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Enhancement of shelf life of tomato by the extract of *Plectranthus amboinicus*

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Abstract
Edible coating has ability to reduce the postharvest losses. Therefore, the aim of this study is to extract the antioxidant from *Plectranthus amboinicus* leaves and optimise the extract using response surface methodology. The extraction was performed by microwave assisted extraction and antioxidant activity of the extract was evaluated. The extract from the leaves were mixed with edible coating solution to prevent water loss, control ripening process, respiration rate, delay oxidative browning and reduce microbial growth. The antioxidant enriched edible coating were applied on the fresh tomatoes. One group of coated and uncoated tomatoes were stored at room temperature and another group at refrigeration temperature. Physicochemical analysis like pH, TSS (Total Soluble Solids) and microbial analysis like bacterial count of tomatoes were done. It was observed that coating delayed the change in pH, TSS and also inhibits the growth of microorganism. The antioxidant based edible coating could be a good alternative for preserving the quality and extending the shelf-life of tomatoes.

Keywords: *Plectranthus amboinicus*, antioxidant, tomatoes, edible coating

1. Introduction

India gets post-harvest losses of fruits and vegetable due to lack of proper storage and processing facilities [1]. Tomato is an essential vegetable crop that is available in most of the tropical countries with seasonal peaks during June-October. It is a climacteric fruit and upholds to ripen after harvest. On ripening, the green pigment chlorophyll degrades and carotenoids are synthesised. It is eatable and berries of the plant *Solanum lycopersicum*, commonly known as a tomato plant. Tomatoes are rich in health associated compounds like antioxidant and polyphenols [2]. For fresh tomatoes, the texture and skin colour are the two important quality attributed to buyers and consumers. There is a high yield of tomatoes on the harvest time, but post- harvest processing and preservation techniques are inefficient. Hence, post-harvest loss of tomatoes occur because of deficiency in preservation and processing [3].

Recently, application of herbal based edible coating has been shown as a tool to improve the quality, extend shelf life and storage of various fruits and vegetables [4]. An edible coating is a thin layer that is deposited on the surface of a fruits [5]. By modulating the transfer of moisture, oxygen, carbon dioxide, aroma and taste compounds in a food system, edible coating improves the quality, prolong shelf life and reduce the microbial growth on products [6]. One such herbal is *Plectranthus amboinicus*, a novel edible coating for fruits and vegetables storage. It is a fleshy, succulent herb famous for its distinct oregano-like flavour and odour [7]. This herbs has therapeutic and nutritional properties to its natural phytochemical compounds which are highly valued in the pharmaceutical industry [8]. It is desirable for curing diseases like skin, oral, urinary diseases, cardiovascular, kidney troubles and respiratory problem [9]. Due to the presence of antioxidant, antimicrobial, anti-inflammatory and anti-bacterial compounds in leaves, it has potential to increase the shelf life and quality of products [10]. Therefore, the aims of this study were: to incorporate *plectranthus amboinicus* extract in edible coating and to evaluate its effect on tomato ripening.

2. Materials and methods

Fresh *Plectranthus amboinicus* leaves were collected from surrounding areas. Simultaneously, tomatoes were procured from farmers.

2.1 Drying of leaves

Plectranthus amboinicus leaves were dried in conventional drying method to obtain the dried sample. The leaves were weighed and subjected to tray drier at 60ºC for 24hrs [11]. The dried leaves were ground into small particles using commercial mixer.

2.2 Extraction

2.2.1 Microwave- Assisted Extraction

Microwave assisted extraction is used for extracting antioxidant from *Plectranthus amboinicus* leaves powder. The factors affecting the extraction are microwave power and time of extraction. The type of solvent chosen was hexane. 6.5 gram of sample is mixed with 65ml of hexane, kept in microwave oven and extracted. Then it is taken out and allowed to cool to the room temperature. The product is filtered using Whatmann filter paper. Then obtained extract is used for the estimation of antioxidant activity. Table 1 shows the independent variable of Central Composite Design which includes the two parameters namely power (A) and time (B) that influence the microwave extraction which were varied in the optimization process. Total of 13 experimental runs were conducted as per Table 2. The temperature maintaining during evaporation is 67ºC. After evaporation the antioxidant activity of leaves extract were analyzed.

Table 1: Independent variables range for microwave-assisted extraction

Factor	Name	Unit	Minimum	Maximum
A	Power	Watt	520	560
B	Time	Minutes	4	8

2.3 Determination of antioxidant activity

The evaluation of antioxidant activity can be performed using chemical based assays especially DPPH assay was used to analyze antioxidant activity. DPPH possess a deep blue purple colour and has a UV-Vis absorption maximum at 517nm. The reducing ability can be assessed by measuring the decrease of its absorbance in control and sample with different concentration can be used to determine its IC50 value. It indicates the required concentration to scavenge free radicals.

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{(Absorbance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control} \times 100$$

2.4 Incorporation of leaves extract in edible coating

The extract was incorporated as a functional ingredient in edible coating to enhance the shelf life of tomato. Edible coating solution were prepared by dissolving the leaves extract with 2% sodium alginate, 50% glycerol and stirred at 55ºC for 10mins[12]. The tomatoes were dipped in the coating solution for 5mins and samples were dried by air drying method. Then the dried tomatoes were divided into two batches and one batch of tomatoes was kept in room temperature and another batch of tomatoes was kept in the refrigeration condition.

2.5 Determination of Tomato shelf life

2.5.1 Estimation of TSS (Total Soluble Solids) and pH

Total Soluble Solids were determined by hand refractometer and the pH of the tomato paste was evaluated by using digital pH meter.

2.5.2 Estimation of Total Bacteria

The plating, incubation and counting method to enumerate bacteria were carried out as per IS 5402: 1969 for bacteria. This analysis was done by serial dilutions method. The sample (1g) was taken from the paste of tomato. Serial dilution were prepared up to 10^{-6}. 1ml of sample was dropped to a plate of nutrient agar for bacteria. The plates were incubated at the temperature of 37ºC for 24 hours. After incubation the plates were observed for typical colonies for each microorganism. The results were recorded as CFU/gm.

3. Result and discussion

By using a Central Composite Design (CCD), the RSM was carried out to optimize the yield of antioxidant extracted from the leaves.

3.1 Optimization of microwave extraction

The result for yield of *Plectranthus amboinicus* extract from the microwave extraction is shown in Table 2.

Table 2: Central Composite matrix and observed response

		Factor 1	Factor 2	Response 1
Std	Run	A: Power	B: Time	Antioxidant activity
		Watt	minutes	%
3	1	520	8	86.25
9	2	540	6	94.17
6	3	568.28	6	81.6
4	4	560	8	91.75
12	5	540	6	94.17
8	6	540	8.83	91.25
1	7	520	4	78.5
2	8	560	4	72.25
7	9	540	3.17	86.4
11	10	540	6	94.17
5	11	511.72	6	83.3
10	12	540	6	94.17
13	13	540	6	94.17

3.2 Effect of Time and Power on Antioxidant activity

As indicated in Figure 1 antioxidant activity is directly proportional to the time and inversely proportional to power. i.e, the antioxidant activity increases when the time increases.

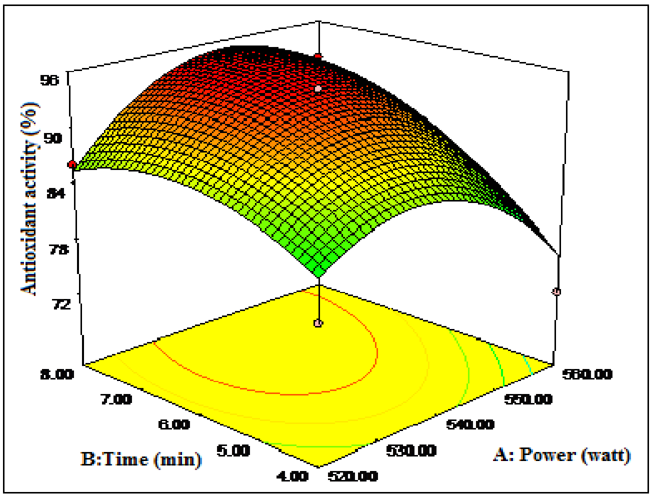

Fig 1: Effect of time and power on antioxidant content

Table 3: Model summary statistics for antioxidant activity

Source	R-Squared	Adjusted R^2	Predicted R^2	Remarks
Linear	0.2359	0.0831	-0.3553	-
2FI	0.2914	0.0553	-1.0740	-
Quadratic	0.8776	0.7901	0.1293	Suggested
cubic	0.9617	0.9081	-1.4506	Aliased

The table 3 shows the models summary statistics for antioxidant activity. From that table it is predicted that quadratic model is suggested for leaves extract because "Model summary statistics" focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

3.3 Analysis of variance

An analysis of variance (ANOVA) was calculated to assess how well the model was fitted to the data ANOVA table for the effect of process variables on antioxidant was tabulated in Table 4.

Table 4: ANOVA for effect of process variables on antioxidant

Source	Sum of squares	df	Mean square	F-value	P-value Prob>5	
Model	545.57	5	109.11	10.03	0.0043	Significant
A-Power	1.24	1	1.24	0.11	0.7452	-
B-Time	145.43	1	145.43	13.37	0.0081	-
AB	34.52	1	34.52	3.17	0.1180	-
A^2	314.38	1	314.38	28.91	0.0010	-
B^2	86.93	1	86.93	7.99	0.0255	-
Residual	76.12	7	10.87	-	-	-
Lack of fit	76.12	3	25.37	-	-	-
Pure error	0.000	4	0.000	-	-	-
Cor total	621.69	12	-	-	-	-

From the Table 4, it is observed that the Model F-values of 10.03 implies the model is significant. There is only a 0.43% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case B, A^2 and B^2 are significant model terms. If the values greater than 0.1000 indicates the model terms are not significant.

Final equation in terms of coded factors. The equation can be used to obtain the optimal value of *Plectranthus amboinicus* extract yield percentage (%) by taking into considerations the parameters of microwave extraction (A: power and B:time) [13].

$$\text{Inhibition} = +94.17 - 0.39 * A + 4.26 * B + 2.94 * A * B - 6.72 * A2 - 3.53 * B2$$

3.4 Optimized condition for extraction

To check the suitability of the surface response methodology model for quantitative predication, experiments were carried out on estimated optimal conditions. Table 5 shows the predicted and experimental results for the variables selected. The predicted results were well matched with the experimental results obtained under optimal extraction conditions which proved the model's validity to describe the process. This results supported the selection of the RSM design, which was shown to be accurate and reliable in predicting the antioxidant ability of *plectranthus amboinicus* extracts.

Table 5: Predicted and experimental values under Optimized Conditions

Factors	Predicted value	Experimental value
Microwave power (watt)	543.94	543.94
Microwave Time (minutes)	7.74	7.74
Antioxidant activity (%)	95.368	94.87

The extract from the optimized condition are further used for the edible coating purpose.

3.5 Effect of antioxidant based edible coatings on tomato shelf-life

3.5.1 PH

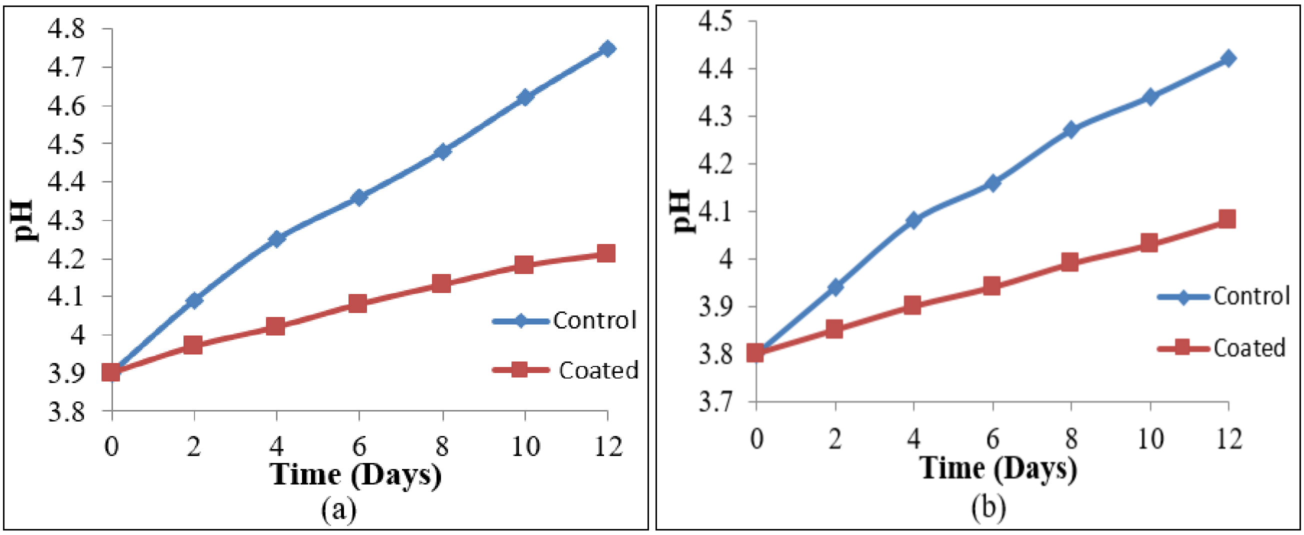

Fig 2: Change of pH value during Storage (a) in room temperature at 23ºC (b) in refrigeration temperature at 4ºC

The changes in the pH of the control and coated tomatoes are shown in Figure 2.The pH increased from 3.9 to 4.75 in control and 3.9 to 4.21 in coated tomatoes respectively on 12th day. Overall, coating solutions restricted changes in pH of tomatoes. The increasing of pH shows the maturity of tomato. While compare to room temperature, the refrigeration temperature restrict to increase the pH [14].

3.5.2 TSS (Total Soluble Solids)

The changes in the TSS of the control and coated tomatoes are shown in Figure 3. In both room and refrigeration temperature, the increase in TSS were significantly high in the control. Control sample showed maximum increase in TSS followed by edible coated sample. The changes in TSS value were prevented by the edible coating. Changes in TSS content are correlated with hydrolytic changes in starch. In tomatoes, the important ripening index is conversion of starch to sugar. The oligosaccharides released by degradation of cell wall polysaccharides influences fruit ripening. [15].

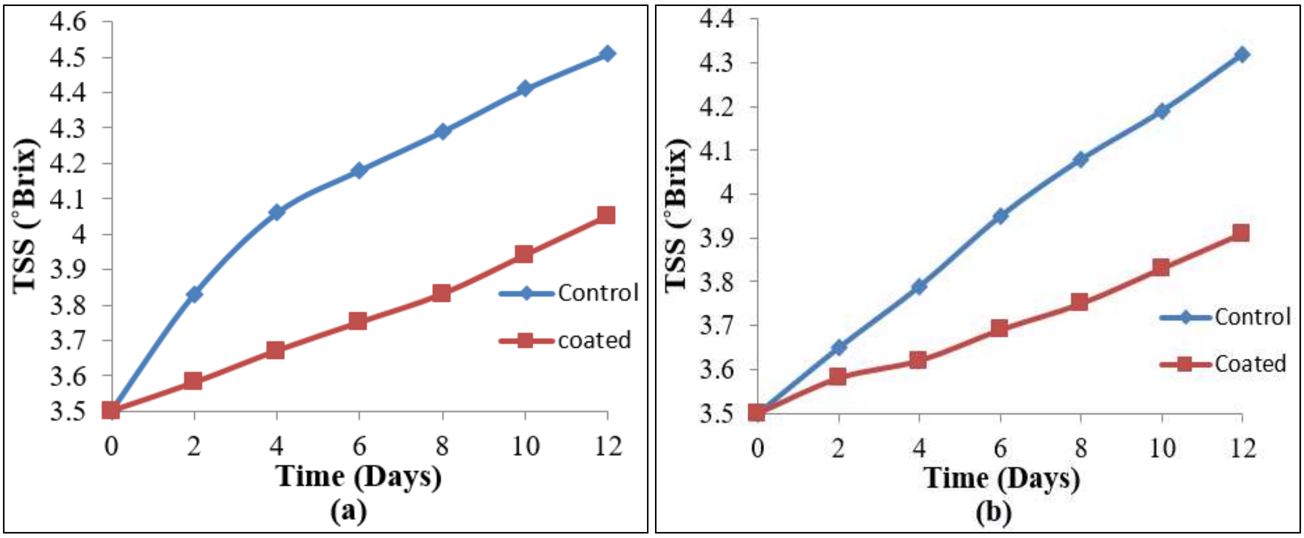

Fig 3: Change of TSS value during Storage (a) in room temperature at 23ºC (b) in refrigeration temperature at 4ºC

3.5.3 Total Bacteria

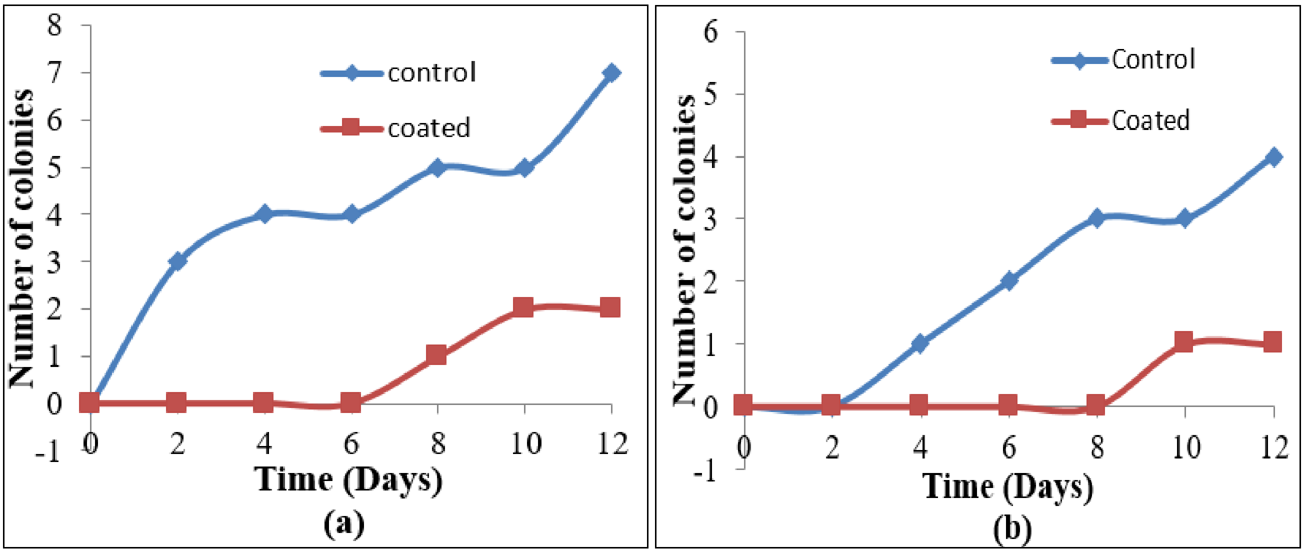

Fig 4: Bacterial counting during storage (a) in room temperature at 23ºC (b) in refrigeration temperature at 4ºC

The leaves extract coating resulted in the best control for the bacterial growth. It is important to note that, with this treatment the tomato were not attacked by microorganism during first 6 days of storage. From day 8th, the presence of microorganism was observed. This could be due to the presence of antioxidant and antimicrobial content in leaves extract. We can extend the shelf-life of the tomato by using *plectranthus amboinicus* extract based edible coating [16].

4. Conclusion

Plectranthus amboinicus were dried in conventional drying method to obtain the dried sample. Then the dried leaves were ground into small particles using commercial mixer for extraction purpose. The optimized conditions for extraction are 543.94 W for 7.74 minutes is optimized by using Response Surface Methodology.

This optimized extract was used as a functional ingredient in edible coating solution to enhance the shelf-life of tomato. The antioxidant based edible coating was most effective for preserving the quality and extending the shelf-life of tomato by delaying the changes of pH, TSS (Total Soluble Solids) and also inhibits the growth of microorganism in tomato.

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