ISSN 2319 – 7757 EISSN 2319 –7765

discovery

Indian Journal of Engineering

Optimization of storage parameters to enhance the shelf life of malta using response surface method

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Publication History
Received: 27 November 2013
Accepted: 13 January 2014
Published: 5 February 2014

Citation
Richa R, Pandey JP, Khan Chand, Shahi NC, Anupama Singh. Optimization of storage parameters to enhance the shelf life of malta using response surface method. *Indian Journal of Engineering*, 2014, 8(18), 11-19

ABSTRACT

In this study, the response surface method and experimental design were applied for optimization of independent variables to enhance the shelf life of four months stored malta. Box-Benkhen design, with, 12 factorial points and 5 replicates at the centre point were used to build a model for predicting and optimizing storage process parameters. The mathematical model equations were developed by using Design-Expert 8.0.6.software. The independent parameters viz scavenger (3, 4 and 5g), polythene thickness (75, 100 and 125 gauge) and fungicide concentration (100, 150 and 200ppm) were selected and analyzed. Statistical checks (ANOVA table, R^2 value, model and F-value) indicated that the model was adequate for representing the experimental data. The dependent parameters measured were titrable acidity and ascorbic acid. The statistical analysis indicated the optimized values of the selected parameters as 5g scavenger, 125gauge polythene thickness and 200ppm fungicide concentration. This revealed that malta could be stored for four months if kept under these conditions and the quality could be maintained.

Key words: Citrus fruits, fungicide, storage studies and shelf life.

1. INTRODUCTION

Malta is having high acceptability due to its attractive color, distinctive flavor and taste. The excellent quality fruits are generally available for only one or two months. However owing to its poor shelf-life, fruits cannot be stored for longer period under ambient conditions and cannot be transported to distant places. Citrus fruits are non-climacteric in nature and their eating quality cannot be improved after harvest. Hence, harvesting should be done when fruits internal quality is at their best. There is need of enhancing the shelf-life of malta for increasing the local raw materials and decreasing the post-harvest losses and for using processed product out of season (Dasmohapatra et al., 2011). In order to store malta fruits active packaging (scavenger) was used prior to fungicide treatment. In view of this, active packaging for storage of malta was investigated. During the storage of packaged fruits and vegetables, the CO_2 is formed, due to respiration reactions, and the ethylene is produced more and more. These gases, accumulated during the time, have to be removed from the package to avoid the food deterioration (i.e. increasing of acidity in fruit, and over maturation) and the packaging destruction so there should be provision of a system which can remove these gases and for this purpose scavenger is a good source (Floros, 1997).

Shelflife of malta depends on three effective factors; scavenger amount, polythene thickness, and fungicide concentration. The statistical method using response surface methodology (RSM) has been proposed to determine the influences of individual factors and the influence of their interactions. RSM is a technique for designing experiments, building models, evaluating the effects of several factors, and achieving the optimum conditions for desirable responses with a limited number of planned experiments RSM helps to demonstrate how a particular response is affected by a given set of input variables over some specified region of interest, and what input values will yield a maximum (or minimum) for a specific response. RSM was initially developed for the purpose of determining optimum operation conditions in the chemical industry, but it is now used in a variety of fields and applications, not only in the physical and engineering sciences, but also in biological, clinical, and social sciences (Khuri and Cornell, 1996). The response surface design was used in this study to: 1) find how weight loss comprising of several levels of shellife factors can be simplified, 2) determine how titrable acidity and ascorbic acid (as responses) are affected by changes in the level of scavenger, polythene and fungicide concentration, 3) determine the optimum combination of scavenger, polythene thickness and fungicide concentration that yields the best storage life. Quantitatively measure the significance and interaction between factors with respect to the optimum weight loss, titrable acidity and ascorbic acid.

2. MATERIALS AND METHODS

Fresh malta fruits were used for storage study. The fresh malta was procured from the market of distt Almora, Uttarkhand; the storage study was carried out for 4 months in the lab. Due to non availability of fresh malta throughout of the year, there are packed in polythene bags of different thickness (permeability) and used for experiments. The malta fruits were analysed for titrable acidity and ascorbic acid as per method prescribed (Rangana, 2005). Malta fruits were treated with azoxystrobin fungicide solution to resist fungus attack. Fruits were dipped in fungicide solution for 1 min and after that fruits were kept under ambient conditions at least 5-6 hrs so that moisture of fungicide solution get evaporated from malta surface and fruit become safe for storage i.e. micro organism could not attack on malta due to moisture. Treated malta fruits were weighed with digital balance and then keeping two mlata in one box. On the base of box, decided amount of scavenger (potassium per magnate and activated charcoal powder) was kept in petri dish. Boxes were packed with polythene of different thickness and stored at room temperature for further analysis. All the treated samples were kept for four months storage. For storage of malta, mill board boxes were used whose dimensions were (18.5×15×10cm). Each box has base in which six perforations were made and each side face has two perforations for the exchange of gases so that accumulation of gases do not take place and fresh air was maintained inside the box. The experimental procedure was described in Figure 1.

2.1. Experimental design

An important assumption satisfy by independent variables that they are measurable, continuous, and controllable by experiments, with negligible errors, the RSM course of action was carried out as follows (Trinh and Kang, 2010):
1) For adequate and reliable measurement of the response of interest a series of experiments were performed
2) A mathematical model of the second-order response surface with the best fit was developed.
3) The optimum response value produced by optimal set of experimental parameters was determined.

Response Surface Methodology was used as it helps to reduce the number of experiments without affecting the accuracy of results and to decide interactive effects of variables on the response. Box-Behnken designs are response surface designs especially made to require only 3 levels, coded as -1, 0, +1. Box-Behnken designs are 3 to 21 factors. They are formed by combining two levels factorial design with incomplete block design. This procedure create design with desirable statistically properties but, most importantly with only the fraction of the experiments required for 3 levels factorial. Because there are only three levels the quadratics model is appropriate (Khuri and Cornell, 1996). A

Table 1

Levels of independent variables in coded and actual form for storage study of malta

Independent variables		Coded Levels		
Name	Code	-1	0	+1
		Actual Levels		
Scavenger (g)	X_1	3	4	5
Polythene thickness (gauge)	X_2	75	100	125
Fungicide concentration (ppm)	X_3	100	150	200

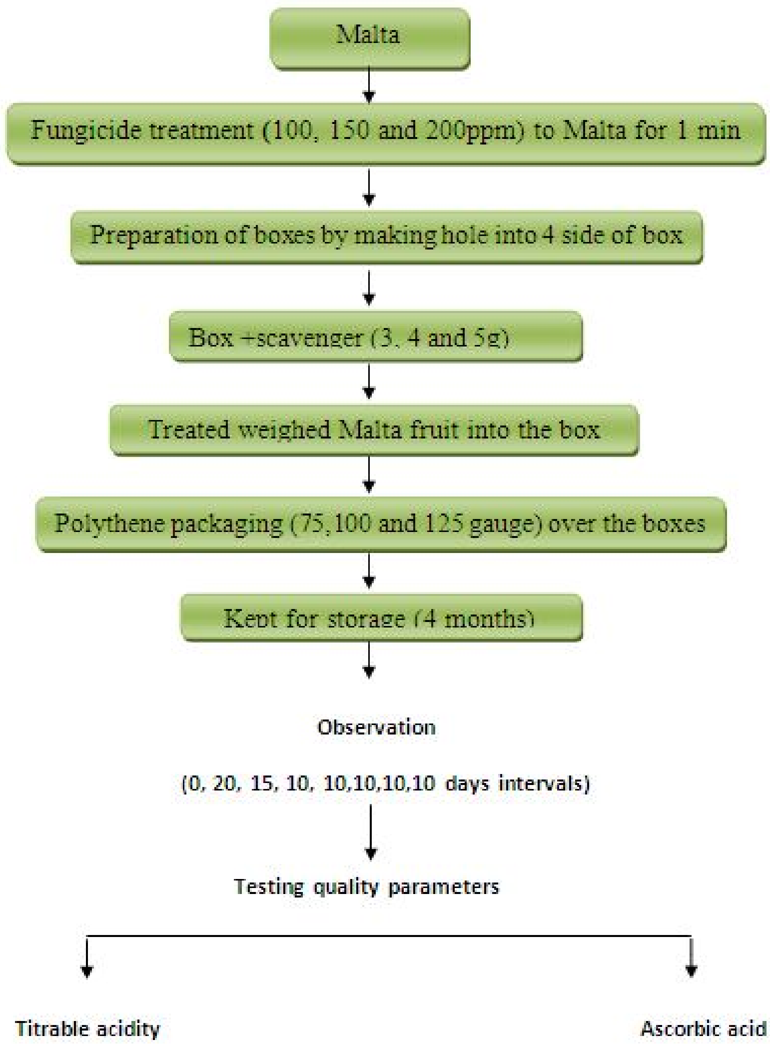

Figure 1

Experimental Procedure for storage of malta

box- behnken design, which is a very efficient design tool for fitting second-order models, was selected for use in this study. Based on literatures and preliminary experiments conducted the level chosen for scavenger, X_1 polythene, X_2 and fungicide, X_3 were X_1: 3, 4 and 5g; X_2:75, 100 and 125 gauge and X_3: 100, 150 and 200ppm for enhancing the shelflife of malta under ambient condition. Once the desired value ranges of the independent variables had been defined, they were coded as ±1 for the factorial points, 0 for the center points. The ranges of variables as in coded and actual form for the design of experiments are shown in Table 1.

2.2. Measurements of quality parameters during storage

2.2.1. Titrable acidity

Titrable acidity was estimated by standard method using visual titration method. Juice was mix thoroughly by shaking and filtering through previously washed and dried muslin cloth.10ml of sample was taken into a beaker. Transfer 10ml of sample accurately in a 100 ml beaker. Dilute the sample with distilled water. Add 1-2 drops of phenolphthalein indicator. 0.1N NaOH was taken into burette. Sample was titrated with 0.1 NaOH till light pink color. Record the volume of 0.1NaOH used to titrate the value (Rangana, 2005).

$$TitrableAcidity(\%) = \frac{\begin{matrix} titre \times Normality \times volume \times equivalent weight of acidity \\ of alkali \quad madeup \end{matrix}}{vol of sample \times vol of aliquot \times 1000} \quad (1)$$

2.2.2. Ascorbic acid

Ascorbic acid was determined by 2, 6- di chloro phenol- indophenols visual titration method recommended by the Association of Vitamin Chemist (Ranganna, 2005). 5ml of standard ascorbic acid solution added to 5 ml of HPO_3. Micro-burette was filled with dye and titrated to pink color which should persist at least 15 sec. dye factor was calculated using the formula for the ascorbic acid content of the sample

$$(Ascorbic\ acid, mg/ml) = \frac{(titre\ x\ dye\ factor\ x\ volume\ made\ up\ x100)}{\begin{pmatrix} aliquot\ of\ extract \\ taken\ forestimat\ ion \end{pmatrix} x \begin{pmatrix} volume\ of\ sample \\ taken\ for\ estimation \end{pmatrix}}$$

(2)

2.3. Second order polynomial model

In order to determine if a relationship existed between the factors and the responses investigated, the collected data were analyzed statistically using regression analyses. A regression design is engaged to model a response as a mathematical function (either known or empirical) of a few continuous factors and 'good' model parameter estimates are most desired (Montgomery, 2001). Each response of *Y* can be represented by a mathematical model that correlates the response surface. The responses can be expressed as second-order polynomial equations, according to Eq. (3):

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \beta_{ii} X_i^2 \quad (3)$$

Where, Y is the predicted response (titrable acidity and ascorbic acid) used as a dependent variable; k the number of independent variables, x_i (i = 1, 2) the input predictors or controlling variables (factors); β_0 the constant coefficient of regression, and β_i, β_{ij} and β_{ii} the coefficients of regression of linear, interaction and quadratic terms, respectively. Multiple linear regression analysis was used for determining the coefficient parameters by employing the software Design-Expert (version 8). Design-Expert was also used to find the 2-D contour plots of the response models.

3. RESULTS AND DISCUSSION

3.1. Model fitting

Seventeen observed response values observed were used to compute the mathematical model using the least square method. The two dependent variables as titrable acidity and ascorbic acid were correlated with the three factors (scavenger amount, polythene thickness and fungicide concentration), using the second-order polynomial, as represented by Eqn 3. After fitting experimental data, quadratic regression models were developed, as shown in Eqn (4) and (5).

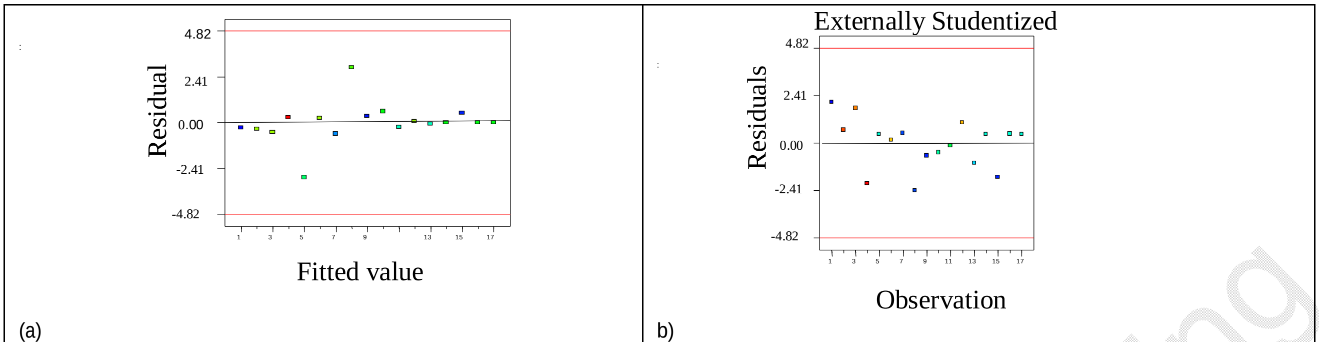

Fig. 2: Residuals vs. fitted values for (a) Titrable acidity (b) Ascorbic acid

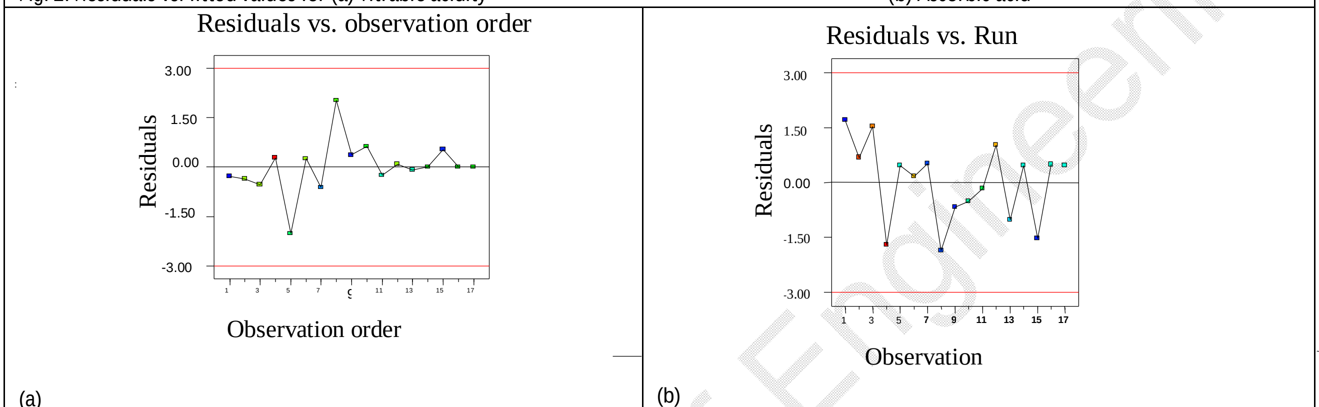

Fig.3: Residuals vs. observation orders of data for (a) Titrable acidity (b) Ascorbic acid

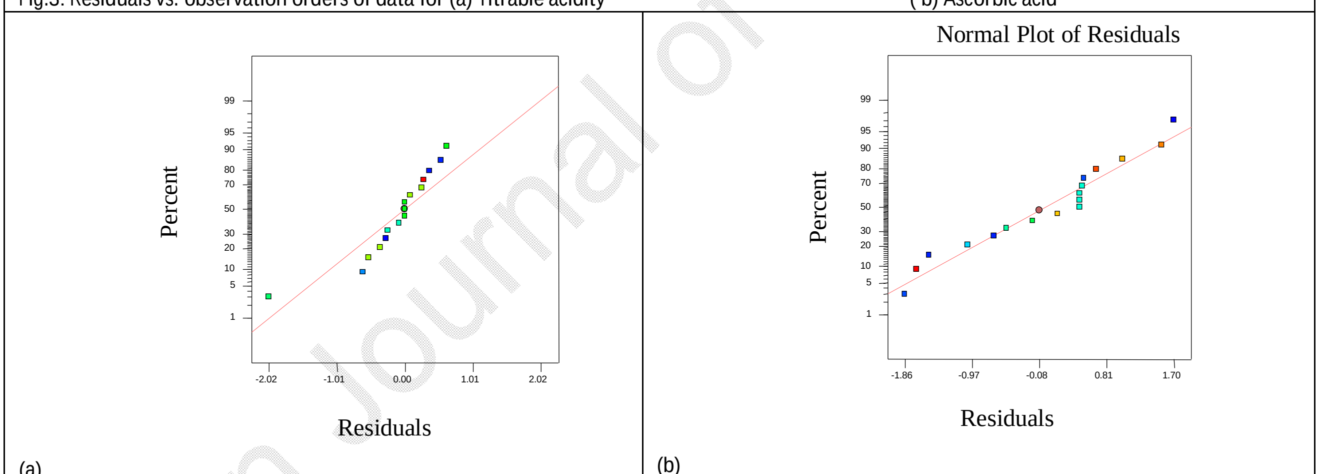

Fig. 4: Normal probability plots for (a) Titrable acidity (b) Ascorbic acid

Titrable acidity $(Y_1) = 0.77+0.064X_1+0.13X_2+0.000X_3-0.032X_1X_2-0.000X_1X_3 + 0.000X_2X_3 +0.016X_1^2-0.080X_2^2-0.016X_3^2$
$(R^2 = 98.66\,\%,\ R^2_{adj} = 96.93\%$ and Pred $R^2 = 96.57\%)$ (4)

Ascorbic acid $(Y_2) = 60.31+2.06X_1+3.63X_2+0.35X_3+1.4RX_1X_2-0.47X_1X_3+0.13X_2X_3+1.00X_1^2 + 0.031X_2^2+2.08X_3^2$
$(R^2 = 99.02\%,\ R^2_{adj} = 90.89\%$ and Pred $R^2 = 64.73\%)$ (5)

Where, X_1, X_2 and X_3 are the scavenger amount, polythene thickness and fungicide concentration. The coefficients with one factor (the ones in front of X_1 or X_2 or X_3) represent the effects of that particular factor, while the coefficients with two factors (the ones in front of X_1X_2 or X_1X_3 or X_2X_3) and those with quadratic terms (the ones in front of X^2_1 or X^2_2 or X^2_3) represent the interaction between the two factors and the quadratic effects, respectively. The positive sign in front of the terms indicates a synergistic effect, while the negative sign indicates an antagonistic effect. Equation (4): Titrable acidity will increase by increasing the value of scavenger; polythene thickness and fungicide. The effect of polythene thickness (0.13) is the highest and is followed by scavenger (0.064). Equation (5): There will be an increase in ascorbic

Table 2

Total effect of individual parameter on titrable acidity for stored malta

SOURCE	DF	SS	MS	F-Value	F cal
Model	9	0.1616	0.0177	56.36***	6.2
Linear	3	0.1510	0.0503	160.19***	8.45
Quadratic	3	0.0083	0.00277	8.79***	8.45
Interactive	3	0.00491	0.00164	5.19**	4.35
Residual error	7	0.00220	0.000314		
Lack of fit	3	0.00015	0.000052	0.0979	
Pure error	4	0.00205	0.000512		
Total	16	0.1638			

***, ** Significant at 1and 5 % level of significance respectively

Table 3

Total effect of individual parameter on ascorbic acid for stored malta

SOURCE	DF	SS	MS	F-Value	F-cal
Model	9	172.97	19.21	18.65***	6.2
Linear	3	140.38	46.79	45.42***	8.45
Quadratic	3	22.493	7.49	7.27**	4.35
Interactive	3	8.928	2.976	2.88	
Residual error	7	7.1783	1.0255		
Lack of fit	3	3.6244	1.2082	1.3598	
Pure error	4	3.5539	0.88848		
Total	16	180.144			

***, ** Significant at 1and 5 % level of significance respectively

acid with increase of all three ingredients. The polythene thickness has got more effect on ascorbic acid will the value of 3.63 followed by scavenger with (2.06) and then by fungicide with (0.35).

3.2. Validation of the Models

The developed quadratic model is usually checked for ensuring it provides an adequate approximation to the real system. Unless the model shows an adequate fit, proceeding with investigation and optimization of the fitted response surface is likely to give poor or misleading results. As a primary tool and confirmation for graphical techniques, graphical and numerical methods were used to validate the models in this study (Trinh and Kang, 2010). The graphical method characterizes the nature of residuals of the models. A residual is defined as the difference between an observed value and its fitted values. The first plot, residuals versus the fitted values, as shown in Figure 2, was used to test the efficiency of the functional part of the developed model. Then, the second plot, residual versus order, as shown in Figure 3, each residual is plotted against an index of observation orders of data, which was used to check for any drift in the process. As previously shown in Figure 2 and 3, the graphical residual analysis indicated no obvious pattern, implying the residuals of the models were randomly distributed. Lastly, in the normal probability plot, shown in Figure 4, the data were plotted against a theoretical normal distribution in such a way that the points should form an approximate straight line, and a departure from this straight line would indicate a departure from a normal distribution, which was used to check the normality distribution of the residuals. As shown in Figure 4, it is necessary that the assumptions of normality were satisfied for the data.

The developed models were then examined using a numerical method employing the coefficient of determination (R^2), adjusted R^2 (R^2adj), and then calculated as shown in Eqn (6) and (7) (Haber and Runyun, 1977), the coefficient of determination R^2 indicates how much of the observed variability in the data was accounted for by the model, while R^2adj modifies R^2 by taking into account the number of covariates or predictors in the model.

$$R^2 = 1 - \frac{SS_{residual}}{SS_{model} + SS_{residual}} \quad (6)$$

$$R^2{}_{adj} = 1 - \frac{n-1}{n-p}(1 - R^2) \quad (7)$$

Where, SS is the sum of the squares, *n* the number of experiments, and *p* the number of predictors (term) in the model, not counting the constant term. The quadratic model of experimental data were developed in this study with the values of R^2 higher than 90%, say 98.66 and 96.02% for titrable acidity and ascorbic acid of stored malta, respectively. Furthermore, an R^2adj very close to the R^2 values for the response titrable acidity than ascorbic acid insures a satisfactory adjustment of the quadratic models to the experimental data and having least residual error in model. Therefore, the regression models explained to check the effect of each response on independent variables well.

3.2.1. Titrable acidity

During storage period the titrable acidity ranged from is 0.96 to 0.588%. The maximum titrable acidity was observed at Experiment No. 4 in which 5 g scavenger (X_1 = +1), under 125 gauge thickness of polythene wrap (X_2 = +1) and 150 ppm fungicide (X_3 = 0) was found. The minimum titrable acidity was observed at Experiment No 1 which had 3 g of

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Table 4

Box- behnken method and results obtained

Expts.	X_1:scavenger	X_2:polythene	X_3:fungicide	Y_1:Titrable acidity	Y_2:Ascorbic acid
	(g)	(gauge)	(ppm)	(%)	(mg/ml)
1	-1	-1	0	0.588	57.92
2	0	1	1	0.832	66.87
3	0	1	-1	0.832	66.35
4	1	1	0	0.96	67.58
5	0	0	0	0.736	60.72
6	1	0	-1	0.832	65.66
7	1	-1	0	0.64	58.62
8	0	0	0	0.8	58.62
9	0	-1	-1	0.595	58.23
10	-1	1	0	0.768	61.23
11	-1	0	1	0.704	62.06
12	1	0	1	0.832	65.85
13	-1	0	-1	0.704	59.99
14	0	0	0	0.768	60.72
15	0	-1	1	0.601	58.23
16	0	0	0	0.768	60.75
17	0	0	0	0.768	60.72

Table 5

Optimum value of parameters for stored malta

Value	Scavenger, g (X_1)	Polythene, gauge (X_2)	Fungicide, ppm (X_3)
Coded	+1	+1	+1
Actual	5	125	200

scavenger (X_1 = -1), 75gauge of polythene thickness (X_2 = -1) and 150 ppm of fungicide (X_3 = 0). ANOVA on titrable acidity model, as shown in Table 2, demonstrates that the models were highly significant (p<0.01), the small value (0.018) of standard deviation of the model indicating that the model fitted well. The predicted R^2 of 96.58% is in reasonable agreement with the adj R^2 of 96.93%. The lack of fit test describes the variation in the data around the fitted model. If the model does not fit the data well, the lack of fit will be significant. The small F-values (0.0979 and 1.3598) of the lack of fit for the titrable acidity and ascorbic acid models, the lack of fit was not significant; implying the models adequately described the data.

During storage period the titrable acidity ranged from 0.96 to 0.588%. Effect of response on scavenger, polythene and fungicide at linear, quadratic and interactive levels are reported in Table 2. It shows that the effect of titrable acidity of four month stored malta at linear and quadratic level was highly significant (P<0.01). The second order model was significant (P<0.01) because it has higher F value than tabulated F value implying least error in data. While the interactive level was significant at 5% level of significance and had less effect on titrable acidity in compare to linear and quadratic level. Malta kept in polythene bag having high thickness showed the minimum change in titrable acidity. Higher polythene thickness has higher retention of titrable acidity. Titrable acidity decreased as storage time increased.

The coefficient of interactive term like polythene and fungicide were negative indicating with the increase or decrease of the level of these variables, the titrable acidity decreased. Negative coefficient of quadratic term indicates that the maximum of titrable acidity is at centre point while positive quadratic give the minimum response. The quadratic term of polythene thickness found to be significant at 1% level of significance and other quadratic terms have no significant effect on titrable acidity.

The range for change in ascorbic acid during storage was from 57.96 to 67.58mg/ml Effect of variables on scavenger, polythene and fungicide on ascorbic acid at linear, quadratic and interactive levels is reported in Table 3**.** It shows that the effect at linear and quadratic level were highly significant at 1% level of significance. The second order model was highly significant at (P<0.01) because it has higher F value than tabulated F value. While the interactive level has no significant effect value due to more residual effect, and least mean square value i.e. no effect on ascorbic acid in compare to linear and quadratic level during storage of malta. The second order model of 18.65 implies the model was highly significant. There is only a 0.04% chance that a model F value this large could occur due to noise. The lack of F value of 1.36 implies the lack of fit is not significant relative to pure error, non significant lack of fit is good to fit the model. The standard deviation of 1.01266 implies the least error. The pred R^2 of 64.73% is not as close the adj R^2 of 90.89% as one might normally expect. This may indicate a large block effect or a possible problem with model or data.

Figure 5 shows the plot of titrable acidity versus ascorbic acid shown in figure 5 not much correlation between these two response noted. Therefore the response of both titrable acidity and ascorbic acid were different and optimum condition for each would also be different. Figure 6 shows the 3 D surface contour plot. The response surface and contour is the graphical representation of regression analysis used to visualize the response between the response and experimental level of each input factor as shown in theses plot. Increase the titrable acidity was observed with slightly increase in polythene thickness and fungicide at optimum condition. An increase in the both

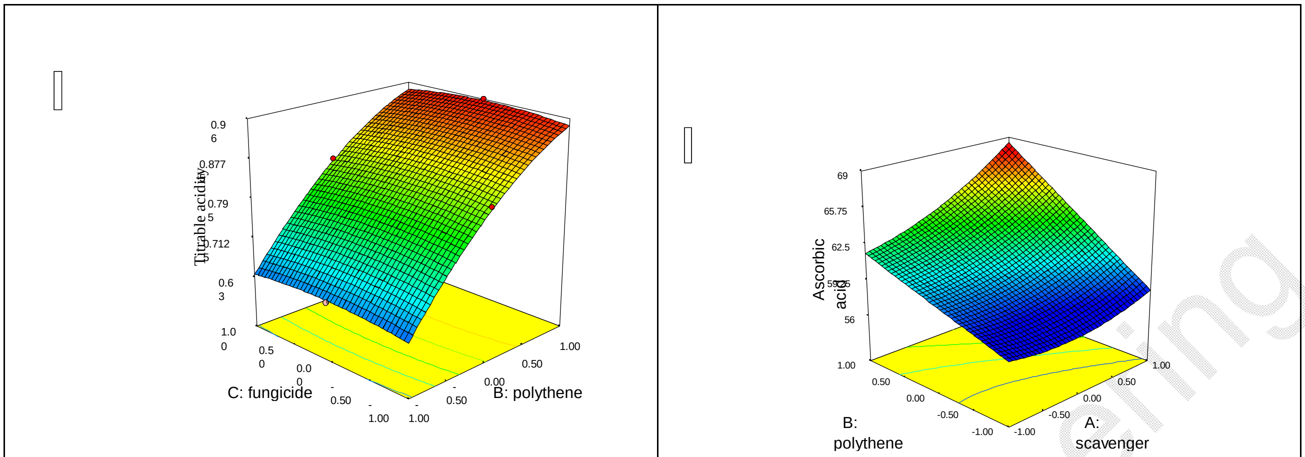

Fig. 6: Three dimensional surface plots for (a) Titrable acidity (b) Ascorbic acid

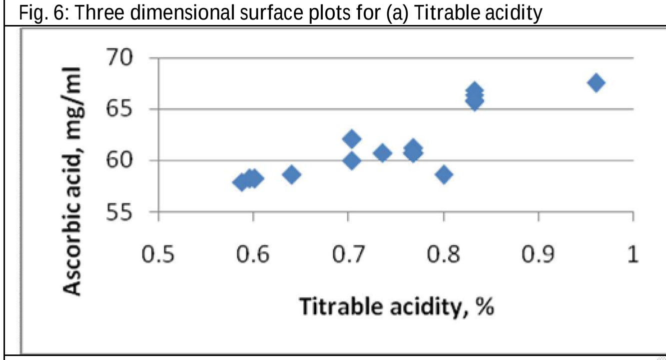

Fig. 5: Plot of Titrable acidity vs. Ascorbic acid

variable beyond the optimum region resulted deteriorate the product. With respect to ascorbic acid as response an increase in the polythene thickness and scavenger was observed. Plot also showed that higher scavenger value were favorable beyond the optimum level decrease in ascorbic acid. An optimum level of independent variables was obtained by analyzing the response surface-contour and the derivative of Eqs. (4) and (5). The optimum conditions were a set of X_1 (scavenger), X_2 (polythene) and X_3(fungicide) where the derivative becomes zero, as shown in Eq. (6):

$$\frac{dY}{dX_1} = \frac{dY}{dX_2} = \frac{dY}{dX_3} = 0 \qquad (8)$$

It is obvious that in the response surfaces which indicated the optimal conditions of variables were located exactly inside the design boundary.

4. CONCLUSIONS

On the basis of experimental and analytical data, it could be concluded that RSM application could be beneficial for seeking the optimal conditions for storage of malta. With the use of appropriate quantity of scavenger (5 g), fungicide (200ppm) and polythene bags (125gauge) malta could be stored upto four months without any losses while the quality attributes Titrable acidity, Ascorbic Acid remains unaffected upto four months.

ACKNOWLEDGEMENTS

This research was carried out in the department of Post Harvest Process & Food Engineering and funded by the Indian Council of Agriculture Research under All India coordinated research project on Post Harvest Technology. We sincerely thanks to Head, PHPFE, Director Research and Dean, College of Technology, G.B.P.U.A. & T., Pantnagar for providing necessary requirements for smooth conducting of the research work.

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