

INFLUENCE OF COATING FORMULATIONS AND PACKAGING PERFORATION ON ENZYMATIC BROWNING OF LITCHI (*LITCHI CHINENSIS* SONN.)

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Abstract–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, three-ingredients, i.e α -Tocopherol, chitosan, and salicylic acid with their varied concentration and perforation percentage of packaging material were taken as independent variables to maintain the polyphenol oxidase activity 5.23 U/ μ g Protein and peroxidase activity 7.45 U/ μ g protein at lower levels during ambient storage conditions.

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is a subtropical fruit that is important in the market because of its tasty, translucent, juicy aril, nutritional value, and refreshing flavor. It is grown in South Africa, China, India, Thailand, and Taiwan (Kumar *et al.*, 2013). Litchi is a fruit that spoils quickly; begins to brown shortly after harvest. The primary causes of litchi fruit's postharvest browning are the rapid degradation of anthocyanin and the oxidation of phenolic compounds by polyphenol oxidase (PPO) and peroxide (POD), which results in brown products (Jaing, 2001). Postharvest treatments like acid dip and sulfur fumigation can effectively inhibit PPO activity and delay the loss of litchi fruit's red skin color (Zauberman *et al.*, 1991). Consumer acceptance and export rejection may sometimes be harmed by these chemicals' residual effects. As a result, there is a constant demand for chemicals that do not harm harvested litchi fruit. All producers place a high priority on efforts to minimize crop losses and preserve the quality of fresh fruits for an extended period of time (Velickova *et al.*, 2013). The edible coating made with chitosan is known to be safe to eat and has excellent antimicrobial and

antioxidant properties (Kumar *et al.*, 2020). On the fruit's surface, chitosan forms a semi-permeable film that slows the rate of respiration and keeps the fruit moist (Badawy *et al.*, 2017). Additionally, the addition of tocopherol to the chitosan films improved both the final quality and the shelf life (Martins *et al.*, 2012). It has been discovered that applying salicylic acid after harvest can delay the senescence of litchi and preserve the health-promoting compounds (Kumari *et al.*, 2015). Numerous researchers have utilized the response surface methodology (RSM), one of the statistical tools for treatment optimization (Tripathi *et al.*, 2016). The aim of this study is to reduce the enzymatic browning of litchi fruits during ambient storage by optimizing coating formulations with RSM.

MATERIALS AND METHODS

Material selection

The experiments used a litchi fruit from the Rose Scented cultivar that was picked when it was ripe (90 to 100 percent of the peel was red). Horticulture Research Centre, G.B. Pant University of Agriculture

and Technology, Pantnagar, US Nagar, India, harvested a large number of selected fruits in the morning of the treatment day. Coating formulation prepared in accordance with the treatment combinations was applied to the graded fruits (Table 1). After that, the fruits were kept at room temperature (25 °C, 85% relative humidity).

Preparation of coating formulation and application on the fruits

The named coating constituents viz., α -Tocopherol, chitosan and salicylic acid, Tween- 20, and other chemicals needed for the trial were bought from Make Sigma Aldrich Chemicals Pvt. Ltd. Mumbai (India). The waterless results of Tween- 20 (2 g L⁻¹) used as surfactant with α -Tocopherol, chitosan, and salicylic acid concertedly prepared as per the experimental plan. During the application of coating formulation, selected samples were dipped into the prepared solution for 5 min (Jiang *et al.*, 2018). At the same time control fruit sample was treated with distilled water. These treated fruits were air-dried at 25 °C keeping in view of ambient temperature and packaged in crannied Low-density polyethylene (LDPE) packets (35 μ m) and stowed in an environmental chamber set at 25°C. 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.

Experimental plan

The preliminary experiments were carried out to select the parameters and their levels for the present experimentation. 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 (Kumar *et al.*, 2020; Kumari *et al.*, 2015). Therefore, four independent variables i.e. α -tocopherol (0.1, 0.2, 0.3, 0.4, 0.5%), Chitosan (0.5, 1.0, 1.5, 2.0, 2.5 %), 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 distinct mixtures of the treatments 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. polyphenol oxidase (PPO) and peroxidase (POD) 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.

Enzymatic analysis

The method of Zauberman *et al.* (1991) was used for determination of polyphenol oxidase activity (PPO activity) by using 4-methylcatechol as a substrate. For this, 6.0 g peel was removed from 5 litchi fruits and homogenized in 30 ml of 0.02 M phosphate buffer (pH 6.8), add 0.6 g of polyvinyl pyrrolidone (insoluble) into the phosphate buffer before homogenization with fruit peel. The homogenate was centrifuged for 20 min at 19,000 g and 4 °C and the supernatant was then collected as crude enzyme extract. Assay of PPO activity was performed using 1.0 ml of 0.1 M phosphate buffer (pH 6.8), 0.5 ml of 0.1 M 4-methylcatechol and 0.5 ml enzyme solution. The increase in absorbance at 410 nm at 25 °C was recorded for 3 min. One unit of enzyme activity was defined as the amount which caused a change of 0.01 in absorbance per minute.

The peroxidase activity was assayed by the method of Zhang *et al.*, 2005 with guaiacol as a substrate. For extraction of peroxidase 2 g of peel was removed from 5 fruits. The representative sample was homogenized with 0.05 M phosphate buffer (pH 7.0) which contains polyvinyl pyrrolidone (insoluble) 10% of the peel by weight. The homogenate was centrifuged for 15 min at 16000 g (4 °C). The supernatant was collected as crude enzyme extract. After that the a reaction mixture was prepared by 2.5 ml of supernatant, 2.73ml of 0.05 M phosphate buffer (pH 7.0), 0.1ml of 1% H₂O₂ and 0.15 ml of 4% guaiacol as substrate. The increase in absorbance, because of the guaiacol oxidation was recorded for 2 min at 470 nm in spectrophotometer. The one unit of enzyme, which caused a change of 0.001 in absorbance per minute.

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 concerted sequel of each independent variable by keeping another two variables at their separate 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:

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

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

RESULTS AND DISCUSSION

The experiment design matrix was used to conduct

the coating of litchi fruits experiments, and the response data in the form of quality parameters were recorded and used for further analysis (Table 1)

Effect of independent variables on polyphenol oxidase activity

The polyphenol oxidase activity increases during ambient storage of litchi with an increase in storage time irrespective of treatments, the range of PPO activity were 5.23 U/ μ g Protein to 9.95 U/ μ g Protein. The highest enzyme activity noted at experiment number 13 with experimental conditions of α -Tocopherol 0.2%, 1% chitosan, salicylic acid 1 mM and perforation 0.2%, whereas the lowest PPO activity (5.23 U/ μ g Protein) was observed in experiment no.2 (Table 1), with experimental conditions of α -Tocopherol 0.5%, 1.5% chitosan,

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 (ml)	Polyethelene perforation (%)	PPO activity (U/ μ g protein)	POD activity (U/ μ g protein)
T1	0.2	2	1	0.2	8.63 ^e	12.45 ^d
T2	0.5	1.5	1.5	0.3	5.23 ^w	7.54 ^q
T3	0.3	1.5	1.5	0.3	7.24 ^{no}	8.56 ^{mn}
T4	0.1	1	2	0.4	6.29 ^s	9.58 ^k
T5	0.4	2	1	0.2	9.89 ^a	10.89 ^h
T6	0.4	1	1	0.2	9.17 ^c	11.43 ^e
T7	0.4	2	2	0.2	7.17 ^{op}	10.44 ⁱ
T8	0.3	1.5	1.5	0.3	7.26 ^{mn}	8.47 ^{no}
T9	0.3	1.5	0.5	0.3	7.24 ^{no}	10.53 ⁱ
T10	0.3	1.5	1.5	0.3	7.33 ^{lm}	8.34 ^{op}
T11	0.4	2	1	0.4	5.64 ^u	9.61 ^k
T12	0.3	1.5	1.5	0.3	7.39 ^{kl}	8.48 ^{no}
T13	0.2	1	1	0.2	9.95 ^a	13.95 ^a
T14	0.2	1	1	0.4	9.57 ^b	12.52 ^d
T15	0.4	2	2	0.4	5.39 ^v	8.14 ^p
T16	0.2	2.5	1	0.4	7.79 ⁱ	11.07 ^{gh}
T17	0.3	2.5	1.5	0.3	6.93 ^q	9.38 ^l
T18	0.3	1.5	1.5	0.3	7.43 ^k	8.23 ^p
T19	0.4	1	2	0.2	7.83 ⁱ	11.45 ^e
T20	0.3	0.5	1.5	0.3	7.33 ^{lm}	11.21 ^{fg}
T21	0.4	1	1	0.4	6.82 ^r	10.12 ^j
T22	0.2	2	2	0.3	8.73 ^d	12.57 ^d
T23	0.5	1.5	1.5	0.3	8.13 ^g	12.87 ^c
T24	0.3	1.5	1.5	0.5	5.39 ^v	8.25 ^p
T25	0.3	1.5	1.5	0.3	7.14 ^p	8.68 ^m
T26	0.3	1.5	1.5	0.3	5.89 ^t	9.74 ^k
T27	0.3	1.5	1.5	0.1	7.94 ^h	11.34 ^{ef}
T28	0.2	0.5	1.5	0.2	7.64 ^j	13.32 ^b
T29	0.2	0.5	1.5	0.4	8.45 ^f	12.83 ^c
T30	0.2	2	1.5	0.4	7.39 ^{kl}	10.95 ^h

Since the P-value In ANOVA table is < 0.05

salicylic acid 1.5 mM and perforation percentage of 0.2%.

The result of regression analysis was represented by equation (2). The coefficient of determination (R^2) for the regression model for total phenolics content was 78.83% and adj R^2 was 59.07%, which ascribe that the model could responsible for 78.83% data. The model was found to be significant at the 1% level of significance with non-significant lack of fit. Therefore, the second-order model was considered to be pertinent for describing changes in total phenolics content with the specified values of independent parameters.

$$\text{PPO Activity} = 7.29 - 0.65A - 0.24B - 0.46C - 0.69D + 0.065AB - 0.069AC - 0.510AD + 0.126BC - 0.296BD + 0.248CD + 0.034A^2 + 0.147B^2 + 0.006C^2 + 0.031D^2 \quad \dots (2)$$

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

Table 2 revealed that at linear levels of α -Tocopherol and perforation percentage affected significantly on change in PPO activity in litchi pericarp at 1% level of significance. Whereas the salicylic acid at the linear level and α -Tocopherol with perforation percentage with interactive levels significantly influenced PPO activity at 5% level of significance. There was no significant effect in remaining interactive and all quadratic levels in

terms of independent variables. Therefore, simplified second order equation of polyphenol oxidase activity becomes,

$$\text{PPO Activity} = 7.29 - 0.65A - 0.46C - 0.69D - 0.510AD \quad \dots (3)$$

The graph (Fig. 1) depicted that polyphenol oxidase activity of the treated fruits decreased sharply with an increase in tocopherol percentage up to the central value. The α -tocopherol is bioactive compound and source of vitamin E it takes part actively in inhibiting PPO activity by competing it for binding of oxygen as the PPO activity depends on atmospheric oxygen (O_2) for (hydroxylation) Tang *et al.* (2020).

The effect of chitosan showed in graph clearly indicates that the decrease in polyphenol activity with an increase in chitosan to the value (1.5%), it becomes stable thereafter. The chitosan formed a thin layer around the pericarp of litchi which prevents the water loss, cracking on the skin of coated fruits and direct contact with atmospheric oxygen leads to reduced PPO activity (Kumar *et al.*, 2020).

The increase in salicylic concentration leads to very minute changes in the polyphenol oxidase activity. Salicylic acid maintained lower pH of coated litchi during storage which prevents the PPO activity by avoiding fixing of substrate for enzyme action; results are confirmed by Kumar *et al.* (2013).

Table 2. Effect of treatments on PPO activity (U/ μ g protein) at ambient storage conditions.

Source	SS	Df	MS	F-value	P-value
Model	36.37	14	2.60	3.99	0.0059***
A- α -Tocopherol	10.34	1	10.34	15.87	0.0012***
B-Chitosan	1.45	1	1.45	2.22	0.1570
C-Salicylic acid	5.29	1	5.29	8.13	0.0121**
D-Perforation	11.72	1	11.72	18.00	0.0007***
AB	0.0689	1	0.0689	0.1058	0.7494
AC	0.0770	1	0.0770	0.1183	0.7357
AD	4.17	1	4.17	6.41	0.0230**
BC	0.2576	1	0.2576	0.3955	0.5389
BD	1.41	1	1.41	2.17	0.1618
CD	0.9851	1	0.9851	1.51	0.2377
A ²	0.0334	1	0.0334	0.0513	0.8239
B ²	0.5959	1	0.5959	0.9151	0.3539
C ²	0.0010	1	0.0010	0.0016	0.9687
D ²	0.0266	1	0.0266	0.0409	0.8425
Residual	9.77	15	0.6512		
Lack of Fit	NS				
R ²	0.7883				
Adj R ²	0.5907				

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

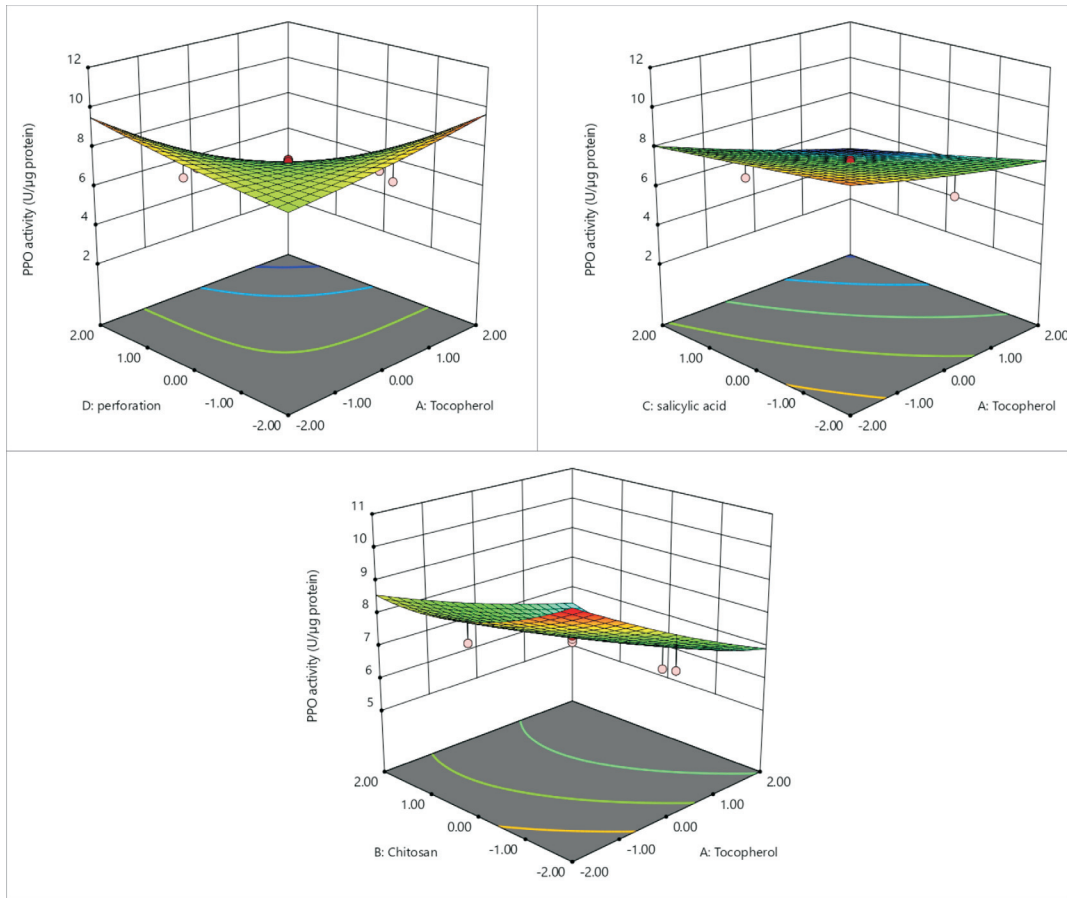


Fig. 1. Effect of independent variables on polyphenol oxidase activity at ambient storage condition.

Table 3. Effect of treatments on POD activity (U/μg protein) at ambient storage conditions.

Source	SS	Df	MS	F-value	P-value
Model	85.47	14	6.10	10.00	< 0.0001***
A-a-Tocopherol	34.22	1	34.22	56.04	< 0.0001***
B-Chitosan	6.76	1	6.76	11.07	0.0046***
C-Salicylic acid	0.7848	1	0.7848	1.29	0.2747
D-Perforation	13.26	1	13.26	21.72	0.0003***
AB	0.2756	1	0.2756	0.4513	0.5119
AC	0.2756	1	0.2756	0.4513	0.5119
AD	0.2116	1	0.2116	0.3465	0.5649
BC	0.0756	1	0.0756	0.1238	0.7298
BD	0.1369	1	0.1369	0.2242	0.6427
CD	0.0484	1	0.0484	0.0793	0.7822
A ²	11.24	1	11.24	18.41	0.0006***
B ²	12.05	1	12.05	19.73	0.0005***
C ²	10.64	1	10.64	17.42	0.0008***
D ²	7.93	1	7.93	12.99	0.0026***
Residual	9.16	15	0.6107		
Lack of Fit	NS				
R ²	0.9032				
Adj R ²	0.8129				

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

The increase in perforation percentage sharply increases the activity of polyphenol oxidase as the oxygen availability is prerequisite for polyphenol oxidase activity.

Effect of independent variables on POD activity

There are two types of peroxidase enzymes in litchi, free and bound initially free peroxidase acts on phenols and reduces the phenols content in litchi, during storage bound peroxidase also released due to cracking and disruption of pericarp tissues, therefore the activity of peroxidase higher at the end of storage (Tang *et al.*, 2020).

The maximum peroxidase activity recorded (13.95%) at experiment number 13 while the lowest (7.54%) at experiment no. 2 (Table 1).

The result of regression analysis was given by equation (4). The coefficient of determination (R^2) for the regression model for this parameter was 82.67% and adj R^2 was 66.49%, which implies that the model could describe of 82.67% data. The model was set up to be significant at 1% level of significance with non-significant lack of fit. Therefore, the second-order model was measured to be satisfactory for explaining the alteration in peroxidase activity with the specified values of independent parameters.

$$\text{POD activity} = 8.46 - 1.19A - 0.53B - 0.18C - 0.74D + 0.13AB - 0.13AC - 0.11AD - 0.068BC - 0.092BD - 0.054CD + 0.64A^2 + 0.66B^2 + 0.62C^2 + 0.537D^2 \dots (4)$$

Where, A- α -Tocopherol, B - Chitosan, C - Salicylic acid and D - Perforation percentage (all in coded form).

Table 3 expressed that at linear levels all independent variables except salicylic acid recorded significant effect with 1% of the level of significance

where as all the independent variables of the quadratic levels had a significant effect on the POD activity at 1% level of significance. There was no significant effect in interactive terms of independent variables. Simplified second order equation of peroxidase activity becomes,

$$\text{POD activity} = 8.46 - 1.19A - 0.53B - 0.18C - 0.74D + 0.64A^2 + 0.66B^2 + 0.62C^2 + 0.537D^2 \dots (5)$$

Response surface plots as shown in Fig. 2 showed the effect of independent variables at the selected range on the peroxidase activity of litchi fruits plunge at the initial augmentation in tocopherol percentage. Whereas the rate of decrease is slightly slower with an increase in chitosan and salicylic acid after middle values (0.3% and 1.5mM respectively) the rate of peroxidase stabilized with the slight increasing trend. The percentage and perforation percentage showed a significant rise in peroxidase activity after middle value (0.3%), while it slightly increased with increase in perforation percentage before middle value.

The chitosan formed a thin layer around the coated litchi which provides supplementary moisture to pericarp during storage at ambient temperature, and leads to a partial inhibition of peroxidase activity (Kumar *et al.*, 2020.).

The α -Tocopherol is bioactive compound and it reduces peroxidase activity by preventing activity of bound peroxidase enzymes during storage (Sarker and Grift, 2021). Ali *et al.* (2016) reported that peroxidase enzyme activity significantly increased in fruit peel tissues, regardless of the treatments.

The results depicted that the perforation percentage affected peroxidase activity significantly and it helps to modify the microclimate around coated litchi fruits reduces enzymatic activity (Kaur

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 15 days				
PPO activity (U/ μ g protein)			5.39	
POD activity (U/ μ g protein)			8.14	

Table 5 Observations recorded under control conditions during ambient storage.

Observations	0 Day	1 Day	2 Days	3 Days
PPO activity (U/ μ g protein)	4.160	6.48	11.31	8.69
POD activity (U/ μ g protein)	7.37	8.69	13.69	10.48

et al., 2014). The salicylic acid recorded nonsignificant effect on peroxidase activity, it might be due to the combined effect of α -Tocopherol and chitosan, which suppress the expression of salicylic acid at given concentration.

Optimization of independent variables

In order to achieve optimal conditions for the coated litchi fruit's maximum quality retention and longer shelf life, the independent variables were optimized. The minimum levels of peroxidase and polyphenol oxidase activity were established as the respective objectives of the selected dependent variables. The importance of all of the responses and independent variables was the same (+++). The criteria that the optimum values should be close to feasible values at the higher desirability were used to select the best optimized solution for optimum values of independent variables among all the optimized solutions given by the software. The optimal coating formulation for litchi fruit is listed in table 4 which contains 0.4% α -Tocopherol, 2% chitosan, 2 mM

salicylic acid, and 0.4% perforation on packaging material.

Response surface methodology (RSM), which is used by numerous researchers (Dhillon *et al.*, 2022; Koprivica *et al.*, 2014) for fruit drying at a low cost. Nevertheless, this is the initial report on litchi fruits.

CONCLUSION

The study includes improving the coating and packaging of litchi in order to extend its shelf life and preserve a greater quantity of health-promoting substances during ambient storage. According to the findings, the combination of tocopherol (0.4 %), chitosan (2.0 %), salicylic acid (2.0 mM), and packaging with perforation (0.4 %) was found to be ideal for ambient storage conditions. Additionally, this combination resulted in a longer shelf life and less browning of the litchi fruits. While the control fruits, which were kept at room temperature, began to deteriorate after the second day and began to spoil on the third.

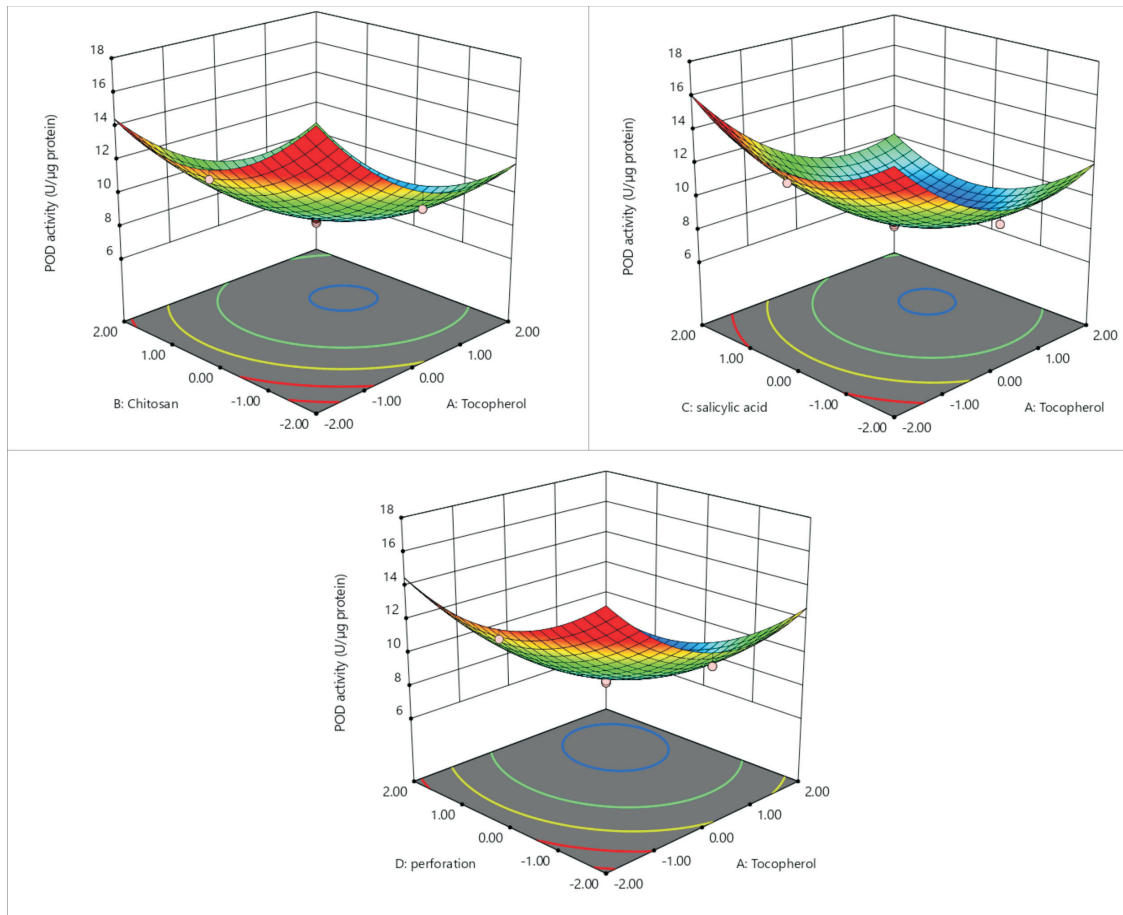


Fig. 2. Effect of independent variables on peroxidase activity at ambient storage condition.

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