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Effect of α-tocopherol based coating formulation on pericarp browning of litchi

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Abstract
The litchi is a perishable fruit and only 10% of the total production is processed due to its poor shelf life, lack of processing and postharvest technologies. The approaches to inhibit the browning reactions is excluding oxygen, adding antioxidants as anti-browning agent or inhibiting the activity of the responsible enzymes. In the present investigation, litchi fruits subjected to 0.4% of α-Tocopherol, 2% of chitosan, 2 mM of salicylic acid, and 0.4% of perforation on packaging material had good retention of total anthocyanin content 8.93 mg/100g of fruit weight with minimum fruit pericarp browning index 52.70 at ambient storage conditions.

Keywords: Anthocyanin, coating, litchi, pericarp browning

1. Introduction
Litchi (*Litchi chinensis* Sonn.) is highly commercialized subtropical fruit. It has a shelf life of only 24-72 hours at ambient storage conditions (Minh *et al*., 2019) [8]. Post-harvest deterioration and browning of pericarp has been identified as a significant barrier to industrial use for further processing and value addition. Longer storage times put a greater strain on the fruits and can lead to increased spoilage. Post-harvest browning of litchi fruit is primarily due to rapid degradation of anthocyanins (Lee and Wicker, 1991) [4]. Therefore, in India, postharvest losses of litchi are estimated to be 20–30% of the harvested fruit and can even be as high as 50% prior to consumption (Kumar *et al*., 2013) [3].
Postharvest treatments, such as sulphur fumigation and acid dip can effectively inhibit PPO activity and thus delay loss of red skin colour of litchi fruit (Zauberman *et al*., 1991) [20]. Sometimes the residual effect of these chemicals may possess an adverse effect on consumer acceptance and export rejection. Also, nowadays this is a serious concern of food safety and regulations agencies at the national as well as international level (Sivkumar *et al*., 2005) [15]. Therefore, there is a continuous demand for alternative chemicals for colour control without toxic effects in harvested litchi fruit. Alternative chemical-free methods, such as irradiation with radioactive elements, have recently been developed but are limited by significant investment, highly skilled labour, and tight norms and regulations (Nicolai *et al*., 2007) [9].
The chitosan-based edible coating is recognized as safe for consumption with excellent antimicrobial and antioxidant capacity (Kumar *et al*., 2020) [17]. Moreover, when α-tocopherol added to the chitosan films enhanced the final quality and shelf-life extension of food products (Martins *et al*., 2012) [7]. Post-harvest application of Salicylic acid has been found to delay the senescence and preserved the health promoting compounds in litchi (Kumari *et al*., 2015) [16]. However, for using any technique, optimization of treatments is essential to get desirable result with desired quality parameters. The response surface methodology (RSM) is one of the statistical tools used for optimization of the treatments have been used by many workers (Tripathi *et al*., 2016) [17]. The present study deals with the optimization of coating formulations using RSM to reduce the pericarp browning of litchi fruits under ambient storage conditions.

2. Material and Methods
2.1 Material selection
A litchi fruit of *Rose Scented* cultivar, harvested at its ripe stage (90 – 100% of the peel exhibiting red colour) was selected for the experiments. A lot of selected fruits were harvested in the morning of the same day of treatment from Horticulture Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar, US Nagar, India.

Trees were planted at 10×10 m distance in a square system of planting and maintained under uniform cultural practices. Immediately after harvesting, fruits were graded for uniformity of shape, colour and size and any blemished or diseased fruits discarded.These graded fruits were then dipped into coating formulation prepared as per the treatment combinations (Table 1). Thereafter, fruits were stored at ambient storage (temperature 25±2 °C, RH 85±5%).

2.2 Preparation of coating formulation and application on the fruits

The selected coating ingredients *viz*., α-Tocopherol, chitosan and salicylic acid, Tween-20, and other chemicals required for the experiment were purchased from Make: Sigma Aldrich Chemicals Pvt. Ltd. Mumbai (India). The aqueous solutions of Tween-20 (2 g L^{-1}) used as surfactant with α-Tocopherol, chitosan, and salicylic acid. The mixture was continuously stirred using a high-speed stirrer to reach at appropriate homogeneity. Selected fruits were dipped into the prepared solution for 5 min (Jiang *et al*., 2018) [1]. At the same time control fruit sample was dipped in distilled water. These treated fruits were air-dried at 25°C keeping in view of room temperature and then packaged in perforated Low-density polyethylene (LDPE) bags (35µm) and stored in an environmental chamber set at 25 °C. The sample was drawn and the response was measured at regular intervals until spoiling or an increase or decrease in any of the response values above the threshold. The first incidence of visible spoilage of coated sample was observed 12 days after storage (DOS) therefore the storage and estimation of dependent parameters were discarded thereafter.

2.3 Experimental plan

The higher and lower ranges of independent variables were selected based on literature and initial trials conducted before designing the final experiment using response surface methodology (Zambrano-Zaragoza *et al*., 2014**;** Kumar, Mukherjee, and Dutta, 2020) [17, 19]. Therefore, four independent variables i.e α-tocopherol (0.1, 0.2, 0.3, 0.4, 0.5 percent), Chitosan (0.5, 1.0, 1.5, 2.0, 2.5 percent), salicylic acid (0.5, 1.0, 1.5, 2.0, 2.5 mM), and perforation percentage (0.1, 0.2, 0.3, 0.4, 0.5) of packing material with five level of each were selected as the independent variables. The actual and coded values of the selected levels are represented in Table 1.The different combinations of the experiments were statistically designed using Central Composite rotatable Design (CCRD). The experimental conditions matrix with its randomized operation order is represented in Table 1. The experiments were conducted as per the run order mentioned in the design matrix. The selected dependent variables i.e. pericarp browning index and anthocyanin were evaluated at each experimental condition, and their data were used for further analysis and interpretations. The optimization of the independent variables was done using Response Surface Methodology (RSM) based on the pre-set goal of selected dependent variables.

2.4 Biochemical analysis

The pericarp browning index of litchi was assessed visually by measuring the extent of total browned area on the pericarp (Kumar *et al*., 2012) [11]. For this, fruits were evaluated on the following scale: 0 = no browning (excellent quality); 1 = slight browning; 2 = <1/4 browning; 3 = 1/4–1/2 browning; 4 = 1/2–1/3 browning and 5 = >1/3 browning (poor quality). The pericarp browning index was calculated using the following formula:

$$\text{Pericarp browning index} = \sum \frac{\text{Browning scale} \times \text{number of fruits in scale}}{\text{Total Number of fruit}}$$

The pH-differential method (Wrolstad *et al*., 2005) [8] using two buffer systems- potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M) used for determination of total anthocyanin content in the litchi fruit pericarp. Samples were diluted in pH 1.0 and pH 4.5 buffers, and absorbance measurements on spectrophotometer were made at 520 and 700 nm using 1 cm path length cuvette. The pigment content was calculated and expressed as mg cyanidin-3glucoside per 100 g fresh weight (FW), using molar extinction coefficient (ε) of 26900 L mol^{-1} cm^{-1} and molecular weight of 449.2 g mol^{-1}.

Absorbance (A) = ($A_{520\ nm} - A_{700\ nm}$) pH 1.0 – ($A_{520\ nm} - A_{700\ nm}$) pH 4.5

$$\text{Total anthocyanin content (mg /100 g)} = \frac{A \times MW \times DF \times 1000}{(\varepsilon \times 1 \times \text{sample wt.})}$$

Where,
A = absorbance; MW = molecular weight; DF = dilution factor; ε = Molar extinction coefficient of pigment.

2.5 Statistical Analysis and Optimization of Variables

A second-order quadratic equation was fitted to the experimental data of selected dependent variable obtained at CCRD designed experimental conditions of independent variables. Multivariate data analysis (ANOVA) and the effect of independent variables at their different interactions on the dependent variables were carried out using Design Expert 8.0.6 software**.** 3-D surface plots of respective dependent variables were also drawn to visualize the individual and combined effect of each independent variable by keeping another two variables at their respective optimum points. A pre-set goal-based multiple response optimization of the independent variables was also carried out through Response Surface Methodology (RSM) technique. The generalized desirability function used for optimization and can be maximized or minimized as per the required goal are as follows:

$$D = [d_1(Y_1).d_2(Y_2)\cdots d_6(Y_6)]^{1/6} \quad (1)$$

Where,
Y_i = predicted value of dependent variable (i=1 to 6)

3. Results and Discussions

The degree of browning of the pericarp is directly proportional to the color of the pericarp and ultimately to the market value of litchi. Experiments on the coating of lychee fruits were conducted according to the experimental design matrix, and relevant data were recorded in the form of quality parameters of coated fruits and used for further analysis (Table 1).

3.1 Effect of independent variables on pericarp browning index

The pericarp browning index of packaged coated litchi fruits increased with time at its ambient storage and it confined within 52.7 to 208.55. The experiment number 15 recorded minimum (52.7) pericarp browning index, where the coating formulation contain α-Tocopherol 0.4%, 2% chitosan, salicylic acid 2 mM and perforation percentage of 0.4%, while the maximum (208.55) recorded in the experiment number 13 (Table 1), with experimental conditions of α-Tocopherol 0.2%, 1% chitosan, salicylic acid 1 mM and perforation percentage of 0.2%.

The data regarding browning index recorded was fitted into full second-order mathematical model equation (1) and the result of regression analysis was denoted by equation (2). The coefficient of determination (R^2) for the regression model for the browning index was 89.90% and adjusted R^2 was 79.10%, which suggest that the model could account for 89.90% data. Model was found to be highly significant at 1% level of significance with non-significant lack of fit. Therefore, second-order model was competently taken into consideration for conveying changes in pericarp browning index with the specified values of independent parameters.

Pericarp browning = 87.41-23.21A-17.65B-14.01C-26.17 D - 1.98AB - 0.28AC -5.85AD + 3.10 BC - 3.89BD - 3.60 CD+ 11.28 A^2+ 17.54B^2 + 4.44 C^2 + 16.67D^2 (2)

Where,
A is α-Tocopherol,
B is chitosan,
C is salicylic acid and
D is perforation percentage (All in coded form).

Table 1 revealed that at linear levels two independent variables α-Tocopherol and perforation percentage had highly significant (p<0.01) effect on browning index. Also chitosan and salicylic acid were effective significantly at 1% level of significance. The major reasons for the pericarp browning are loss of water from pericarp, oxidase enzymes activity and unfavourable pH conditions, but as the coating formulations were prepared from anti-browning agent α-Tocopherol it played a vital role in minimization of enzymatic activity and hence prevented the pericarp browning (Zambrano-Zaragoza *et al*., 2014) [19].

The chitosan and salicylic acid maintained the moisture of pericarp and its pH respectively, ultimately reduces the pericarp browning. The perforation percentage might decide the activity of oxidizing enzymes as the oxygen is prerequisite for oxidase enzymes like polyphenol oxidase and peroxidase, therefore the rate of browning is directly proportional to the perforation (Lin *et al*., 2011) [5].

There was no any significant effect of independent variables on pericarp browning index of coated litchi fruits at interactive levels. The interactive effect might be nullified due to mutual dilution of effect in combination. In quadratic levels, α-Tocopherol effected significantly at 5% level of significance, while the chitosan percent and perforation percentage were affected significantly at 1% level of significance. The results were in accordance with (Kumari *et al*., 2015) [13]. Therefore, simplified second order equation (3) of pericarp browning index becomes,

Pericarp browning = 87.41 - 23.21A - 17.65B - 14.01C - 26.17D+11.28A^2+17.54B^2+16.67D^2 (3)

Response surface plots as shown in Fig. 1 clearly displayed that pericarp browning of the treated fruit decreased steeply at the initial increase in tocopherol percent and afterward its increases slightly with an increase in tocopherol. A very little effect of salicylic acid on pericarp browning index was displayed in the graph. The increase in perforation percentage up to its central value tends to decrease in pericarp browning index, while it was increasing at higher rate thereafter with an increase in perforation (Sivakumar and Korsten, 2006) [16], it might be due to the fact that equilibrium gets disturbed and presence of further oxygen promoted polyphenol oxidase and peroxidase activity.

3.2 Effect of independent variables on anthocyanin

The coated litchi fruits stored at ambient storage condition were analyzed for anthocyanin content on the 12th day of storage and the maximum value obtained at experiment number 15 with experimental conditions of α-Tocopherol 0.4%, 2% chitosan, salicylic acid 2 mM and perforation percentage of 0.4%, whereas the minimum anthocyanin content (2.87 mg/100g FW) observed in experiment no.13 (Table 1) with experimental conditions of α-Tocopherol 0.2%, 1% chitosan, salicylic acid 1 mM and perforation 0.2%.

Anthocyanin content data was fitted into full second-order mathematical model equation (1) and the result of regression analysis was represented by equation (4). The coefficient of determination (R^2) for the regression model for anthocyanin content was 82.48% and adj R^2 was 66.14%, which refers that the model could render for 82.48% data. The model was found to be significant at 1% level of significance with non-significant lack of fit. Therefore, second-order model was considered to be appropriate for describing changes in anthocyanin content with the specified values of independent parameters.

Anthocynin content = 7.85 + 0.89A + 0.67B + 0.76C + 0.56D + 0.001AB + 0.20AC + 0.06AD + 0.05BC + 0.10BD + 0.04CD - 0.48A^2- 0.48B^2 - 0.46C^2 - 0.42D^2 (4)

Where,
A is α-Tocopherol,
B is chitosan,
C is salicylic acid and
D is perforation percentage (all in coded form).

Table 2 revealed that at linear levels all independent variables affected anthocyanin content significantly with 1% of the level of significance except perforation percentage which is significant at 5% level of significance. There was no any significant effect in interactive terms of independent variables, whereas at quadratic levels anthocyanin content was affected at 5% level of significance. Therefore, simplified second order equation of anthocyanin content becomes,

Anthocynin content = 7.85 + 0.89A + 0.67B + 0.76C + 0.56D - 0.48A^2- 0.48B^2 - 0.46C^2 - 0.42D^2 (5)

The response surface plots predicted the effect of independent variables as shown in Fig. 2. It is clear from the graph that with increasing rate of tocopherol and chitosan to some extent

the anthocyanin levels in stored litchi pericarp was maintained at its higher levels. With the increase in salicylic acid and perforation percentage it slightly increases up to its central values i.e.1.5mM and 0.3% respectively, while it was decreased slightly thereafter.

The results are consistent with, the levels of anthocyanin in the pericarp of coated litchi were maintained higher level by the salicylic acid and perforation percentage up to a certain limit afterward rise creates somewhat negative effect on anthocyanin and eventually colour of litchi Sivakumar and Korsten (2006) [16]. According to Ruenroengklin *et al*., (2009) [14], polyphenol oxidase accelerated anthocyanin degradation by directly oxidising (-)- epicatechin, which was the oxidative by product of epicatechin-catalyzed anthocyanin degradation in litchi. The polyphenol oxidase is inhibited by tocopherol, which helps to retain higher anthocyanin levels in coated litchi fruits during storage by lowering its activity.

The chitosan formed the modified atmosphere around coated litchi which reduces the rate of respiration and tocopherol content might act as a protective substance which results in maintenance of a higher level of anthocyanin during ambient storage Zhang and Quantick, (1997) [21]. The pH is also responsible for degradation of anthocyanin as an increase in pH make it more unstable resulting in the development of colorless chromanol, while it can be stored at the stable form of flavylium at pH 3.0 and below (Jurd, 1972) [2]. The salicylic acid reduces pH which results in high anthocyanin content after 12 days of storage.

3.3 Optimization of independent variables

The respective goals of the selected dependent variables were set likewise maximum anthocyanin content with a minimum pericarp browning index. All the responses and independent variables were given similar (+++) importance. Among all the optimized solutions are given by the software, the best optimized solution for optimum values of independent variables was selected based on the criteria that the optimum values should be close to viable values at higher desirability. The optimum results of coating formulation applied on litchi fruit include 0.4% of α-Tocopherol, 2% of chitosan, 2 mM of salicylic acid, and 0.4% of perforation on packaging material given in the table 4.

Table 1 Experimental data on optimization of coating and packaging perforation of litchi at ambient storage condition (12th Day).

Observations Treatments	Tocopherol (ml)	Chitosan(g)	Salicylic acid (mm)	Polyethylene perforation (%)	Pericarp browning index	Anthocyanin content (mg/100g FW)
T1	0.2	2	1	0.2	201.06^{b}	4.1^{v}
T2	0.5	1.5	1.5	0.3	56.89^{s}	8.48^{c}
T3	0.3	1.5	1.5	0.3	87.09^{nop}	7.87^{fg}
T4	0.1	1	2	0.4	86.69^{op}	7.20^{j}
T5	0.4	2	1	0.2	145.2^{i}	5.4^{p}
T6	0.4	1	1	0.2	186.58^{d}	4.58^{u}
T7	0.4	2	2	0.2	149.28^{h}	7.35^{i}
T8	0.3	1.5	1.5	0.3	87.87^{nop}	7.74^{h}
T9	0.3	1.5	0.5	0.3	106.27^{l}	5.65^{n}
T10	0.3	1.5	1.5	0.3	89.64^{n}	7.93^{ef}
T11	0.4	2	1	0.4	101.16^{m}	6.71^{k}
T12	0.3	1.5	1.5	0.3	88.71^{no}	7.77^{h}
T13	0.2	1	1	0.2	208.55^{a}	2.86^{x}
T14	0.2	1	1	0.4	180.78^{e}	3.74^{w}
T15	0.4	2	2	0.4	52.7^{t}	8.93^{a}
T16	0.2	2.5	1	0.4	143.07^{i}	4.93^{s}
T17	0.3	2.5	1.5	0.3	81.23^{q}	8.39^{d}
T18	0.3	1.5	1.5	0.3	85.6^{p}	7.82^{gh}
T19	0.4	1	2	0.2	151.54^{h}	6.2^{l}
T20	0.3	0.5	1.5	0.3	195.44^{c}	5.59^{no}
T21	0.4	1	1	0.4	137.07^{j}	5.09^{r}
T22	0.2	2	2	0.3	163.29^{g}	5.33^{q}
T23	0.5	1.5	1.5	0.3	169.75^{f}	5.52^{o}
T24	0.3	1.5	1.5	0.5	81.89^{q}	8.68^{b}
T25	0.3	1.5	1.5	0.3	85.55^{p}	7.97^{e}
T26	0.3	1.5	1.5	0.3	65.64^{r}	8.49^{c}
T27	0.3	1.5	1.5	0.1	187.88^{d}	5.80^{m}
T28	0.2	0.5	1.5	0.2	178.61^{e}	4.04^{v}
T29	0.2	0.5	1.5	0.4	143.79^{i}	4.69^{t}
T30	0.2	2	1.5	0.4	122.56^{k}	6.27^{l}

Since the p-value in ANOVA table is $p<0.05$

Table 2: Effect of treatments on pericarp browning index (%) at ambient storage conditions

Source	SS	Df	MS	F-value	P-value
Model	58268.19	14	4162.01	8.84	< 0.0001***
A-α-Tocopherol	12936.56	1	12936.56	27.47	< 0.0001***
B-Chitosan	7480.63	1	7480.63	15.89	0.0012***
C-Salicylic acid	4711.68	1	4711.68	10.01	0.0064***
D-Perforation	16446.54	1	16446.54	34.93	< 0.0001***

AB	63.18	1	63.18	0.1342	0.7193
AC	1.32	1	1.32	0.0028	0.9585
AD	548.32	1	548.32	1.16	0.2976
BC	154.35	1	154.35	0.3278	0.5755
BD	243.33	1	243.33	0.5167	0.4833
CD	207.89	1	207.89	0.4415	0.5165
A^2	3495.23	1	3495.23	7.42	0.0157**
B^2	8440.60	1	8440.60	17.92	0.0007***
C^2	542.48	1	542.48	1.15	0.3001
D^2	7631.01	1	7631.01	16.20	0.0011***
Residual	7063.60	15	470.91		
Lack of Fit			NS		
R^2			0.8919		
Adj R^2			0.7910		

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

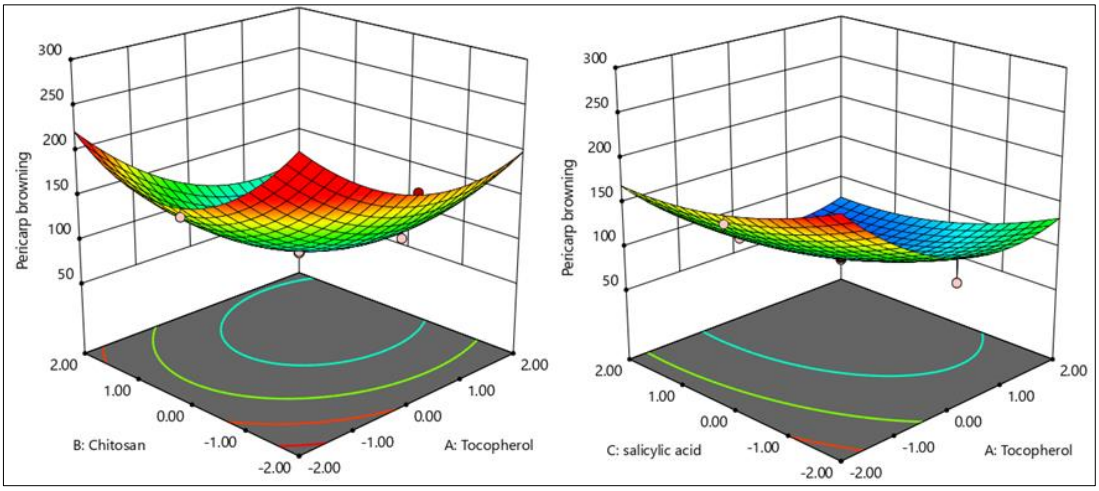
Table 3: Effect of treatments on anthocyanin (mg/100g FW) at ambient storage conditions

Source	SS	Df	MS	F-value	P-value
Model	69.03	14	4.93	5.05	0.0018***
A-α-Tocopherol	19.16	1	19.16	19.61	0.0005***
B-Chitosan	10.93	1	10.93	11.18	0.0044***
C-Salicylic acid	13.93	1	13.93	14.25	0.0018***
D-Perforation	7.55	1	7.55	7.72	0.0140**
AB	0.0000	1	0.0000	0.0000	0.9965
AC	0.6415	1	0.6415	0.6564	0.4305
AD	0.0761	1	0.0761	0.0779	0.7840
BC	0.0483	1	0.0483	0.0494	0.8271
BD	0.1669	1	0.1669	0.1708	0.6852
CD	0.0258	1	0.0258	0.0265	0.8730
A^2	6.36	1	6.36	6.50	0.0222**
B^2	6.40	1	6.40	6.54	0.0218**
C^2	5.92	1	5.92	6.05	0.0265**
D^2	4.86	1	4.86	4.97	0.0414**
Residual	14.66	15	0.9772		
Lack of Fit			NS		
R^2			0.8248		
Adj R^2			0.6614		

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

Table 4: Optimum value of parameters for coating formulation applied on litchi fruit

Value	α-Tocopherol,% (X_1)	Chitosan,% (X_2)	Salicylic acid, mM (X_3)	Perforation,% (X_4)
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 12 days				
Pericarp browning index (%)			52.7	
Anthocyanin (mg/100g Fruit weight)			8.93	



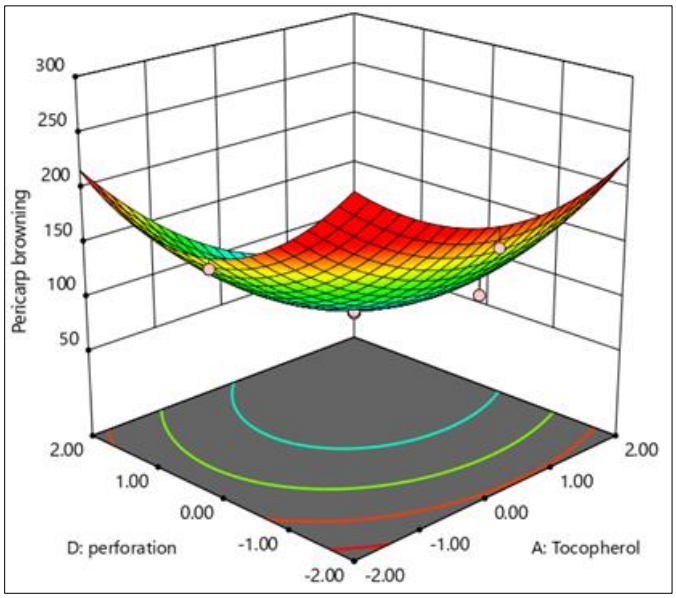
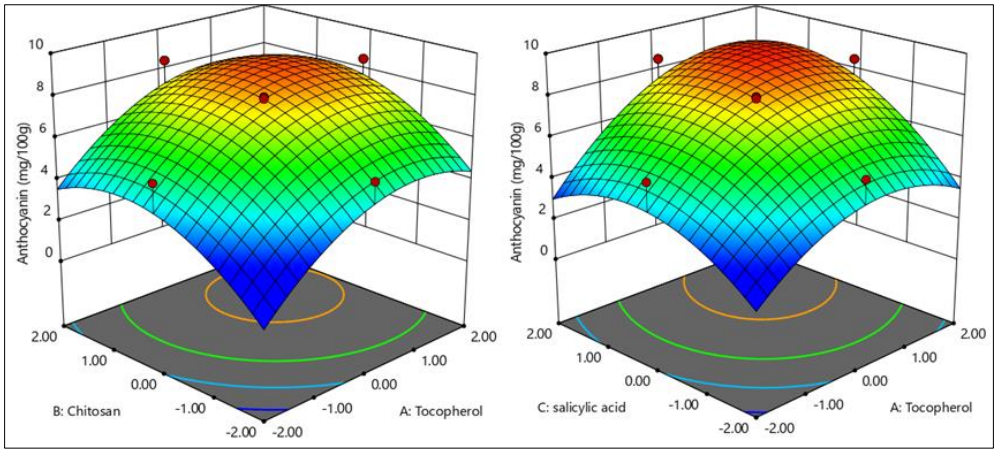


Fig 1: Effect of independent variables on pericarp browning index at ambient storage condition.



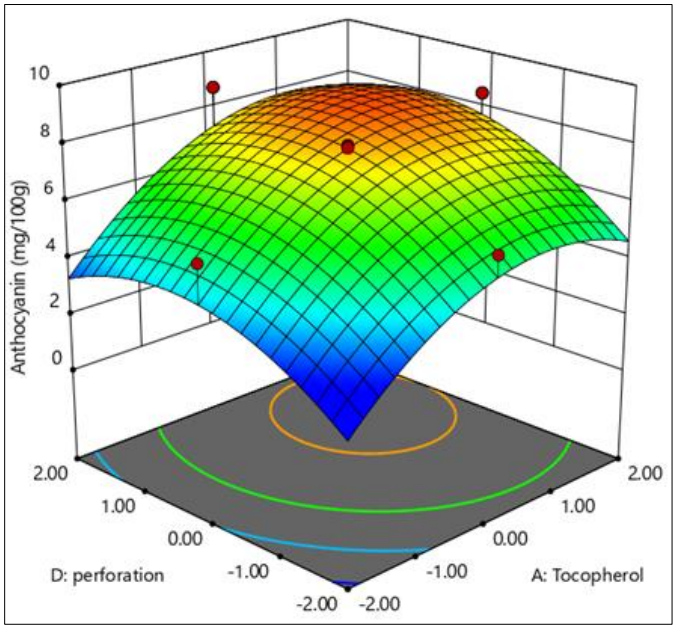


Fig 2: Effect of independent variables on total anthocyanin content ambient storage condition.

4. Conclusion

The study includes optimization of coating and packaging of litchi for maintaining better shelf life at ambient conditions. No incidence of spoilage was observed up to the 12th day of storage of coated fruits. The combination of α-Tocopherol of 0.4%, chitosan of 2.0%, salicylic acid of 2.0 mM, and

packaging with perforation of 0.4% were found optimum for ambient storage conditions and yields better shelf life with lesser browning of litchi fruits.

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