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Research Article

Statistical Optimization of Modified Atmospheric Packaging Conditions of Minimally Processed Red Amaranth (*Amaranthus tricolor* L.) Leaves

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Abstract

Packaging is a crucial aspect of food processing, ensuring both fresh and processed agricultural products are safely handled and delivered from manufacturers to consumers. The main objectives of packaging technology are to preserve quality, enhance safety, and reduce waste from post-harvest losses. Modified Atmosphere Packaging (MAP) is frequently used to pack fruits and vegetables. This technique involves replacing the air in the packaging's headspace with specific atmospheric gases that differ in proportion to normal air. To extend the shelf life of minimally processed Red Amaranth (Amaranthus tricolor L.) leaves using MAP technology, a statistical approach was employed. A Box-Behnken design was utilized to optimize the packaging conditions regarding time, temperature, and the ratio of the packet size and the weight of the samples. The results aligned well with the proposed regression model. The maximum shelf life of the leaves was determined to be 12 days at 19 ˚C, with the optimum ratio of packet size to sample weight being 0.0888221. Under normal storage conditions, the total phenolic content, antioxidant activity, and the color of Red Amaranth leaves significantly deteriorated after just 3 days. However, the samples stored under MAP exhibited a four-fold increase in shelf life and overall acceptability.

Keywords: *Amaranthus tricolor* L; Antioxidant Activity; Color; Total Phenol Content; RSM

Introduction

Fresh vegetables have gained the attention of both consumers and scientists due to their natural antioxidants. Regularly consuming these antioxidants has been shown to reduce the risk of heart disease [1,2], making them a valuable addition to the diet. Antioxidants can be categorized into three groups: vitamins, phenolics, and carotenoids, all of which play a role in providing protective properties to fruits and Vegetables. While carotenoids are lipophilic antioxidants, vitamin C and phenolics are hydrophilic antioxidants [3].

Modified atmosphere packaging (MAP) is a preservation technique that involves manipulating the levels of O2 and CO2 in the atmosphere surrounding fresh vegetables, without the addition of preservatives. This technique helps to reduce the respiration rate, ethylene production, and physiological changes in vegetables, making it ideal for transportation with minimal loss. Red amaranth leaves, for instance, are an excellent source of nutrition, including significant amounts of vitamin C, β-carotene, anthocyanin, and antioxidants. As such, this study aims to increase the shelf life and utilization of leafy vegetables [4-6]. This study will help in increasing the self- life, utilization of leafy vegetables [7].

The Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques used to develop, improve, and optimize processes. RSM requires fewer trial experiments to establish a relationship between different parameters, making it less time-consuming and more efficient. In the food industry, RSM has been widely used to optimize processes. Specifically, in this study, RSM was used to optimize the modified atmospheric packaging of red amaranth leaves. The aim was to investigate the effect of modified atmospheric packaging parameters on the nutritional quality of the vegetables and optimize these parameters to develop an effective modified atmospheric packaging process that would produce a higher-quality final product.

Materials

For the investigation, only analytical grade chemicals were used. Gallic acid from S.d Fine Chem. Ltd. Mumbai, India, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) from Himedia, Mumbai, India, Sodium carbonate, Acetic acid, Iron (III) Chloride 6-hydrate, Iron (II) Sulphate 7-hydrate, Sodium acetate, Ethanol, Hydrochloric acid, and Folin-Ciocaltue's Phenol from Merck (Germany) were all utilized. The Red Amaranth Leaves sample was identified by the Botanical Survey of India, Ministry of Environment, Forest and Climate Change (Government of India).

Methods

Moisture Content

To determine the moisture content of the sample, we followed the procedure developed by Ranganna S. in 1986[8]. First, we accurately weighed a 10g sample in a dry Petri dish. Next, we placed the dish in an oven and dried the sample at 105˚C until it reached a constant weight, indicating that all the moisture had evaporated. By using this method, we were able to determine the amount of moisture present in the sample.

Determination of total polyphenolic content (TPC)

The total phenol content in a sample can be determined using the Folin-ciocalteu's reagent, as described by Singleton and Rossi in 1965 [9]. To do this, mix 200µL of the sample extract with 1.5mL of Folin-ciocalteu reagent (previously diluted tenfold with distilled water) and let it sit for 5 minutes at room temperature. Then, add 1.5mL of a sodium bicarbonate solution (60gm/L) to the mixture. Vortex the mixture, cover it, and allow it to stand for 120 minutes in a dark place. Take triplicate measurements and measure the absorbance using a spectrophotometer (Hitachi U-2000) at 765nm against a blank containing all the reagents except the sample. Use a Gallic Acid standard curve for calculation. The results should be expressed as Gallic Acid Equivalents/gram of dry sample.

Determination of total antioxidant content (FRAP)

The FRAP assay was conducted based on the method outlined by Benzie and Strain in 1996 [10]. To prepare the FRAP reagent, a mixture of sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution (dissolved in 40 mM HCl), and 20 mM iron (III) chloride solution was used in a ratio of 10:1:1 (v/v), respectively. Freshly prepared FRAP reagent (3ml) was combined with 100 µl of the sample solution and then heated in a water bath at 37°C. After four minutes, the absorbance was measured at 593 nm using a spectrophotometer. The results were then expressed as µmol Fe (II)/g dry sample, and a standard calibration curve of FeSO4 solution was used for plotting.

Sample preparation

The process of preparing fresh, matured red amaranth leaves involves careful selection and thorough cleaning. The leaves were carefully selected from the Jadavpur market near Jadavpur University, ensuring that only the undamaged ones were purchased. Once the leaves were brought home, they were washed thoroughly in tap water to remove any foreign matter like mud, dirt, chaff, and immature leaves. After washing, the leaves were carefully dried using a muslin cloth to remove any surface moisture. Finally, they were air-dried at room temperature, which helped to retain their freshness and nutrient content.

Packaging material

Low density polyethylene (LDPE) pouch was used for packaging material, the thickness of the package is 0.1mm and the package size is 0.022m^2.

Optimization of freshness of red amaranth leaves using Box-Behnken Response surface methodology

The study aimed to extend the shelf-life of minimally processed red amaranth leaves, which are known for their nutritional and medicinal properties. The researchers employed a statistical approach to optimize the conditions of modified atmospheric packaging, which is crucial for maintaining the quality and safety of these leaves.

To analyze the packaging conditions, the researchers used the Box-Behnken design, which is a popular statistical method for optimizing experimental conditions. The design factors included time, temperature, and the ratio between the size of the packet and the weight of the leaves. The researchers investigated the impact of these factors on the experimental responses, including total phenol content, antioxidant activity, and color.

In the preliminary stage of the study, the researchers statistically optimized the range of experimental variables using RSM, a popular optimization technique. They used the optimized values as the Box-Behnken design of RSM and developed a 17 preliminary test plan. Each factor was tested at three distinctive coded levels: low (-1), middle (0), and high (+ 1). The researchers used a quadratic polynomial model to represent the function in the range of interest, enabling them to predict the reaction and assess the model's sufficiency.

The response surface design helped the researchers examine the reaction across the entire factors space and identify the region where it reached its ideal value. They used the reaction surface plot to identify the combination of factors that gave the best reaction, leading to the optimization of the modified atmospheric packaging conditions.

Overall, the study showed that a statistical approach can be used to optimize the packaging conditions of minimally processed red amaranth leaves, leading to an extension of their shelf-life. This approach can be applied to other similar products to enhance their preservation and maintain their nutritional and medicinal properties.

Statistical analysis

An 'F' test was conducted to establish the statistical significance of an equation. The quality was evaluated using the coefficient of determination R2 value of a polynomial model. The optimization process involved conducting repeated searches to identify the best combination of factor levels, ensuring that all response and factor requirements were met. Mathematical and graphical techniques were employed to select the optimal objectives for each factor and reaction in this study. All statistical analyses, from experimental design to optimization, were performed using statistical software Design Expert (Version 12.0; STATEASE INC.; Minneapolis, MN).

Results and Discussion

Optimization of environmental parameters for extension of shelf life of red amaranth leaves through Box-Behnken Response surface Methodology

The optimal level of the key factors and the effect of their interactions on modified atmospheric packaging of red amaranth leaves were explored by the Box-Behnken Response surface Methodology. Modified atmospheric packaging of red amaranth leaves was described by the following established second order polynomial equation put in multiple regression analysis on the experimental data:

Equation in expression of coded factors

Total Phenol Content (TPC) $Y_1 = -4425.86250 + 115.54187A + 389.15562B + 7314.68750C + 3.00188AB - 15.62500AC + 11.31250BC - 8.24937A^2 - 10.95375B^2 - 54931.25000C^2$

Antioxidant activity (FRAP) $Y_2 = -972.60562 + 32.76875A + 81.11250B + 1920.75000C + 0.393750AB - 25.62500AC + 43.62500BC - 1.89562A^2 - 2.29437B^2 - 18218.75000C^2$

Color $Y_3 = -5224.41937 + 356.42375A + 318.11625B + 7044.75000C + 1.22750AB + 407.50000AC - 57.50000BC - 17.42437A^2 - 8.47812B^2 - 84431.25000C^2$.

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space. Where Y_1, Y_2, and Y_3 were the predicted yields of total phenol content (TPC), Antioxidant activity (FRAP) and color respectively. A,B,C were the coded values of time, temperature and s/w ratio correspondingly. The coefficient of determination R^2 was used to fixed out the decacy of fit of the model equation. Statistical suggestion of that equation was future studied by Fisher's test. The R^2 was measured by the degree of fit. The R^2 was conceivately enhanced the empirical representation fits of the real values [6,11]. The model F-value suggested that the model is notable with the total phenol content, antioxidant activity and color. Values were 34.46, 28.65 and 70.88. A chance of arising was 0.01% of Model-F-value for the noise. The p-esteems are utilized as a device to check meaning of every factor, which likewise show the connection strength between every independent factor. P-values less than 0.05 in this study indicate the model expressions are considerable. In this case all the coded values are important model expressions. The coefficient of variation R^2, from the equation 3,4 and 5 were found 0.9779,0.9736 and 0.9891 indicated excellent contormity connecting observed and predicted results and suggested that the numerical model is trustworthy for self-life extension of modified atmospheric packaged red amaranth leaves in this study. Simultaneously the lack of fit measurement, which is utilized to test the ampleness of the model, show that the P-value of time, temperature and S/W ratio were not significant. No irregularity was seen from the findings of residuals. To test the importance of the model's second order polynomial equation for experimental data ANOVA was supervised (Figure 1-18, Table 1-5). From ANOVA data it could be accomplished that the predicted R^2 is in coherent conformity with the adjusted R^2. Using this statistical software (Design expert version 12.0.1.0) desirability and regression analysis was done. The independent variable optimized with assist of desirable task criteria existing in this software plan was to boost total phenol content, antioxidant activity and color turn over preserving the factors in their applicable investigational scale. So, utilizing this desirability of rule was hold on to highest i.e.0.9495, 0.9396 and 0.9752. Desirablity (d = 1) if activity ≥ high value, (d = 0) if enzyme activity < low value, 0 ≤ d ≤ 1 as activity varies from low to high [12]. Thus, it can be concluded that the model was statistically sound. The contour plots shown depict the relationship between two variables by maintaining the other variables of their zero value for extension of self-life of red amaranth leaves. Model of response plane indicate the character and scope of the interaction between different factors. Less prominent or negligible interactions are generally shown by the circular nature of the contour plots, while comparatively prominent interactions were otherwise shown by the elliptical nature of the contour plot showed that the most extreme anticipated worth is demonstrated by the surface restricted in the littlest oval in the graph.

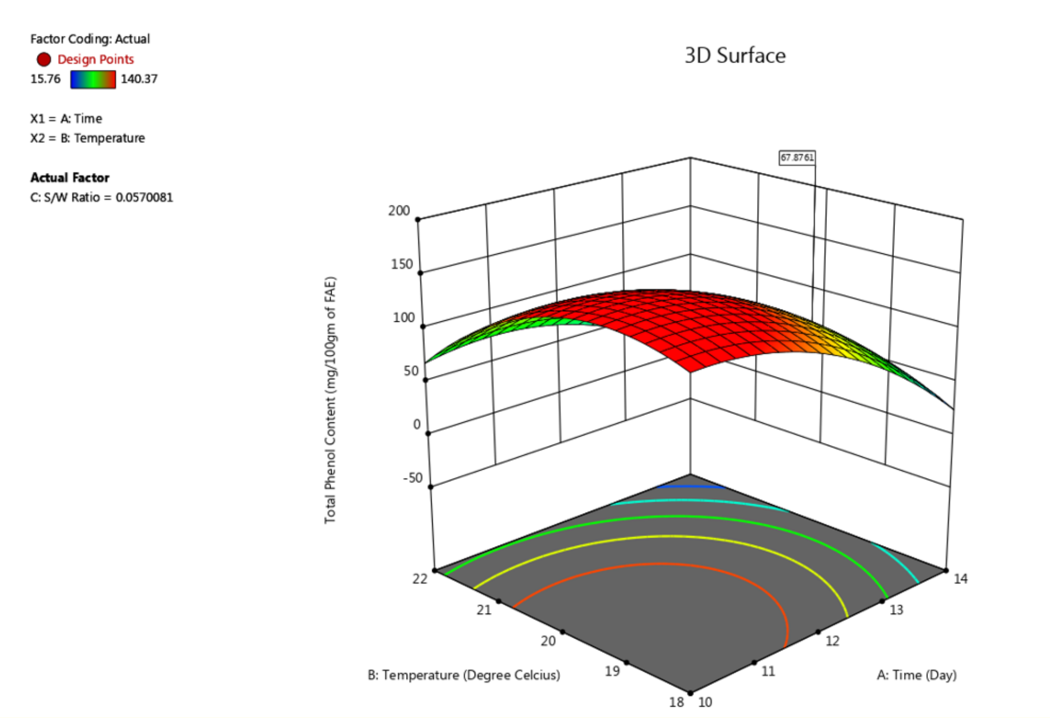


Figure 1: 3D response surface plot of interactive effect of total phenol content on Time and temperature.

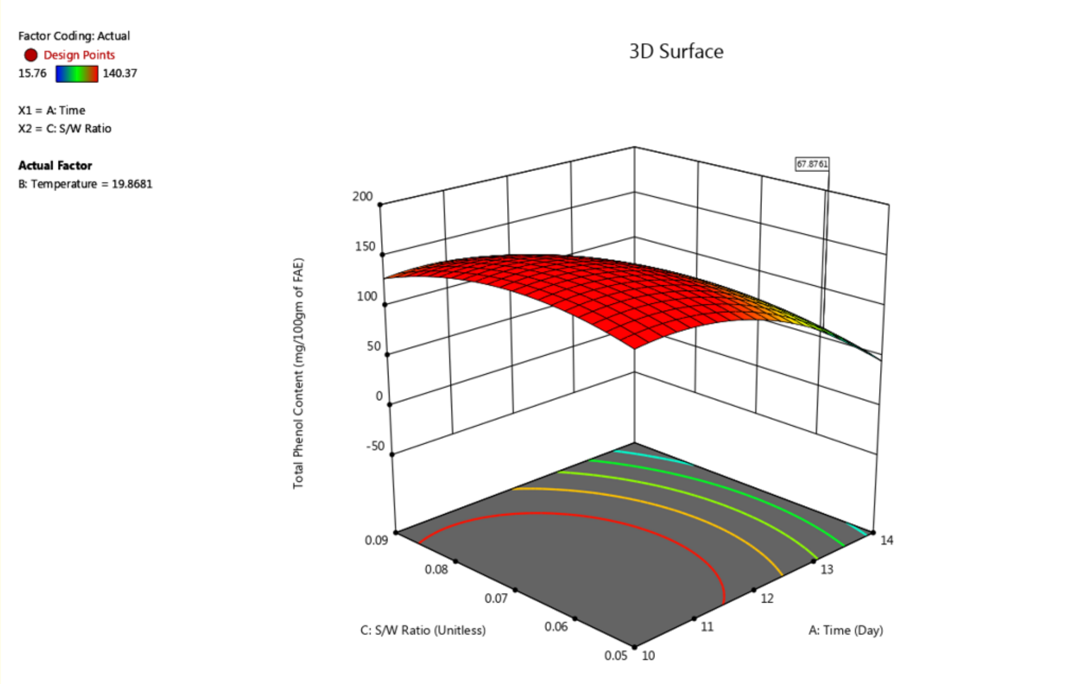


Figure 2: 3D response surface plot of interactive effect of total phenol content on Time and S/W ratio.

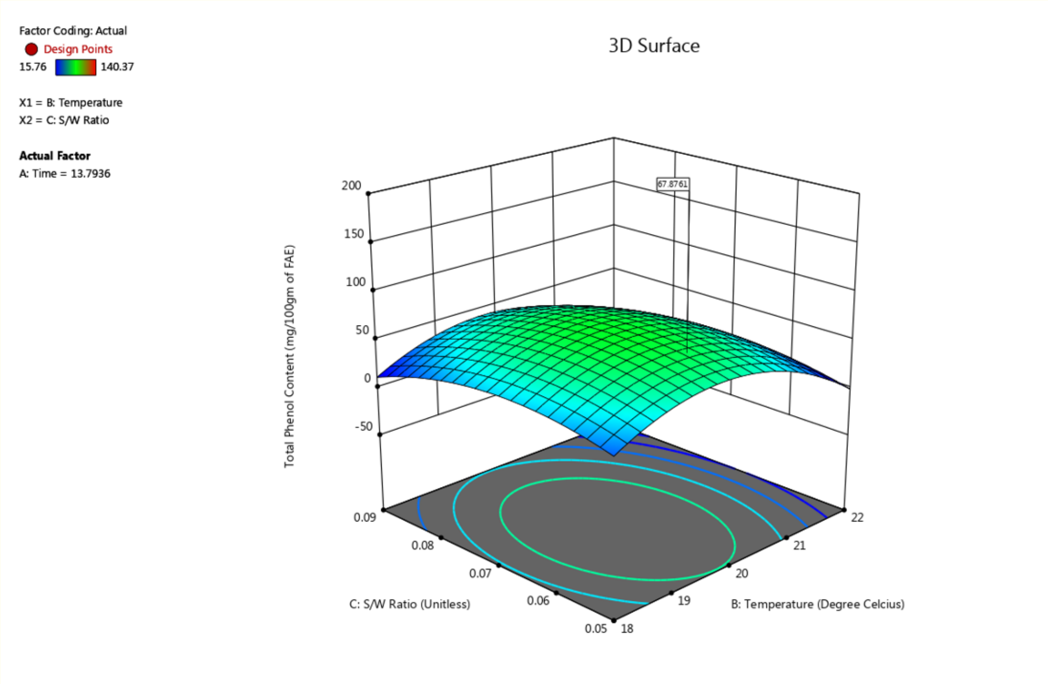
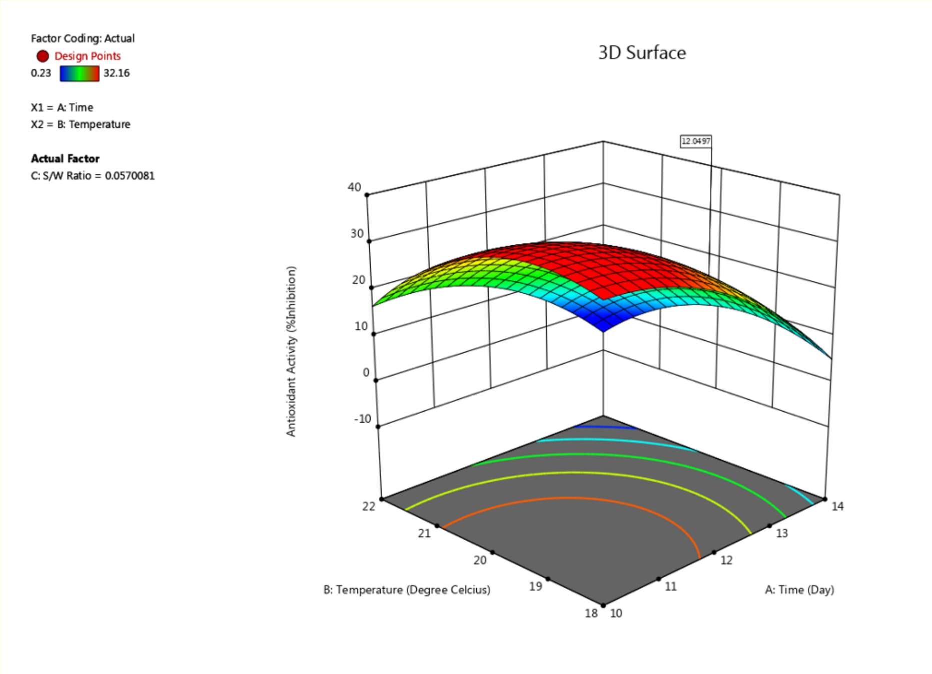


Figure 3: 3D response surface plot of interactive effect of total phenol content on Temperature and S/W ratio.



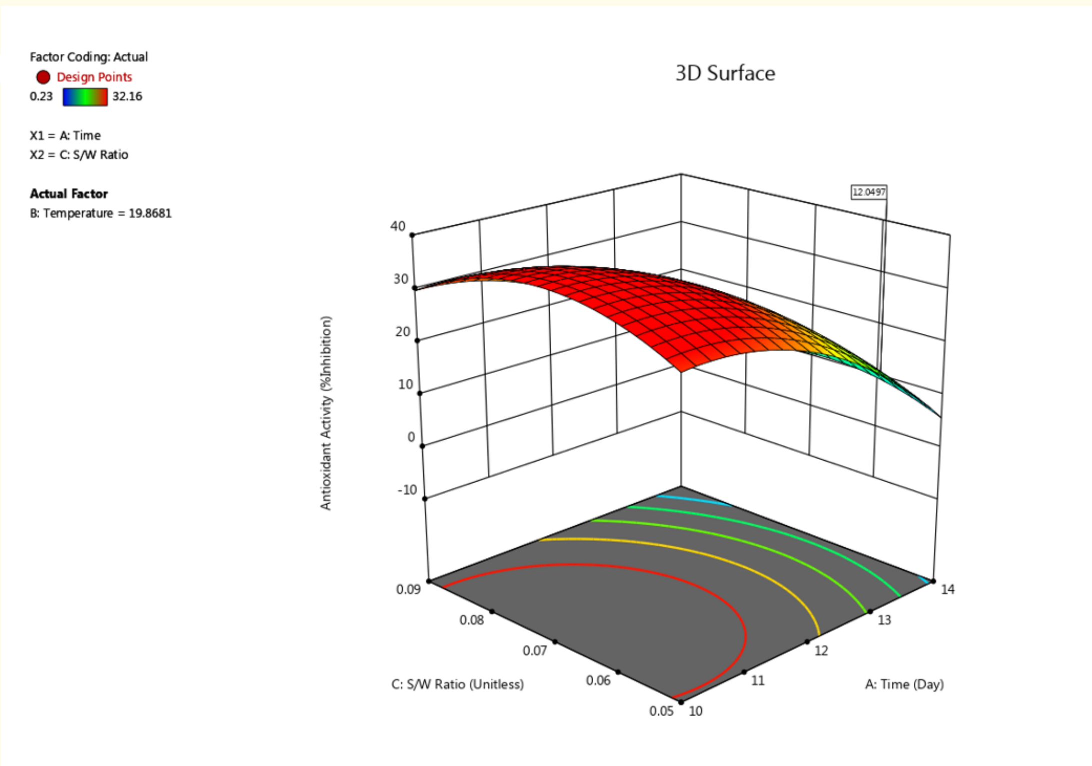


Figure 5: 3D response surface plot of interactive effect of antioxidant activity on time and S/W ratio.

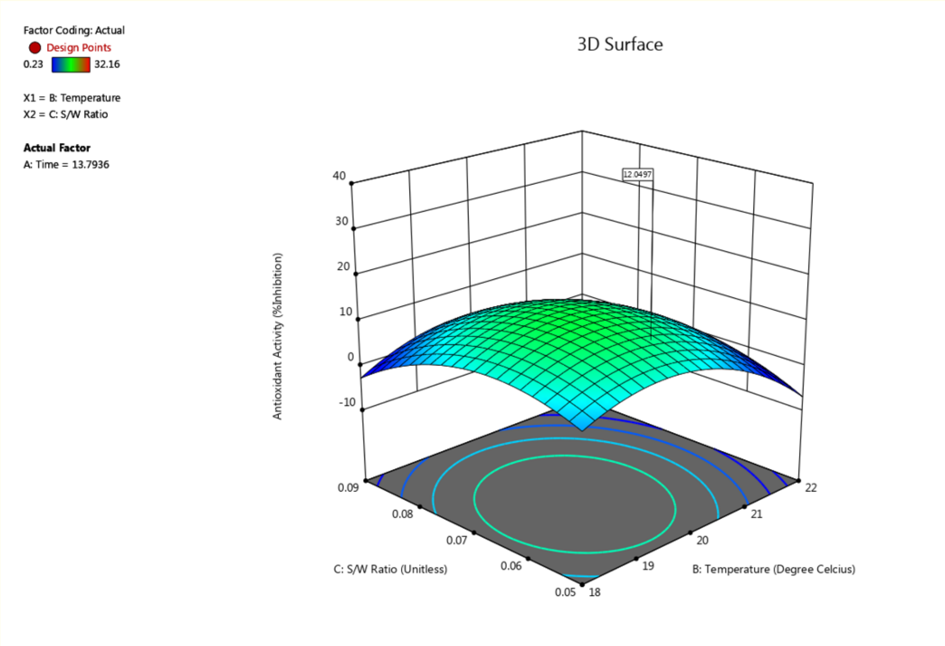


Figure 6: 3D response surface plot of interactive effect of antioxidant activity on temperature and S/W ratio.

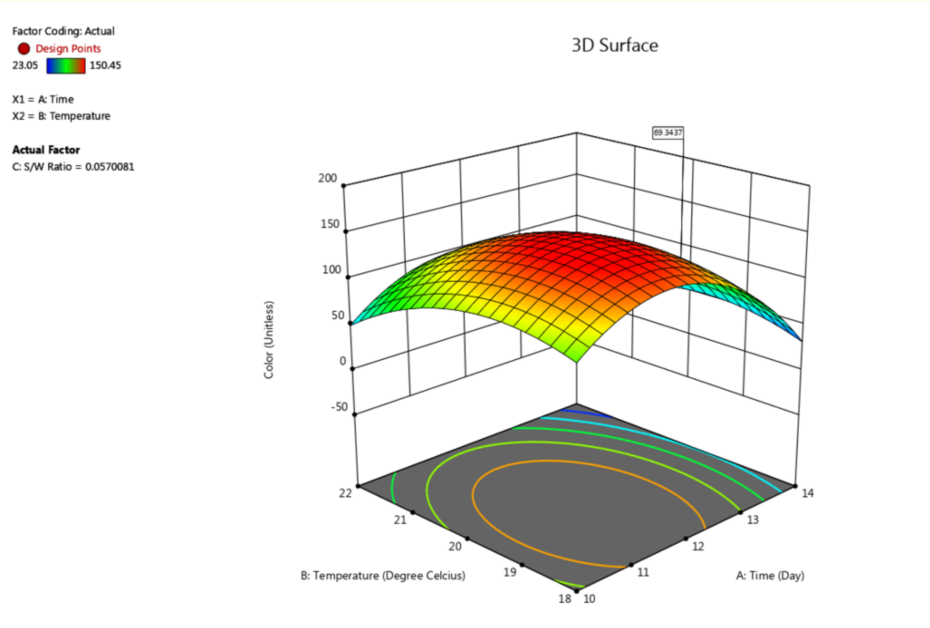


Figure 7: 3D response surface plot of interactive effect of color on time and temperature.

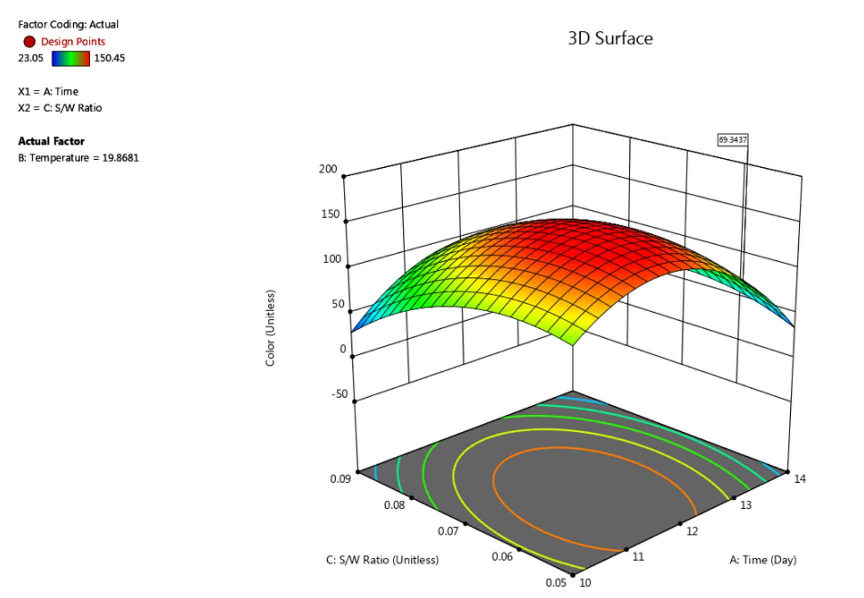


Figure 8: 3D response surface plot of interactive effect of color on time and S/W ratio.

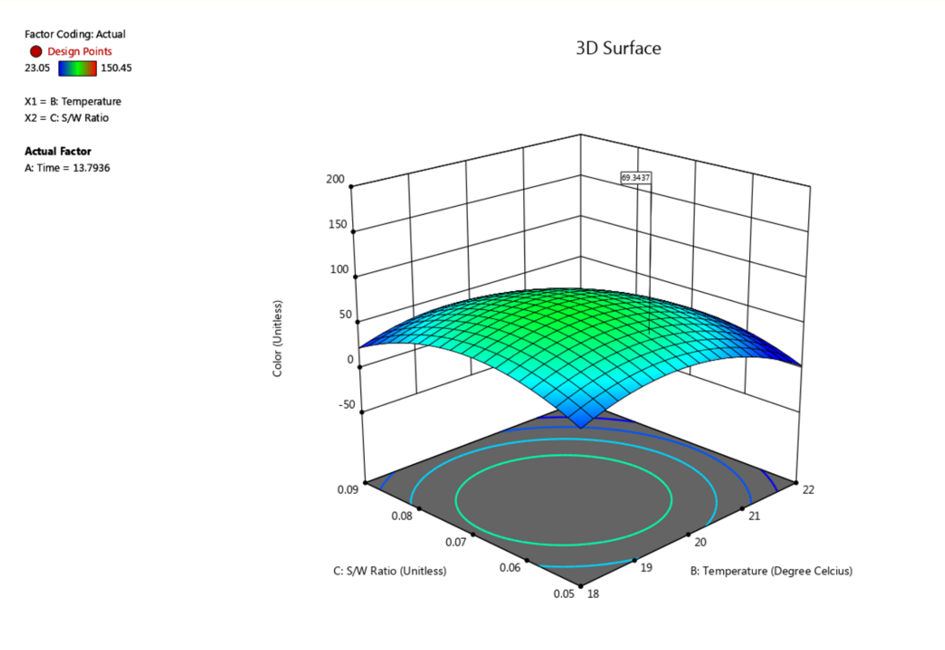


Figure 9: 3D response surface plot of interactive effect of antioxidant activity on temperature and S/W ratio.

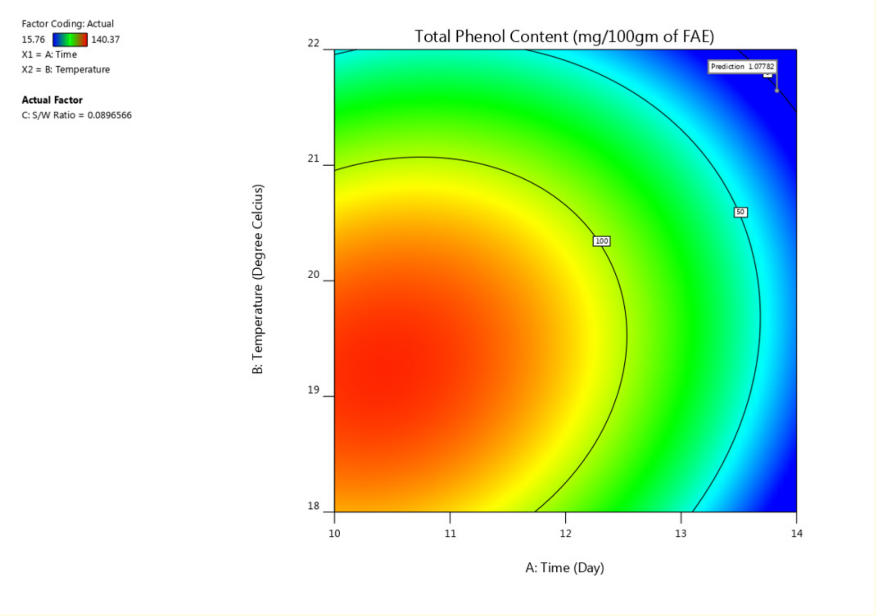


Figure 10: Contour plot of interactive effect of total phenol content on time and temperature on stability of fresh product.

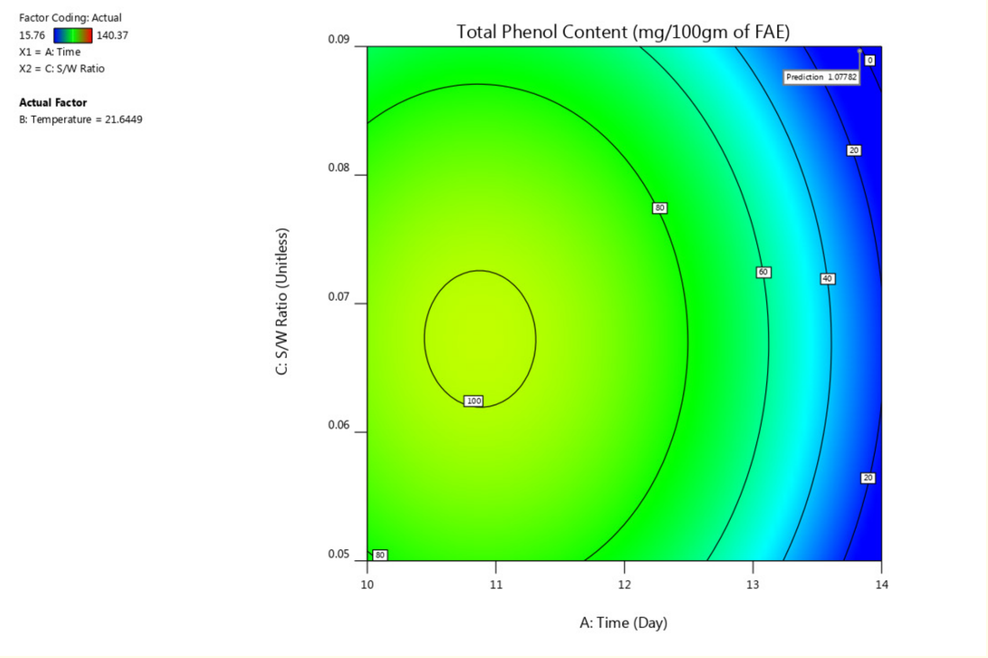


Figure 11: Contour plot of interactive effect of total phenol content on time and temperature on stability of fresh product.

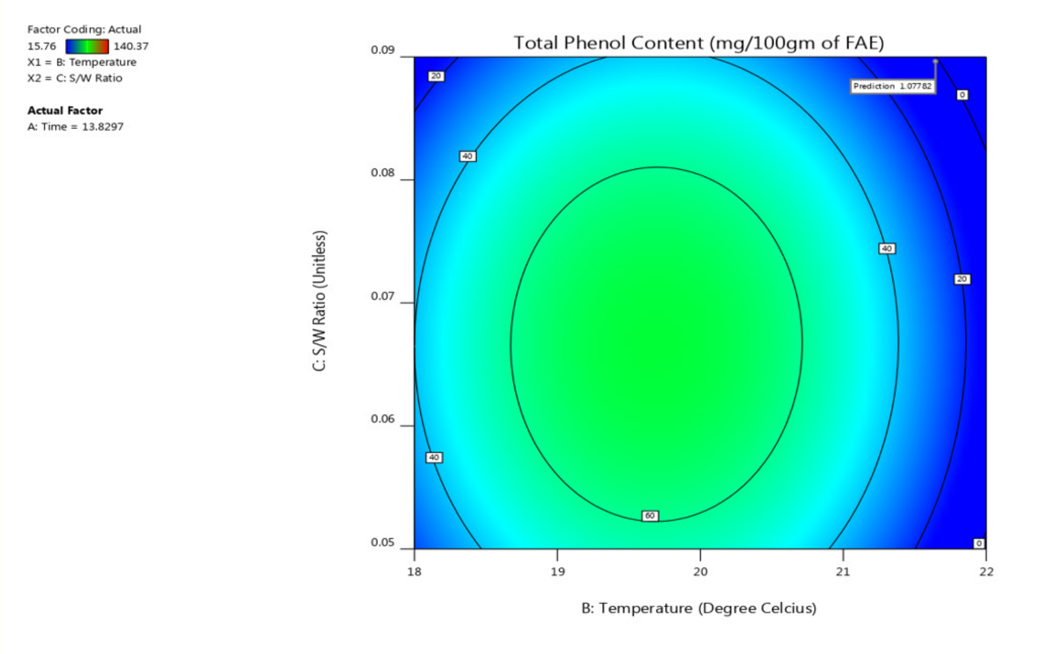


Figure 12: Contour plot of interactive effect of total phenol content on temperature and s/w ratio on stability of fresh product.

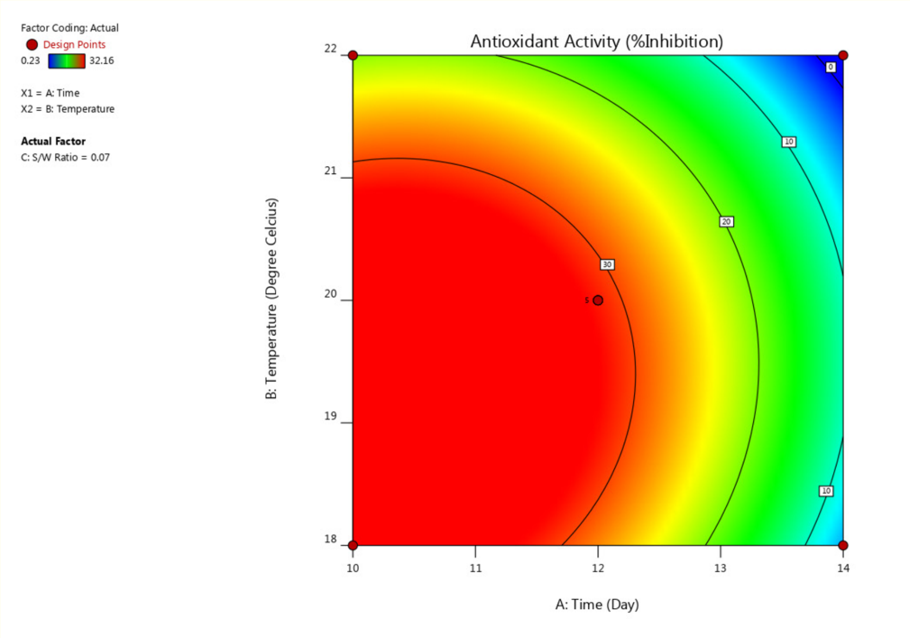


Figure 13: Contour plot of interactive effect of antioxidant activity on time and temperature on stability of fresh product.

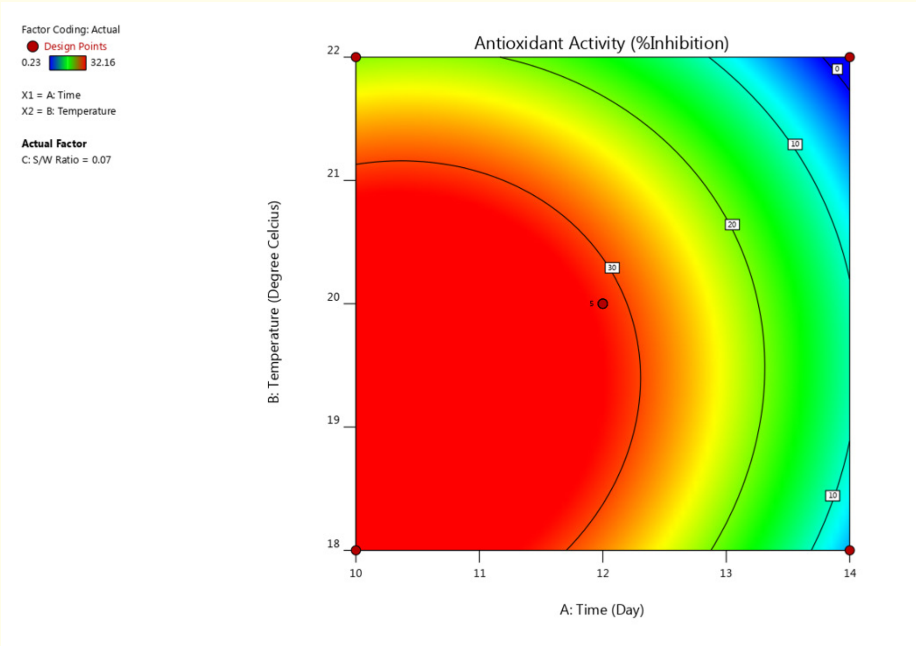


Figure 14: Contour plot of interactive effect of antioxidant activity on time and s/w ratio on stability of fresh product

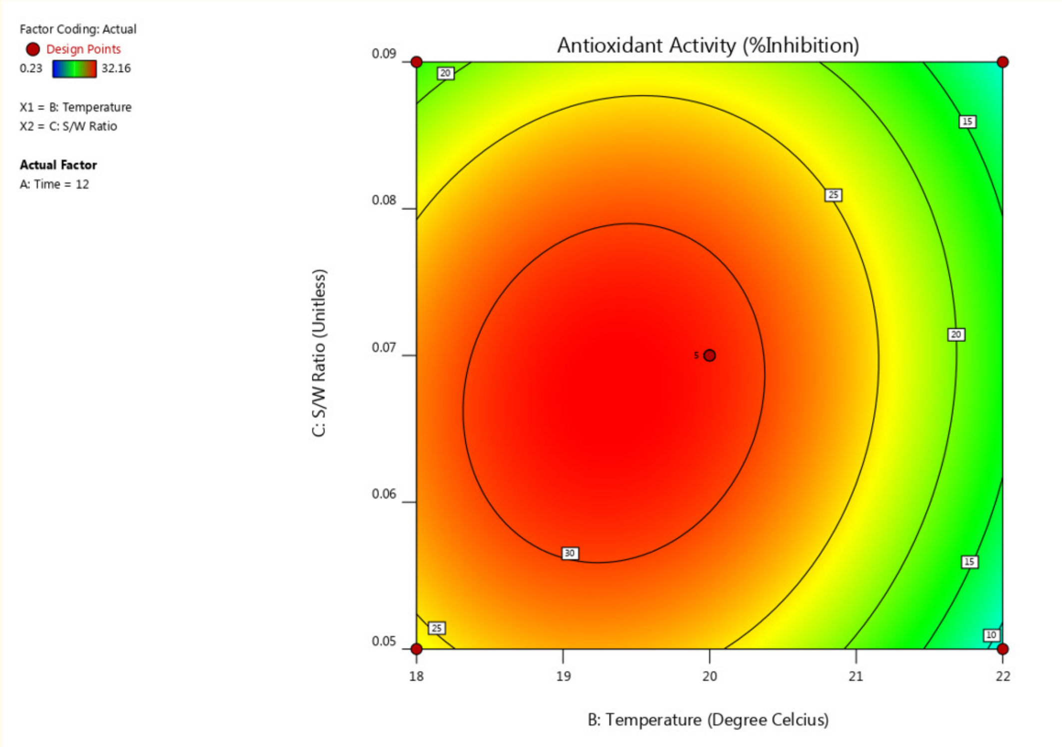


Figure 15: Contour plot of interactive effect of antioxidant activity on temperature and s/w ratio on stability of fresh product.

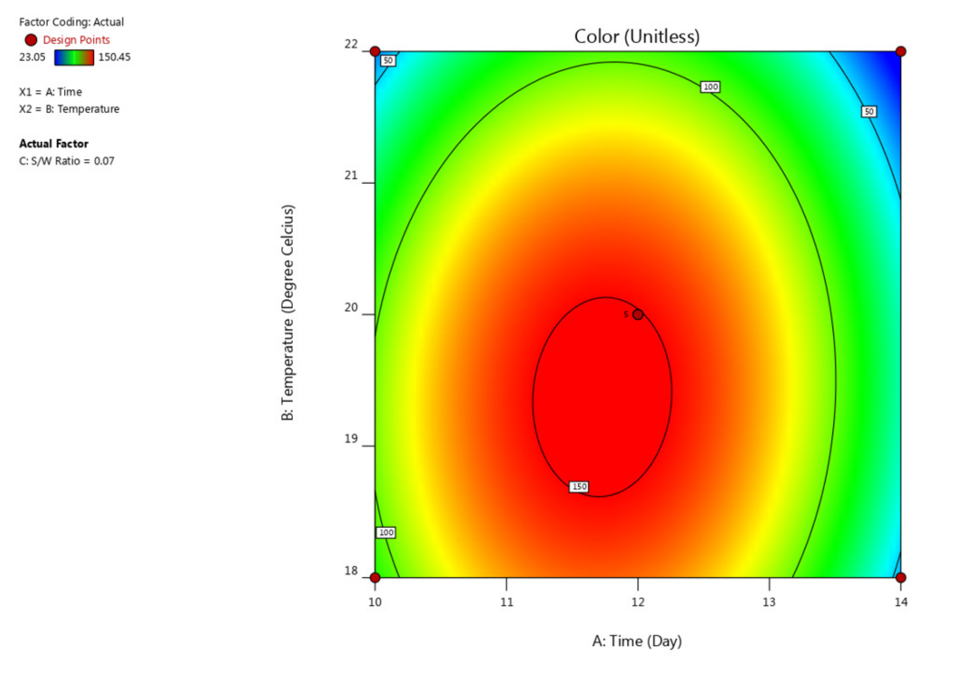


Figure 16: Contour plot of interactive effect of color on time and temperature on stability of fresh product.

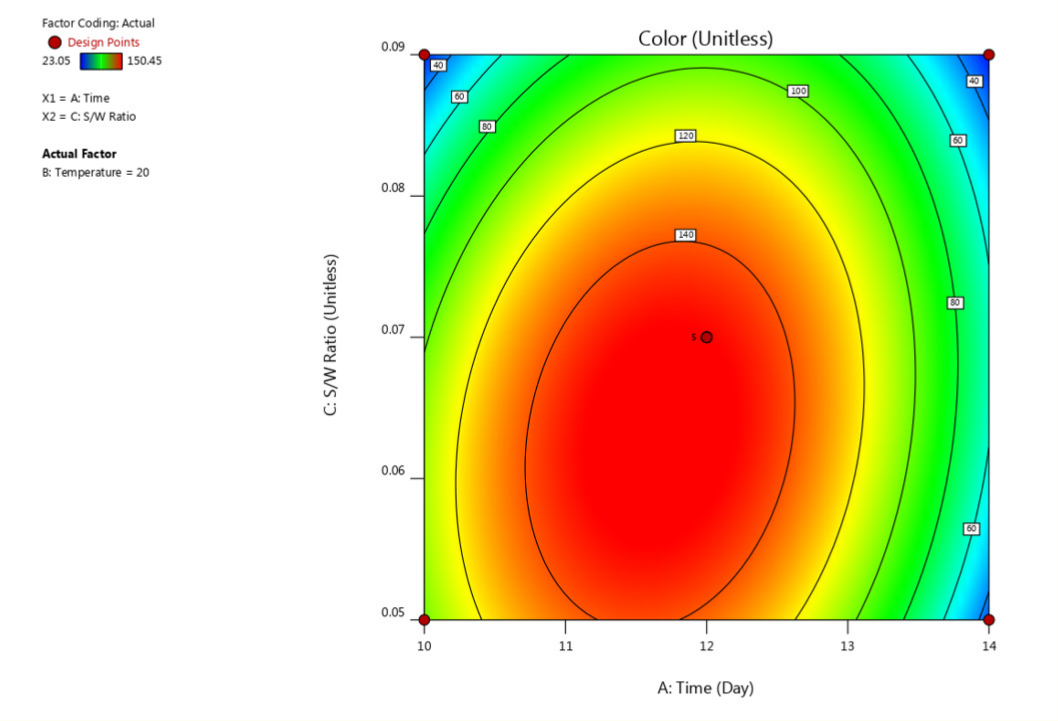


Figure 17: Contour plot of interactive effect of antioxidant activity on time and s/w ratio on stability of fresh product.

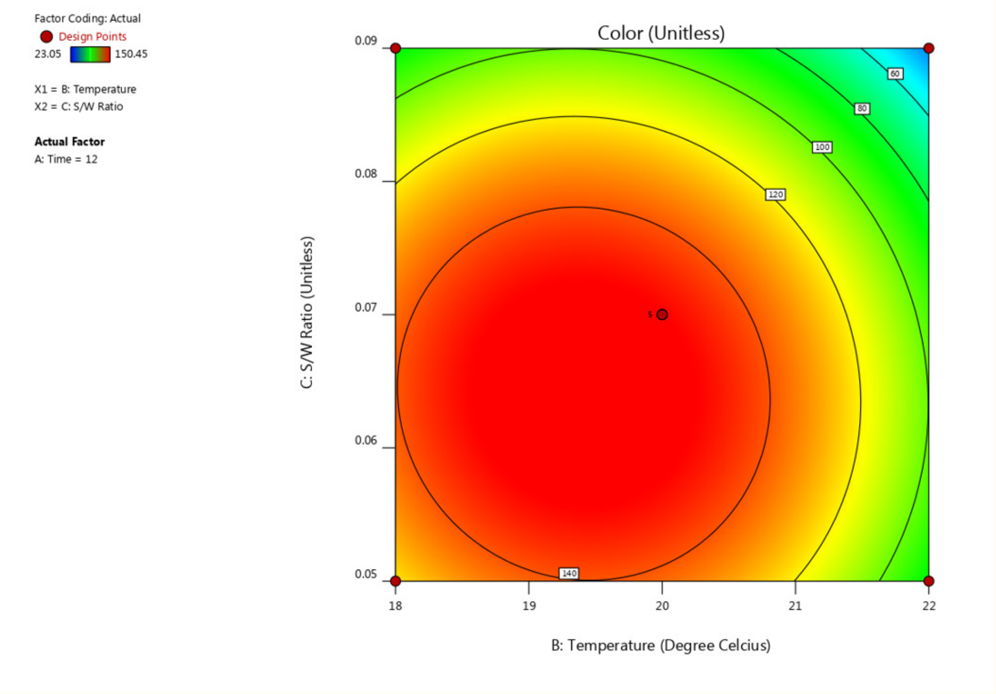


Figure 18: Contour plot of interactive effect of antioxidant activity on temperature and s/w ratio on stability of fresh product.

Independent variable	Units	Coded	Coded variable level		
			-1	0	+1
Time	Day	A	10	12	14
Temperature	Degree Celsius	B	18	20	22
S/W Ratio	Unitless	C	0.05	0.07	0.09

Table 1: The denoted level of varied conditions selected for this experiment.

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Std	Run	A:Time	B:Temperature	C:S/W Ratio	Total Phenol Content	Antioxidant Activity	Color
		Day	Degree Celsius	Unitless	mg/100gm of FAE	%Inhibition	Unitless
15	1	12	20	0.07	140.37	31.38	150.45
16	2	12	20	0.07	140.37	31.38	150.45
3	3	10	22	0.07	82.32	23.45	43.78
5	4	10	20	0.05	136.76	30.13	101.56
4	5	14	22	0.07	15.76	0.23	23.05
8	6	14	20	0.09	32.79	0.84	25
9	7	12	18	0.05	119.44	26.76	130.87
6	8	14	20	0.05	40.59	5.39	30.02
17	9	12	20	0.07	140.37	31.38	150.45
14	10	12	20	0.07	140.37	31.38	150.45
2	11	14	18	0.07	20.78	2.64	40.08
10	12	12	22	0.05	50.13	5.76	79.89
12	13	12	22	0.09	30.63	6.56	30.06
11	14	12	18	0.09	98.13	20.58	90.24
13	15	12	20	0.07	140.37	31.38	150.45
7	16	10	20	0.09	131.46	29.68	31.34
1	17	10	18	0.07	135.37	32.16	80.45

Table 2: Box-Behnken experiments design matrix with experimental variables and its responses for self-life extension of red amaranth leaves.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	39622.36	9	4402.48	34.46	< 0.0001	significant
A-Time	17671.06	1	17671.06	138.30	< 0.0001	
B-Temperature	4747.28	1	4747.28	37.15	0.0005	
C-S/W Ratio	363.29	1	363.29	2.84	0.1356	
AB	576.72	1	576.72	4.51	0.0712	
AC	1.56	1	1.56	0.0122	0.9151	
BC	0.8190	1	0.8190	0.0064	0.9384	
A^2	4584.57	1	4584.57	35.88	0.0005	
B^2	8083.18	1	8083.18	63.26	< 0.0001	
C^2	2032.80	1	2032.80	15.91	0.0053	
Residual	894.41	7	127.77			
Lack of Fit	894.41	3	298.14			
Pure Error	0.0000	4	0.0000			
Cor Total	40516.76	16				

Table 3: ANOVA for quadratic model table for second order polynomial curve of Total phenol content.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2634.26	9	292.70	28.65	0.0001	Significant
A-Time	1412.99	1	1412.99	138.32	< 0.0001	
B-Temperature	266.11	1	266.11	26.05	0.0014	
C-S/W Ratio	13.47	1	13.47	1.32	0.2886	
AB	9.92	1	9.92	0.9713	0.3572	
AC	4.20	1	4.20	0.4114	0.5417	
BC	12.18	1	12.18	1.19	0.3110	
A^2	242.08	1	242.08	23.70	0.0018	
B^2	354.64	1	354.64	34.72	0.0006	
C^2	223.61	1	223.61	21.89	0.0023	
Residual	71.51	7	10.22			
Lack of Fit	71.51	3	23.84			
Pure Error	0.0000	4	0.0000			
Cor Total	2705.77	16				

Table 4: ANOVA for quadratic model table for second order polynomial curve of Antioxidant activity.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	43458.65	9	4828.74	70.88	< 0.0001	Significant
A-Time	2414.43	1	2414.43	35.44	0.0006	
B-Temperature	3397.35	1	3397.35	49.87	0.0002	
C-S/W Ratio	3432.06	1	3432.06	50.38	0.0002	
AB	96.43	1	96.43	1.42	0.2729	
AC	1062.76	1	1062.76	15.60	0.0055	
BC	21.16	1	21.16	0.3106	0.5947	
A^2	20453.65	1	20453.65	300.25	< 0.0001	
B^2	4842.35	1	4842.35	71.08	< 0.0001	
C^2	4802.45	1	4802.45	70.50	< 0.0001	
Residual	476.86	7	68.12			
Lack of Fit	476.86	3	158.95			
Pure Error	0.0000	4	0.0000			
Cor Total	43935.51	16				

Table 5: ANOVA for quadratic model table for second order polynomial curve of Color.

Factor name	Optimized level	Total phenol content(TPC)		Antioxidant activity(FRAP)		Color	
		Observed	Predicted	Observed	Predicted	Observed	Predicted
Time	12.15	142.37	140.37	31.38	31.38	150.45	150.45
Temperature	19.42						
s/w ratio	0.089						

Table 6: Optimum condition suggested by Box-Behnken Response Surface Methodology model.

Conclusion

Red amaranth leaves are a highly valued leafy vegetable due to their rich bioactive compounds. However, this vegetable exhibits a highly perishable nature that limits its shelf life. To address this issue, Modified Atmosphere Packaging (MAP) has been explored as a viable solution for storing the vegetable without the use of chemical preservatives.

The study aimed to investigate the effectiveness of MAP in preserving the quality of red amaranth leaves. The results indicated that after three days of storage under normal atmospheric conditions, the total phenol, antioxidant activity, and color of the red amaranth leaves decreased significantly. However, samples stored under MAP exhibited a four-fold increase in shelf life and acceptability. This technique is effective in delaying the degradation of leaf pigment, which is responsible for the color and quality of the leaves.

The experimental results were in good agreement with the proposed regression model with a p-value of less than 0.05. The study found that MAP is a promising technology that can extend the shelf life of red amaranth leaves while maintaining their quality. This technology provides a sustainable solution for growers and suppliers in the commercial food industry.

In conclusion, the study findings provide a viable solution for extending the shelf life of red amaranth leaves without the use of chemical preservatives. The use of MAP technology provides a sustainable solution for the commercial food industry, which can benefit both growers and suppliers. Further research in this area can provide additional insights into the effectiveness of MAP technology for other perishable vegetables.

Acknowledgement

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Authors’ contributions

Both the authors have carried out the research work and participated in manuscript preparation.

- **Conflict of Interest:** Authors do not have any conflict of interests to declare.
- **Ethical Issues: “**None”.

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