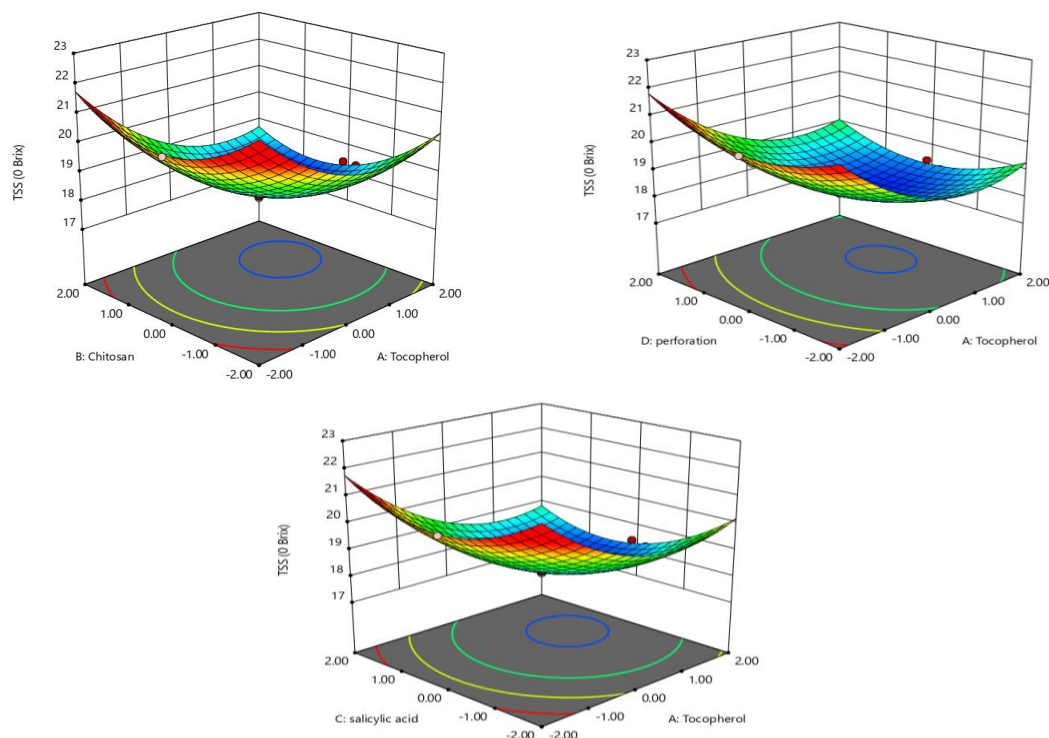
**Table 4** Effect of treatments on TSS (°Brix) under ambient storage conditions

Source	SS	Df	MS	F-value	P-value
Model	19.02	14	1.36	3.08	0.0191**
A- $\alpha$ -Tocopherol	8.17	1	8.17	18.53	0.0006***
B-Chitosan	1.37	1	1.37	3.11	0.0980
C-Salicylic acid	0.8588	1	0.8588	1.95	0.1831
D-Perforation	0.0451	1	0.0451	0.1022	0.7536
AB	0.1089	1	0.1089	0.2470	0.6264
AC	0.0870	1	0.0870	0.1974	0.6632
AD	0.0342	1	0.0342	0.0776	0.7843
BC	0.5852	1	0.5852	1.33	0.2673
BD	0.8742	1	0.8742	1.98	0.1794
CD	0.0324	1	0.0324	0.0735	0.7900
A <sup>2</sup>	2.62	1	2.62	5.95	0.0276**
B <sup>2</sup>	2.86	1	2.86	6.49	0.0223**
C <sup>2</sup>	2.71	1	2.71	6.14	0.0256**
D <sup>2</sup>	1.52	1	1.52	3.45	0.0831
Residual	6.61	15	0.4408		
Lack of Fit	NS				
R <sup>2</sup>	0.7421				
Adj R <sup>2</sup>	0.5014				

\*\*\* 1% level of significance, \*\*5% level of significance

Response surface plots as shown in **Figure 1** confirmed that total soluble solids of the treated fruits decreased at initial stage with an increase in tocopherol percentage (0.3%) and then it was increased at the same rate with increase in tocopherol. The increase in chitosan, salicylic acid, and perforation percentage showed increasing trend in total soluble solids. However, it was nearly stable to its central values (1.5%), (1.5mM) and (0.3%) respectively, Similar findings have been reported by [1], [5], and [6].

In case of the non-climacteric fruits like litchi, the TSS increase during storage due to the continued action of sucrose phosphate synthase, the results depicted that the TSS of coated litchi fruits increase continuously during storage [7]. The  $\alpha$ -Tocopherol is a source of vitamin E which might help to maintain the physiochemical properties of litchi during storage to some extent. The  $\alpha$ -Tocopherol prevent the action of cell wall degrading enzymes like pectin methylesterase, polygalacturonase, and cellulase [8]. The treated fruits maintained TSS in litchi fruits by preventing activity of cell wall degrading enzymes and water loss, it might be synergistic effect of coating and packaging which maintain sensory quality of fruits up to 15 days during ambient storage condition [9].



**Figure 1** Effect of independent variables on total soluble solids under ambient storage condition

### Effect of independent variables on Titratable acidity

Fruit ripening and senescence leads to the changes in sensory properties which affect eating qualities. The acidity of litchi fruits decrease during storage, irrespective of the treatment [8]. Acidity of coated packaged litchi fruits stored at ambient storage condition varied in the range of 0.12 to 0.27%. The maximum value obtained at experiment number 15 with experimental conditions of  $\alpha$ -Tocopherol 0.4%(A=1), 2% chitosan (B= 1), salicylic acid 2 mM (C=1) and perforation 0.4% (D=1), while minimum (0.12) with experimental conditions of  $\alpha$ -Tocopherol 0.2%(A=-1), 1% chitosan (B=-1), salicylic acid 1 mM (C=-1) and perforation percentage of 0.2% (D=-1) and  $\alpha$ -Tocopherol 0.2% (A=-1), 1% chitosan (B=-1), salicylic acid 1 mM (C=-1) and perforation percentage of 0.4% (D=1) at experiment no.13 and 14 respectively (Table 3). The full second-order mathematical model equation (1) was used to fit data of Titratable acidity. The coefficient of determination ( $R^2$ ) for the regression model for this parameter was 36.44% and adj  $R^2$  was 26.26%, which implies that the model could account for only 36.44% data. Therefore the second order equation is inadequate to represent the behaviour of change in titratable acidity. Therefore, linear polynomial equation which represent the change in titratable acidity at selected range of independent variables are represented by equation 4.

$$\text{Titratable acidity} = 0.1783 + 0.0166 A + 0.0125B + 0.0125C + 0.0091D \quad (4)$$

Where, A is  $\alpha$ -Tocopherol, Chitosan, C is salicylic acid and D is perforation percentage (all in coded form).

**Table 5** expresses the individual effect of each term in second-order quadratic equation fitted to the experimental data. It is revealed that at linear levels only one independent variable  $\alpha$ -tocopherol effected significantly with 5% of the level of significance while there were non-significant effect of remaining independent variables [10]. It might be due to the superimpose effect of  $\alpha$ -tocopherol at minute levels in maintaining organic acids by improving antioxidant capacity. Therefore Eq.5 was modified as

$$\text{Titratable acidity} = 0.1783 + 0.0166 A \quad (5)$$

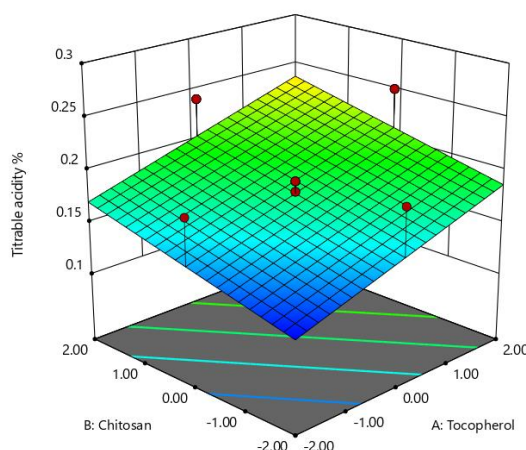
The  $\alpha$ -Tocopherol prevents the action of enzymes responsible for utilization of organic acids during respiration, maintained higher amount of ascorbic acid during storage [4]. The salicylic acid application reduces pH and maintain acidity.

**Table 5** Effect of treatments on Titratable acidity (%) under ambient storage conditions

Source	SS	Df	MS	F-value	P-value
Model	0.0162	4	0.0040	3.58	0.0193**
A- $\alpha$ -Tocopherol	0.0067	1	0.0067	5.90	0.0226**
B-Chitosan	0.0037	1	0.0037	3.32	0.0804
C-Salicylic acid	0.0038	1	0.0038	3.32	0.0804
D-Perforation	0.0020	1	0.0020	1.79	0.1935
Residual	0.0282	25	0.0011		
Lack of Fit	NS				
R <sup>2</sup>	0.3644				
Adj R <sup>2</sup>	0.2626				
Pred R <sup>2</sup>	0.0129				

\*\*\* 1% level of significance, \*\*5% level of significance

The **Figure 2** showed that there is direct correlation between acidity and  $\alpha$ -Tocopherol percentage. This might be responsible for the higher titratable acidity (0.27%) in fruits coated with combined formulations of  $\alpha$ -Tocopherol, chitosan and salicylic acid [6]. The acidity increases during storage due to the fermentation. The increase in CO<sub>2</sub> concentration around fruits may leads to the fermentation of fruits, but it might be restricted by perforation during packaging, though it doesn't given any significant effect on acidity of litchi during storage under ambient conditions results are affirmed the findings of [11].



**Figure 2** Effect of independent variables on Titratable acidity under ambient storage condition

### Optimization of independent variables

To optimize the process parameters, the goal was fixed to be maximize or minimize **Table 6**.

**Table 6** Constraints for optimization coating formulation for ambient storage of litchi.

Name	Goal	Lower limit	Upper limit
$\alpha$ -Tocopherol	is in range	-2	2
Chitosan	is in range	-2	2
salicylic acid	is in range	-2	2
perforation	is in range	-2	2
TSS	is in range	17.78	20.87
Titratable acidity	is in range	0.12	0.27

All the responses and independent variables were given similar (+++) importance. If the response selected to be maximum, upper limit was taken as maximum value of the response and lower limit was taken as the 25% less of the upper limit of response. If the response required is minimum, lower limit was considered as minimum value of response and upper limit was 25% more of the lower limit.

The optimum results of coating formulation applied on litchi fruit includes 0.4% of  $\alpha$ -Tocopherol, 2% of chitosan, 2 mM of salicylic acid and 0.4% of perforation on packaging material. Whereas the fruits under control condition starts losing its quality from second day onwards on the day 3<sup>rd</sup> most of the fruits were not suitable for marketing and becomes brown.

**Table 7** Optimum value of parameters for coating formulation applied on litchi fruit

Value	$\alpha$ -Tocopherol, % (X <sub>1</sub> )	Chitosan, % (X <sub>2</sub> )	Salicylic acid, mM (X <sub>3</sub> )	Perforation, % (X <sub>4</sub> )
Coded	1.0	1.0	1.0	1.0
Actual	0.4	2	2	0.4
Observations recorded at optimum levels of independent parameters during ambient storage for 15 days				
TSS (° Brix)			17.78	
Titratable acidity (%)			0.27	

**Table 8** Observations recorded under control conditions during ambient storage.

Observations	0 Day	1 Day	2 Days	3 Days
TSS (° Brix)	16.32	17.46	19.38	21.44
Titratable acidity (%)	0.56	0.45	0.37	0.21

## Conclusion

The results concluded that the combination of  $\alpha$ -Tocopherol (0.4 %), chitosan (2.0 %), salicylic acid (2.0 mM) and packaging with perforation percentage (0.4) were found optimized for ambient storage conditions for better shelf life with better maintenance of TSS and acidity compounds.



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