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Effect of various coating formulations and perforated packaging on ascorbic acid content of litchi cv. Rose scented (*Litchi chinensis* Sonn.) fruits

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Abstract
Litchi (*Litchi chinensis* Sonn.) deteriorates very fast after harvest. Pericarp browning is a major post-harvest problem, which renders the fruit unacceptable in market. Browning is associated with desiccation. Peroxidase activity coupled with ascorbic acid oxidation enhances anthocyanin degradation. Techniques to reduce browning and maintain the red colour and prolonged storage life include sulphur treatment and packaging in perforated plastic bags, storage under cold conditions and coating with bioactive compound. Aril breakdown or softening of the aril involves a loss of turgidity and translucency where fruits become blunt in taste. To prevent losses during storage coating of bioactive compound α-Tocopherol along with chitosan and salicylic acid were done and coated fruits were packaged in perforated polythene film. The major loss of ascorbic acid during storage is due to the oxidation and utilization of it in scavenging superoxide and hydrogen peroxides generated due to stress of temperature and respiration. The higher ascorbic acid content tends to improves shelf life with prevention of health promoting substances during storage. Since the chemical is being restricted from use, alternative methods like coating with natural compounds are desirable. Amongst the various combination of coating material and perforation percentage α-Tocopherol (0.4%), chitosan (2%), salicylic acid (2mM) and perforation percentage of (0.4%) found effective in maintaining higher amount of ascorbic acid (20.57 mg/100 g fruit weight). Use of perforated polythene bags have also been reported to increase shelf-life. Thus, to have better post-harvest life of litchi fruits, coating formulation with bioactive compounds and packaging in polythene with different perforation were studied in this experiment.

Keywords: Litchi; ascorbic acid; coating; packaging; storage

Introduction
The litchi (*Litchi chinensis* Sonn.) is a wonderful fruit with plenty of health benefits belongs to family Sapindaceae. The most widely cultivated fruit trees in this family other than litchi are rambutan (*Nephelium lappaceum* L.) and longan (*Dimocarpus longan* Lour.). The main centre of origin of litchi is believed to be between latitudes 23^0 and 27^0 north in the subtropical parts of southern China, northern Vietnam, and Malaysia. In India litchi is cultivated mainly in subtropical zone starting from Uttarakhand to the West Bengal, because of its strict subtropical climatic requirements. The litchi fruit is best known for its juicy and translucent arils with plenty of health benefits, including its ability to aid in weight loss, protect the skin, boost the immune system, prevent cancer, improve digestion, build strong bones, lower blood pressure, defend the body against viruses, improve circulation, and optimize metabolic activities. The litchi fruit is packed with health benefits and they come from the vitamins, minerals, and nutrients in the fruit, including vitamin C, vitamin B6, niacin, riboflavin, folate, copper, potassium, phosphorus, magnesium, and manganese. Furthermore, litchi is a great source of dietary fiber, protein, and a good source of proanthocyanidins and polyphenolic compounds. The vitamin C is highly volatile and oxidized fastest rate during storage, its role in preventing loss of health promoting substances were clear through glutathione cycle and antioxidant systems. It has many biological activities in human body, including reduction in levels of C-reactive protein (CRP), a marker of inflammation and possibly a predictor of heart disease. More than 85% of vitamin C in human diets is supplied by fruits [1, 2]. Biological function of l-ascorbic acid can be defined as an enzyme cofactor, a radical scavenger, and as a donor/acceptor in electron transport at the plasma membrane.

Ascorbic acid is able to scavenge the superoxide and hydroxyl radicals, as well as regenerate α-tocopherol [1].

Material and Methods

Litchi fruits (cv. Rose Scented) were harvested at fully mature stage (90–100% of the pericarp showing red colour) from the orchard of Horticultural Research Centre, Pattharchatta, Department of Horticulture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (India). The fresh fruits of uniform size, shape, colour and free from diseases, pests or physical injury were selected for the experiment. A total of 250 fruits were taken for the experiment and divided into 5 lots having 50 fruits in each treatment.

Experimental Plan

During experimentation α-tocopherol concentration, chitosan concentration, salicylic acid concentration and perforation percentage of polythene bag with its five level each were taken as independent variables. The various combinations of the experimental conditions with its experiment matrix was designed by using Central Composite Rotatable Design (CCRD) of statistics. The actual and coded values of the levels of independent variables were shown in table1. After conducting the coating experiment at particular experimental condition, the effect of various coating treatments on the ascorbic acid content in aril of litchi fruit was estimated. The average value of triplicate response data were used for regression analysis. The regression analysis for studying the effect of independent variables on the response and optimization of the selected independent variables was done with the help of Response surface methodology (RSM).

Experimental Procedure

For conducting the experiment, selected fresh fruits were treated with aqueous solutions of α-Tocopherol, chitosan and salicylic acid in combination at particular experimental conditions and then packaged in polythene bag with specified perforation. For giving the treatment to the individual fruit, 10 L treatment solution containing Tween-20 (2 g L^{-1}) as surfactant was prepared at 25 °C for 5 min. The selected fruits were dipped in prepared solution in such way that coating solution formed uniform layer on brown pericarp of the fruit. After treatment application, fruits were air-dried and stored at room temperature for 15 days. At every 3 days interval, ten fruits from each treatment (each replicate contained five fruit) were sampled at random and analysed for ascorbic acid on fresh weight basis.

Ascorbic acid content in the edible part of fruit was quantified by 2,6-dichlorophenol indophenol method [3]. Five grams of fruit sample were crushed and diluted to 100 ml with 3% metaphosphoric acid solution. The sample was centrifuged and the supernatant was titrated with the dye (2, 6-dichlorophenol indophenol) to a pink end point (persisting for 15 s). The ascorbic acid content was calculated from the titer value and expressed as mg 100 g^{-1}.

Optimization of independent variables

Full second order model was fitted to ascorbic acid data obtained at various experimental conditions. Data analysis and optimization were carried out by using Design-Expert 8.0.6 software. Effect of independent variables on the ascorbic acid content was interpreted using the second order polynomial models. Optimization of the independent variables were carried out by selecting and setting the appropriate goals of parameters and response.

For evaluation the relationship between the response and independent variables the generalized polynomial model was used as below:

$$Y = 0 + ixi + iixi2 + ijxixj \ldots\ldots \text{Equation (1)}$$

The goal seeking began at a random starting point and proceeded up and down the steepest slope on the response surface for a maximum or minimum value of the response, respectively. All the responses and independent variables were given similar (+++) importance.

Result and Discussion

Nutritional value of many fruits have been mainly evaluated as the content of ascorbic acid. The ascorbic acid is quite unstable and thus it is also an indication of fruit freshness. Upcoming generation is more health conscious with knowledge of functional elements in their daily diet, the dieticians also recommended food containing higher amount of vitamin C and high antioxidant capacity for stressful life of 21st century. Therefore it is important to study the effect of coating and packaging on ascorbic acid one of the component of vitamin C. Ascorbic acid content of coated litchi fruits stored at ambient storage condition varied in the range of 6.97 to 20.57 mg/100 g fruit weight (Table 1). The maximum value obtained at experiment number 15 with experimental conditions of LD α-Tocopherol 0.4% (A=1), 2% chitosan (B=1), salicylic acid 2 mM (C=1) and perforation percentage of 0.4% (D=1), while minimum (6.97) with experimental conditions of LD α-Tocopherol 0.2% (A=-1), 1% chitosan (B= -1), salicylic acid 1 mM (C= -1) and perforation 0.2% (D=-1) at experiment no.13 (Table 1).

Ascorbic acid content data obtained from coated litchi fruit stored under ambient storage condition was fitted into full second-order mathematical model equation (1) and the result of regression analysis was represented by equation (2). The coefficient of determination (R^2) for the regression model for this parameter was 77.05% and adj R^2 was 55.64%, which implies that the model could account for 77.05% 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 adequate for describing the change in ascorbic acid content with the specified values of independent parameters.

$$\text{Ascorbic Acid Content} = 14.46 + 2.29A + 1.26B + 0.97C + 0.22D + 0.30AB + 0.85AC - 0.70AD + 0.52BC + 0.78BD + 0.66CD - 0.51A^2 - 0.28B^2 - 0.72C^2 - 0.56D^2$$

…… Equation (2)

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

Table 1: Effect of coating and packaging perforation on ascorbic acid content at the end of storage (15th day) in ambient condition.

Standard Order	Run Order	α-Tocopherol	Chitosan	Salicylic Acid	Perforation Percentage	Ascorbic Acid	
3	1	-1	1	-1	-1	10.24	α-Tocopherol -2=0.1% -1=0.2% 0=0.3% 1=0.4% 2=0.5%
18	2	2	0	0	0	17.61	
30	3	0	0	0	0	14.2	
14	4	1	-1	1	1	11.23	
4	5	1	1	-1	-1	14.24	
2	6	1	-1	-1	-1	12.58	Chitosan -2=0.5% -1=1.0% 0=1.5% 1=2.0% 2=2.5%
8	7	1	1	1	-1	17.41	
25	8	0	0	0	0	14.86	
21	9	0	0	-2	0	12.02	
29	10	0	0	0	0	14.59	
12	11	1	1	-1	1	11.45	
27	12	0	0	0	0	14.07	
1	13	-1	-1	-1	-1	6.97	Salicylic acid -2=0.5 mM -1=1.0 mM 0=1.5 mM 1=2.0 mM 2=2.5 mM
9	14	-1	-1	-1	1	7.56	
16	15	1	1	1	1	20.57	
11	16	-1	1	-1	1	9.54	
20	17	0	2	0	0	16.52	
28	18	0	0	0	0	14.17	
6	19	1	-1	1	-1	14.83	
19	20	0	-2	0	0	13.85	
10	21	1	-1	-1	1	10.04	
7	22	-1	1	1	-1	7.06	Perforation percentage -2=0.1% -1=0.2% 0=0.3% 1=0.4% 2=0.5%
17	23	-2	0	0	0	10.93	
24	24	0	0	0	2	15.5	
26	25	0	0	0	0	14.89	
22	26	0	0	2	0	14.85	
23	27	0	0	0	-2	12.66	
5	28	-1	-1	1	-1	8.32	
13	29	-1	-1	1	1	7.49	
15	30	-1	1	1	1	13.54	

Table 2 expresses the individual effect of each term in second-order quadratic equation fitted to the experimental data. It is revealed that at linear levels Tocopherol effected ascorbic acid content significantly with 1% of the level of significance, while chitosan and salicylic acid effected at 5% level of significance.

There was no any significant effect in interactive terms of independent variables as well as quadratic levels of ascorbic acid content. The results were in accordance with 4, 5, 6. Therefore, simplified second order equation of ascorbic acid content becomes,

Ascorbic Acid Content = 14.46 + 2.29A + 1.26B + 0.97C …….. Equation (3)

Table 2: ANOVA for Ascorbic acid content (mg/100g fruit weight)

Source	SS	Df	MS	F-value	P-value
Model	255.15	14	18.22	3.59	0.0095***
A-Tocopherol	125.99	1	125.99	24.87	0.0001***
B-Chitosan	38.43	1	38.43	7.58	0.0147**
C-Salicylic acid	22.99	1	22.99	4.53	0.0500**
D-Perforation	1.23	1	1.23	0.2443	0.6282
AB	1.53	1	1.53	0.3023	0.5904
AC	11.61	1	11.61	2.29	0.1507
AD	7.99	1	7.99	1.57	0.2281
BC	4.39	1	4.39	0.8686	0.3660
BD	9.81	1	9.81	1.93	0.1842
CD	7.08	1	7.08	1.39	0.2551
A^2	7.27	1	7.27	1.43	0.2492
B^2	2.24	1	2.24	0.4440	0.5152
C^2	14.37	1	14.37	2.83	0.1127
D^2	8.68	1	8.68	1.71	0.2101
Residual	75.967	15	5.064		
Lack of Fit	NS				
R^2	0.7705				
Adj R^2	0.5564				

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

It is clearly showed in Fig. 1 that the ascorbic acid content of coated litchi fruits during storage decreases with time irrespective of the treatment. The significant changes were recoreded from 12th day of storage. The ascorbic acid decreases rapidly during storage due to its convertion into monodehydroascorbate, it is recycled into ascorbic acid through glutathione cycle in living sysytem of plant, it might be due to the utilization of ascorbic acid in fruit during storage and failure of its recycycling 7, 8.

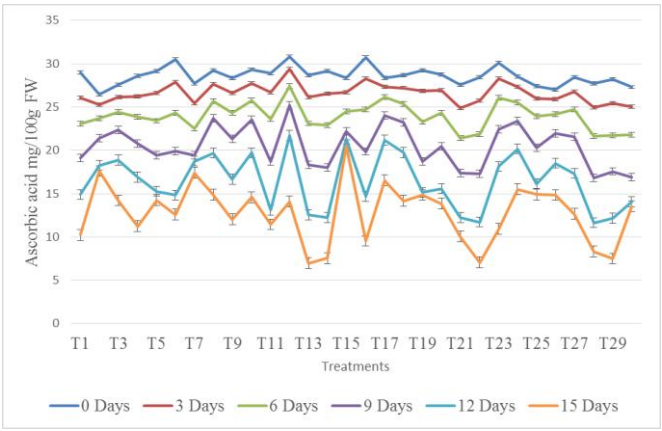

Fig 1: Effect of coating and packaging perforation on ascorbic acid content of litchi during ambient storage.

The effect of independent variables was affirmed by Response surface plots as shown in Fig. 2, 3 & 4 at the selected range on the ascorbic acid content of litchi fruit stored at ambient condition.

It is clear from the graph (Fig.2) that ascorbic acid content of the treated fruit increased rapidly at the initial increase in tocopherol percentage and chitosan to some extent and afterward it becomes stable. The stress in form of temperature, oxygen, chilling or biotic stress creats reactive oxygen species, as these are highly reactive, if not reduced within short period it stolen electron from cell membrane which disintegrate stability of membrane and cell becomes succeptible for pathogen attack, breaking of compartmentation, loss of water and electrolyte leakage.

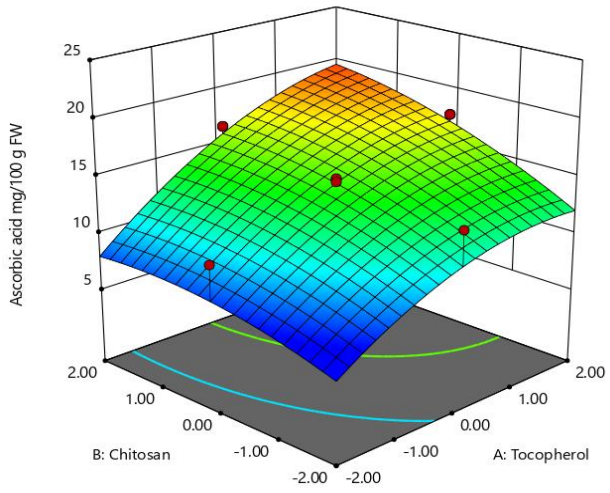

Fig 2: Response surface plot for ascorbic acid content as a function of tocopherol and chitosan.

The reactive oxygen species are convereted into water molecules through ascorbate glutathione cycle the enzyme monodehyadroascorbate reductase (MDHAR) convert ascorbic acid into monodehyadroascorbate (MDHA) and dehydroascorbate recycled into ascorbic acid through dehydroascorbate reductase along with conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG), [9]. The addition of α-Tocopherol in coating might be beneficial to prevent enzymatic browning, scavenging of hydrogen peroxide and superoxide and alleviating lipid peroxidation, which ultimately maintained ascorbic acid levels at equilibrium in coated litchi fruits. The α-Tocopherol from coating formulation acts as inhibitor of reactive oxygen species, it might be penetrate into the pericarp preventing antioxidant capasity and loss in ascorbic acid. The α-Tocopherol is bioactive compound source of vitamin E utilized during mehler reaction and might prevent degradation of ascorbic acid into monodehydroascorbate [10].

The chitosan effected ascorbic acid content significantly at 5% level of significance. One of the reasons for the loss of ascorbic acid was nonenzymatic browning, which was prevented by the chitosan this might be helpful to maintain ascorbic acid in coated litchi at a higher level,findings were affirmed by [11] confirmed that retention of higher amount of ascorbic acid in litchi fruits treated with salicylic acid in combination with chitosan. Whereas, ascorbic acid in coated litchi fruits declined during storage irrespective of treatments. The maximum ascorbic acid (25.46 mg/100 g fruit weight) was retained in SA (1.0 mM) + chitosan (2%) treated litchi fruits. Higher radical scavenging activity due to higher retention of bioactive compounds *viz*. phenolics, flavonoids, anthocyanin and ascorbic acid, have also been reported earlier in litchi [12].

The Fig.3 showed that with increase in salicylic acid and ascorbic acid slightly increase up to its central values i.e.1.5mM while it was decreased slightly thereafter.

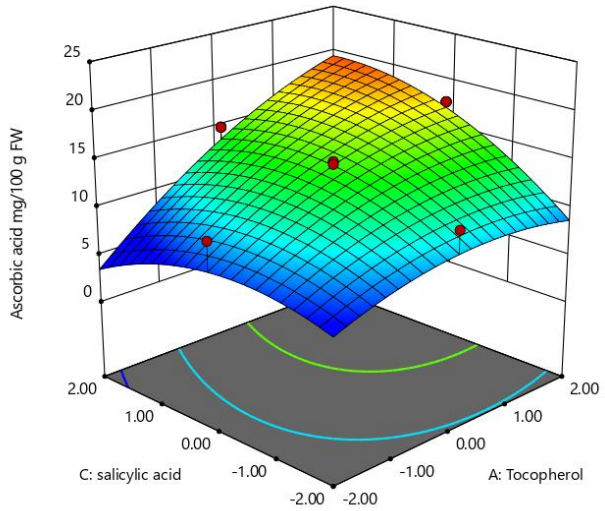

Fig 3: Response surface plot for ascorbic acid content as a function of tocopherol and saliclic acid.

The saicylic acid is natural antisenscencing agent prevents production of ethylene and rate of respiration, which leads to maintainance of higher ascorbic acid during storage under ambient condition. The major natural antioxidants are phenolic compounds, tocopherols, flavonoids, carotenoids as well as ascorbic acid [13, 14, 15]. Phenolic compound and ascorbic acid are correlated with enzymatic browning, phenolic coumpounds are converted into highly unstable quinones which turn into black, brown pigments [16]. Similar findings have been reported (17), the 0.25% L- cysteine was

used for treatment of litchi fruits as antibrowning agent and stored at 5 °C with 95% relative humidity (RH) for twenty eight days. The treated litchi fruits maintained higher levels of ascorbic acid and activities of enzymes like, superoxide dismutase and catalase during storage.

The Fig.4 indicated that the certain level increase in the perforation percentage, the level of ascorbic acid increases, afterward it did not showed any significant effect on ascorbic acid content of coated litchi fruits at 15th day of storage in ambient condition. It might be due to higher oxygen levels helpful for maintaining ascorbic acid as the anaerobic respiration were prevented. [18] The ascorbic acid content of sliced mango fruit fall continuously during seven days of storage. The fruit treated with chitosan had a greater ascorbic acid content.

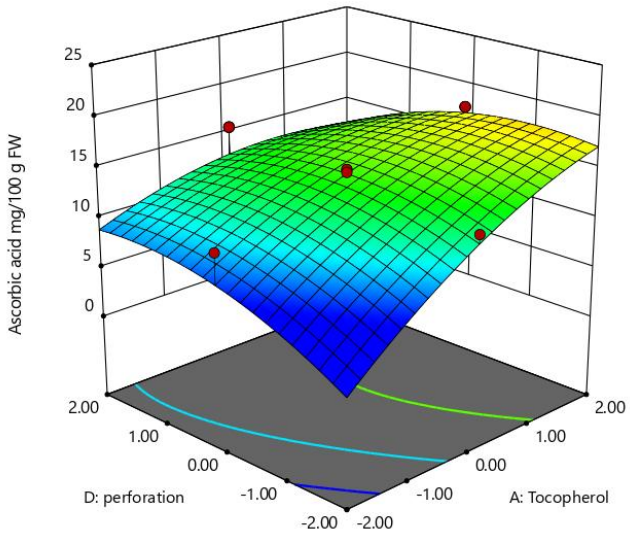

Fig 4: Response surface plot for ascorbic acid content as a function of tocopherol and perforation.

The coating formulation with chitosan effectively prevent the enzymatic activity to some extent by forming thin layer around fruits, this might be one of the reasons that the increase in concentration of chitosan reduces loss of ascorbic acid and higher level of ascorbic acid maintained in coated litchi fruits. The external application of ascorbic acid prevents browning in litchi under ambient condition with higher anthocyanin content up to 9 days [19].

Optimization of independent variables: The objective of the study reveals that, to get the optimized conditions for maximum retention of ascorbic acid content in the aril of litchi fruit during selected storage conditions. The goal of all independent variables at "in range" condition while ascorbic acid at maximum condition was set for optimization. Based on mentioned criteria, the optimization was carried out. During optimization, 30 solutions were obtained, out of which the one that suited the criteria most was and having highest desirability (0.865) were selected. The optimum results of coated litchi fruit was obtained when α- tocopherol concentration is 0.4%, chitosan concentration is 2%, salicylic acid concentration is 2 mM and perforation percentage of polythene bag is 0.4%.

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