

## **Optimization of Ethanol and Potassium Sorbate Treatment for Minimizing Postharvest Losses in Onion Bulbs (*Allium Cepa* L. cv. *Sukhsagar*)**

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### **Abstract**

Response surface methodology (RSM) was used to optimize treatment combination of ethanol and potassium sorbate for minimizing physiological loss in weight ( $PL_w$ ) and rotting of onion bulbs during storage. Based on Central composite rotatable design (CCRD),  $PL_w$  and rotting percent as responses, 13 treatment combinations were selected involving four factorial points, four axial points and five central points. Optimum treatment combination of model generated by RSM was ethanol (71.08 percent; v/v) and potassium sorbate (0.76 percent; w/v). It was observed that this combination of ethanol and potassium sorbate had significantly reduced  $PL_w$  and rotting percent (8.57 and 1.38 percentage, respectively) during storage up to 90 days. Two-tailed t-test (assuming unequal variances) statistics ( $P < 0.05$ ) showed no significant difference between RSM-predicted and experimental values of the two responses. Hence, optimum treatment combination generated using CCRD/RSM was validated and confirmed from this study.

**Keywords:** Onion, shelf-life,  $PL_w$ , rotting, optimization, ethanol, potassium sorbate.

### **1. Introduction**

Onion, scientifically known as *Allium cepa* L., is a member of class Liliaceae and family Alliaceae, widely distributed and grown in tropical, sub-tropical and temperate climatic zones (Fritsch and Friesen, 2002). It is a biennial crop (Bohanec, 2003), and in

India it is grown twice (Rabi and Kharif season) in a year due to its wide consumer demand (NHRDF, 2009). Besides its culinary values, onion provides variety of health promoting compounds (Slimestad et al, 2007). However, productivity of onion bulbs in India is comparatively very low (16.41 tons/ha), particularly due to disease associated during its cultivation and postharvest processing, handling and storage system (NHRDF, 2009). Therefore, good storage techniques become essential. The main purpose of onion bulb storage is to maintain reasonably stable price throughout the year whilst maintaining the product quality during extended shelf-life period (Chope et al, 2005). So far as extension of shelf-life is concerned, Qadir et al (2007) has reported the delay of sprouting, rooting and decay with ethanol vapour treatment on bulbs (cv. Tazan and Lyomante) during storage for three months. Karabulut et al (2005) achieved an effective control of postharvest disease of grapes with pre-storage treatment of ethanol in combination with potassium sorbate.

On the other hand, controlled atmosphere, cold storage, storage at ambient temperature with controlled relative humidity, pre-harvest and postharvest application of costly chemicals have been reported to control losses of bulbs due to sprouting, rooting and rotting during storage in temperate climate (Gubb and MacTavish, 2002; Downes et al, 2009). However, these methods of onion storage are costly and therefore, are not sustainable at commercial level. Hence, cheap alternative approaches for storage becomes inevitable. In view of the above, the objective of this study was fixed to evaluate the effect of ethanol in combination with potassium sorbate on  $PL_W$  and rotting of bulbs during storage, and to achieve the above objective, optimization and generation of regression model for treatment effect was carried out with CCRD and RSM, respectively (Senanayake and Shahidi, 2002; Zahid et al, 2011).

## 2. Materials and Methods

CCRD was used to generate experimental trials based on combination of different concentrations of factors (ethanol and potassium sorbate), and its responses ( $PL_W$  and rotting percentage) were studied. The effect of factors on responses was reported using RSM. Onion bulbs of cv. Sukhsagar with uniform size ( $55 \pm 10$  mm diameter) were procured from a commercial supplier and sorted for use as experimental material. For giving treatment to the bulbs for extending shelf-life, a hand sprayer with a fine nozzle was used. Mixture of different concentrations of ethanol and potassium sorbate were sprayed (three kg onion per treatment) until entire surface area of bulbs was wet. Thereafter, the bulbs were air dried and placed in cartons and stored at room temperature ( $30 \pm 2$  °C) in the dark for 90 days. Relative humidity in the storage room ranged from  $73 \pm 2$  % during the period of experimentation.

Observations were recorded on the physical parameters namely,  $PL_W$  and rotting percentage at the end of the storage period of 90 days. For recording  $PL_W$ , weight of the bulbs was recorded at the end of 90 days after storage (DAS) using an electronic balance (having least count of 0.1 g). The cumulative loss in weight of bulbs were

calculated and expressed in percentage using the formula given below (Koraddi and Devendrappa, 2011):

$$\text{PLW (\%)} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100$$

Observations were also recorded on rotting percentage at the end of 90 DAS. It was calculated by the following formula given below (Kukanoor, 2005):

$$\text{Rotting (\%)} = \frac{\text{Weight of rotted bulbs}}{\text{Initial weight of bulbs}} \times 100$$

## 2.1 Experimental design for determination of optimum combination of ethanol and potassium sorbate

A five-level with two variables Central composite rotatable design was used in the present study. The design required 13 experiments including four factorial points, four axial points, and five replications at the central point. Five replications of the central point provided sufficient degrees of freedom for estimating the experimental error. In CCRD, low actual value and high actual value of ethanol (50-75%) and potassium sorbate (0.5-1%) was fixed based on preliminary trials made. The factors and their levels in the experimental design are shown in Table 1. The statistical analysis of the data was performed using Design-Expert 7.0 (Stat-Ease, Inc., Trial version) and the results are shown in Tables 2. The second-order quadratic regression equation of the following form developed for PL<sub>w</sub> and rotting percentage is given below:

$$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{12}x_1x_2 \quad (1)$$

Where,  $Y$  is predicted response,  $\beta_0$  is a constant,  $\beta_1$  and  $\beta_2$  represents regression coefficient of linear terms,  $\beta_{11}$  and  $\beta_{22}$  represents, regression coefficient of quadratic terms and  $\beta_{12}$  represents regression coefficient of interaction terms, and  $x_1$  and  $x_2$  are the coded values of an independent variables i.e. PL<sub>w</sub> and rotting percent respectively.

**Table 1:** Central composite rotatable design (CCRD) for the factors (actual and coded levels).

Run#	Independent Variables			
	Actual level		Coded level	
	Ethanol (%)	Potassium sorbate (%)	x1	x2
1	75.00	0.50	+1	-1
2	75.00	1.00	+1	+1
3	50.00	1.00	-1	+1
4	50.00	0.50	-1	-1
5	80.18	0.75	+1.414	0
6	62.50	1.10	0	+1.414
7	44.82	0.75	-1.414	0

8	62.50	0.40	0	-1.414
9	62.50	0.75	0	0
10	62.50	0.75	0	0
11	62.50	0.75	0	0
12	62.50	0.75	0	0
13	62.50	0.75	0	0

### 3. Results and Discussion

#### 3.1 Model fitting and statistical analysis of response

The experimental responses with respect to variation of two factors were evaluated by generating the best model equation for the particular response. The quadratic models were chosen for both the responses, as it gave higher precision which are presented in the equations given below with respect to coded factors. Therefore, the simplified second-order regression equations for PL<sub>W</sub> and rotting percent in terms of coded factors were determined with the following equation:

$$Y_1 = +9.05 - 1.14x_1 - 0.66x_2 + 0.66x_1^2 + 0.71x_2^2 + 0.55x_1x_2 \quad (2)$$

$$Y_2 = +1.55 - 0.67x_1 - 0.34x_2 + 0.62x_1^2 + 0.70x_2^2 + 0.22x_1x_2 \quad (3)$$

where, independent variables  $x_1$  and  $x_2$  are ethanol (v/v with water) and potassium sorbate (w/v with water), respectively, and Responses  $Y_1$  and  $Y_2$  are responses in terms of PL<sub>W</sub> (%) and rotting (%) at the end of 90 DAS respectively. The model significance (F and  $P$ -value), determination coefficient ( $R^2$ ), coefficient of variation (C.V %), and lack of fit criteria were used to judge the goodness of fitted model. Factor with most significant effect in regression equation for PL<sub>W</sub> (%) was the linear term of ethanol ( $x_1$ ), followed by quadratic term of potassium sorbate ( $x_2^2$ ), linear term of potassium sorbate ( $x_2$ ), and quadratic term of ethanol ( $x_1^2$ ), respectively. Their respective  $P$ -value proved that they are highly significant ( $P < 0.01$ ). These suggested that ethanol ( $x_1$ ) have more direct relationship with PL<sub>W</sub> percent than potassium sorbate ( $x_2$ ). In regression equation for rotting (%), the linear term of ethanol ( $x_1$ ), followed by quadratic term of potassium sorbate ( $x_2^2$ ) was most significant ( $P < 0.01$ ) factor. Unlike, regression equation for PL<sub>W</sub> (%), quadratic term of ethanol ( $x_1^2$ ) was found to be more significant factor over linear term of potassium sorbate ( $x_2$ ) in regression equation for rotting (%). This suggested that quadratic term of potassium sorbate is more related to rotting than linear term of potassium sorbate. However, interactive terms ( $x_1x_2$ ) of regression equation for PL<sub>W</sub> (%) is more significant at 0.01 level, than interactive terms of regression equation for rotting (%), which is significant at 0.05 level. Regression model obtained from CCRD was found to be highly significant ( $P < 0.0001$ ) for PL<sub>W</sub> and rotting with coefficient of determination,  $R^2$  of 0.978 and 0.980, respectively. The large F-values (PL<sub>W</sub> percent 63.11, rotting percent 71.14) and small  $P$ -value ( $P < 0.01$ ) suggested that the models were statistically significant at 0.01 level

(Myers et al. 2002). F-values and P-values enlisted in Table 2, indicates the significance of each regression coefficient. Predicted  $R^2$  of 0.90 and 0.88 is in reasonable agreement with adjusted  $R^2$  of 0.96 and 0.97 for PLW and rotting percent models, respectively (Joglekar and May, 1987). Coefficient of variation percent (CV) for PL<sub>W</sub> percent (2.61), and rotting percent (7.11) was found to be less than 10%, which indicated better precision and reliability of the experiments conducted. The insignificant lack of fit value for the quadratic model ( $P= 0.3607$  and  $P= 0.1022$  for PL<sub>W</sub> and rotting percent, respectively) suggested statistical acceptability of the regression model for screening experimental design to predict both the responses.

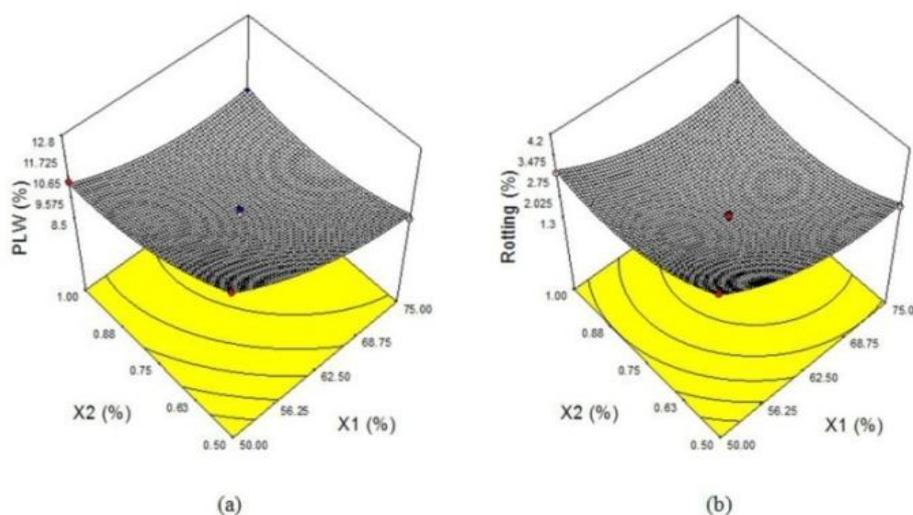
**Table 2:** Significance level of regression coefficient for response variables.

Source	PLW (%)				Rotting (%)			
	Regression coefficient	S.E.	F-value	P-value	Regression coefficient	S.E.	F-value	P-value
$\beta_0$	+9.05*	0.12	63.11	<0.0001	+1.55*	0.07	71.14	<0.0001
Linear								
$\beta_1$	-1.14*	0.09	157.72	<0.0001	-0.67*	0.06	126.71	<0.0001
$\beta_2$	-0.66*	0.09	52.17	0.0002	-0.34*	0.06	32.73	0.0007
Quadratic								
$\beta_{11}$	+0.66*	0.09	46.18	0.0003	+0.62*	0.06	94.90	<0.0001
$\beta_{22}$	+0.71*	0.09	53.02	0.0002	+0.70*	0.06	119.20	<0.0001
Interaction								
$\beta_{12}$	+0.55*	0.13	17.88	0.0039	+0.22*	0.08	6.69	0.0361**
Lack of Fit			1.42	0.3607NS			4.13	0.1022NS
R <sup>2</sup>	0.978				0.980			
Adj. R <sup>2</sup>	0.963				0.967			
Pred. R <sup>2</sup>	0.904				0.889			
C.V. %	2.61				7.11			

Subscripts: 1=Ethanol; 2=Potassium sorbate; Adj= adjusted; Pred= predicted; C.V= coefficient of variation; S.E= standard error; \*significant at 0.01 level. \*\*significant at 0.05 level. <sup>NS</sup>=non-significant.

### 3.2 Effect of chemicals on PL<sub>W</sub> and rotting percent at the end of storage study

The three dimensional response curve plots were generated using the regression equations to assess the effect of ethanol and potassium sorbate on the PL<sub>W</sub> and rotting percent. The significance of each variables and its interaction were assessed by evaluating three-dimensional response plots described in Figure 1.

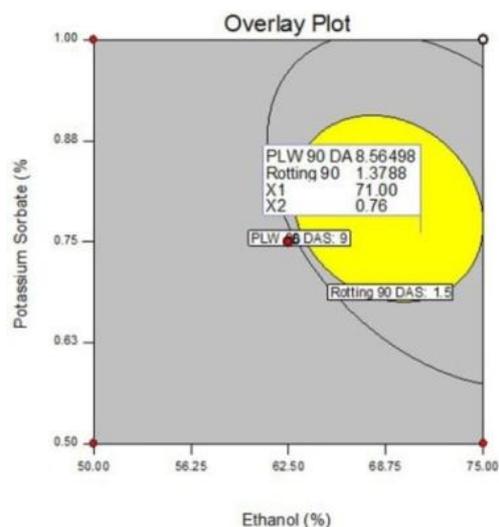


**Figure 1:** Surface plots showing variation in (a) PL<sub>W</sub> and (b) Rotting with ethanol and potassium sorbate.

### 3.3 Optimization

For obtaining optimum combination of ethanol and potassium sorbate in reducing PL<sub>W</sub> and rotting during storage, multiple response optimizations applying desirability approach in response surface methodology was done. According to the objective of the experiment, criteria for responses (PL<sub>W</sub> and rotting percent) were minimized and factors were kept in range i.e. 50-75% for ethanol and 0.5-1.0% for potassium sorbate. As reported by Myers et al (2002) each response ( $Y_i$ ) was converted into an individual desirability function ( $d_i$ ) that varied over a range of  $0 \leq d_i \leq 1$ , and nature of responses were studied by using value of the desirability functions.

With these conditions set, 30 different solutions for numerical optimization were generated. Out of those, solution (71.08% ethanol; 0.76% potassium sorbate) with predicted score of 8.57% for PL<sub>W</sub> and 1.38% for rotting was chosen based on above considerations and small variation between different solutions obtained. Therefore, the optimum combination of ethanol and potassium sorbate to reduce PL<sub>W</sub> and rotting percent during storage was identified as ethanol (71.08 percent, v/v) and potassium sorbate (0.76 percent, w/v) having desirability index of 1.00, which is considered as satisfactorily good value. Hence, the treatment combination has been selected for further validation of the optimized combination obtained by the RSM. An overlay plot in Figure 2 was obtained from graphical optimization, showed the optimum concentrations of ethanol and potassium sorbate as 71% ethanol and 0.76% potassium sorbate with predicted value of responses as 8.57% and 1.38% for PL<sub>W</sub> and rotting percentage, respectively. Hence, the predicted values of responses obtained from numerical and graphical optimization were closely situated.



**Figure 2:** Overlay plot showing optimum region for responses of two treatments.

#### 4. Model Verification

Model verification was done with additional three sets of experiments with obtained numerically optimized combination from CCRD/RSM model to obtain the actual values of responses (PL<sub>W</sub> and rotting percent). The actual values of responses obtained after storage of 90 days in dark, at ambient room temperature is presented in Table 3. The predicted value suggested by the RSM model was 8.57 % for PL<sub>W</sub> and 1.38 % for rotting. Two-tailed t-test (assuming unequal variances) statistics ( $P < 0.05$ ) was performed to find the difference between means of experimental and predicted values of PL<sub>W</sub> and rotting percent values. From t-test it was found that means of experimental value and predicted value of responses (PL<sub>W</sub> and rotting percentage) were not significantly different from each other. Hence, the results of validation parameters were satisfactory.

**Table 3:** Experimental values of responses 90 DAS of onion bulbs treated with optimized combination of ethanol and potassium sorbate.

Independent Trials	a Experimental Values of PL <sub>W</sub> (%) 90 DAS	b Experimental Values of Rotting (%) 90 DAS
Trial 1	8.25 ± 0.11	1.42 ± 0.01
Trial 2	8.68 ± 0.15	1.40 ± 0.05
Trial 3	9.15 ± 0.08	1.39 ± 0.02

<sup>a, b</sup> values expressed as mean ± S.E., significant at 0.05 level.

## 5. Conclusion

In this study, response surface methodology has been used to minimize two major postharvest losses of onion bulbs during storage ( $PL_w$  and rotting percent) as a function of pre-storage ethanol and potassium sorbate treatment. The optimum combination of ethanol and potassium sorbate was found to be 71.08 % (v/v) and 0.76 % (w/v), respectively. Under this optimum combination, the predicted and actual values of  $PL_w$  and rotting percent were found to be in close agreement and also were not significantly different from each other.

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