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Optimization of button mushroom browning inhibition using response surface methodology

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ABSTRACT

Post-harvest browning of fresh button mushroom (*Agaricus bisporus*), was inhibited by conducting a trial using three independent process variables *viz*. citric acid (0.1, 1.0 and 1.5%), *aloe vera* gel (50, 60 and 70%) and drying time (6, 7 and 8 h). Drying of harvested mushroom was done in a cabinet dryer at 60 ± 3ºC. Response surface methodology was adopted for experimental design and optimization of process variables. Five minutes dip of mushroom in 60% aloe vera gel along with 1% citric acid and its subsequent drying at 60°C for 7 hours was found best in retaining the desirable traits *viz*. color, pH (6.52), TSS (6.38ºBrix), ascorbic acid (6.92 mg/100g) and moisture content (12.16%). Results showed that the data were adequately fitted into second-order polynomial models developed by response surface methodology.

Keywords: *Agaricus bisporus*, dehydration, citric acid, *aloe vera* gel, drying time.

INTRODUCTION

Mushrooms are extremely perishable in nature and may not be kept for more than one day after harvesting at ambient conditions. Various physiological and morphological changes occur after harvest, which make these mushrooms unacceptable for consumption. Among different varieties, the button mushroom (*Agaricus bisporus*) is the most widely cultivated and consumed mushroom in the world and it contributes around 40% of the total world production of mushroom. Mushrooms are liked for their delicious flavor, low calorific value, high protein contents (20-40%), vitamins and minerals (Walde *et al.*, 5). In view of the highly perishable nature, the fresh mushroom has to be processed to extend their shelf life for off-season use.

Browning phenomenon which occurs during drying is ascribed to the activity of phenolic compounds, oxygen and poly phenol oxidase (PPO), which reduces the sensory quality and commercial value of dried mushrooms (Kotwaliwale *et al.,* 3; Kumar *et al.,* 4). Though application of advanced drying methods, e.g. microwave drying and freeze-drying, can alleviate this problem, it needs expensive equipments and high production cost. Application of color protecting agents is a practical way to inhibit browning in mushrooms while drying. Reducing agents such as EDTA, cysteine and ascorbic acid inhibit the activity of PPO and browning. Dehydrated mushrooms are valuable ingredients in a variety of food formulations such as instant soups, sauces, snacks, pizzas, meat and rice dishes. More specifically, the duration and drying temperature are the important factors affecting the properties such as colour, texture, density, porosity and sorption characteristics of the dehydrated materials. Thus, the aim of this study is to investigate the inhibitory effects of reducing agents on the activity of PPO and browning in mushroom during hot air drying.

MATERIALS AND METHODS

Fresh mushrooms were obtained from the semi-permanent poly house structure developed under AICRP (PET) scheme and stored at 4±1°C until used. Harvested mushrooms were sorted and washed with tap water to remove the impurities. Further slices of 2 mm thickness were prepared using a sharp knife. To inhibit the browning, various pre-treatments were tried out which includes, seven levels of glutamic acid (0.25, 0.5, 0.70, 0.75, 1, 1.25 and 1.5%), five levels of sodium chloride (0.5, 1, 1.5, 2 and 2.5%), five levels of benzoic acid (0.25, 0.50 and 0.75), ten levels of citric acid (0.25, 0.50, 0.75, 1, 1.25, 1.50, 1.75 , 2 and 2.5%), two levels of ascorbic acid (0.5 and 1%) and three level of aloe vera jel (25, 50 and 75%). The slices were dipped in different treatments for 2-4 minutes while stirring the solution thoroughly. Then, the slices were removed and spread on blotting paper for five minutes for removal of surface moisture. The slices were further subjected for drying in a cabinet dryer at 60±3°C. After the desired moisture content of 10-12% weight basis was attained, the dried samples

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were removed and packed in suitable packaging material and stored till further use. Moisture content of mushroom was calculated by standard oven method (AOAC, 2). Colour of dried mushroom slices was measured using handy colorimeter NR 3000 (M/S Nippon deshkhon, Japan). The degree of browning was expressed by the changes in the lightness (L) value. Titratable acidity was determined by the method suggested by AOAC, 1. Ascorbic acid content was determined against 2, 6 dichlorophenol-indophenol dye solution (AOAC, 1).

Box-behnken design was adopted using three process variables and three levels which resulted in 17 experimental run including five central point experiments. Lack-of-fit test of each model was calculated. R^2 values, standard error (SE) estimate, significance of F-test and the derived *p* values were the criteria used for eliminating a variable from the full regression equation.

Preliminary trials suggested that treatment with aloe vera along with citric acid gave promising result. Therefore, for conducting further trials, three concentrations each of aloe vera and citric acid along with three levels of drying time were selected. The coded and actual levels are shown in Table 1.

Table 1. Coded and actual level of process variables.

Factor	Coded and actual level		
Aloe vera (%), X_1	50 (-1)	60 (0)	70 (1)
Citric acid (%), X_2	0.50 (-1)	1(0)	1.50 (1)
Drying time (h), X_3	6 (-1)	7 (0)	8 (1)

RESULTS AND DISCUSSION

A second order polynomial equation was fitted with the experimental data. Estimated regression coefficients of the second order polynomial models for all the responses and their statistical validity defining values are reported in Table 2. Lower values of coefficient of determination (R^2) for TSS, TA and pH suggested no significant effect of process variables and hence were excluded for regression analysis. Quadratic model was fitted to the experimental data and statistical significance for linear, quadratic and interaction terms was calculated for all the responses. The multiple regression analysis resulted in the following prediction equation pertaining to L value: $L = 81.27 + 6.35 X_1 + 0.68 X_2 - 0.72 - 1.66 X_1X_2 - 0.057 X_1X_3 + 1.03 X_2X_3 - 3.94 X_1^2 + 1.56 X_2^2 + 2.95 X_3^2$

The R^2 value was 0.89 showing good fit of model to the data. The model F value of 6.23 implies that the model is significant (*P< 0.01*). The linear terms (X_1, X_2 and X_3) are significant (*P<0.01*). The 'lack of fit' was not significant which indicates that the developed model was adequate for predicting the response. Moreover the predicted coefficient of determination (R^2= 0.75) was in reasonable agreement with adjusted R^2 of 0.74. Therefore, this model could be used to navigate the design space.

The 3D response (Fig. 1) was generated for the fitted model to visualize the combined effect of two variables on the whiteness, while keeping third variable at its central value. The lightness increased

Table 2. ANOVA and regression coefficients of the second order polynomial models of the various responses.

Response	*L*	*a*	*b*	Moisture (%)	pH	Ascorbic acid (mg/100 g)	Protein (%)
Intercept	81.276	45.69	36.04	12.89	5.95	6.62	160.71
X_1	-6.35***	5.54**	-0.44	-0.10	-0.10	0.45***	-2.67
X_2	0.68	-1.60	-0.24	0.08	0.38***	-0.0025	0.62
X_3	-0.72	1.8	4.96***	-0.91***	0.07	-0.17***	-14.52***
X_1* X_2	-1.66	4.55	-2.37**	-0.097	0.025	0.01	-2.56
X_1* X_3	-0.057	0.082	2.42**	0.23**	0.05	0.11*	0.0025
X_2* X_3	1.03	-3.23	-0.79	0.002	0.13	-0.085	14.89***
X_1^2	-3.94**	-2.11	2.88**	-0.026	-0.05	-0.99***	-3.14
X_2^2	1.56	-8.91***	0.63	-0.14	0.19	0.11*	-2.009
X_3^2	2.95*	-6.92**	1.33	0.43***	0.005	0.21***	-3.37
R^2	0.89	0.86	0.93	0.97	0.75	0.98	0.86
Model F value	6.23	4.67	11.31	25.82	2.39	49.35	4.78
Lack of fit (p value)	0.0002	0.5714	0.2834	0.1930	0.4613	0.2903	0.0683
CV%	3.49	13.22	4.44	1.42	4.44	1.88	5.13

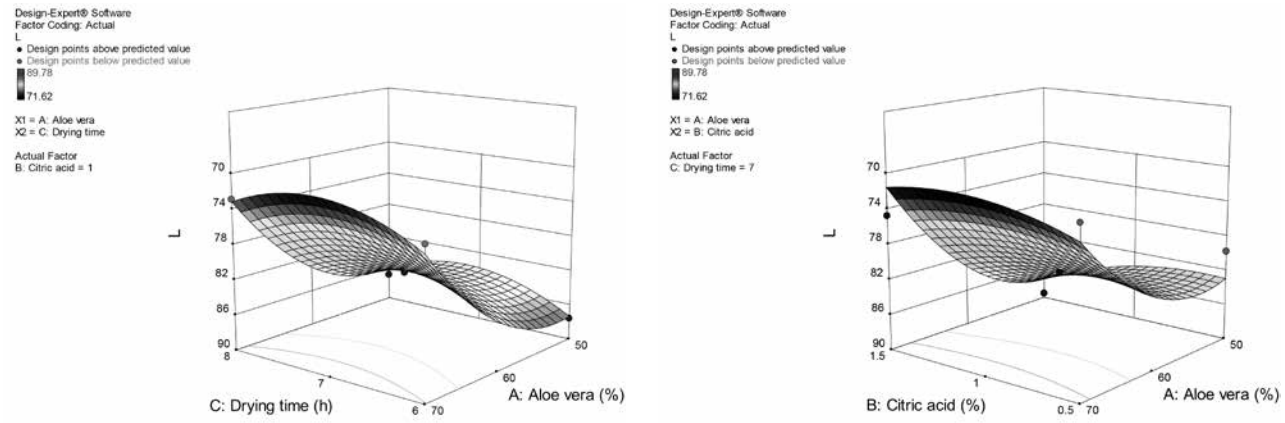

Fig. 1. Response surface plots showing the effect of process variables on *L* value of button mushroom.

positively with increase in citric acid and aloe vera concentration, whereas lower values of drying time reduced the degree of browning.

High value of coefficient of determination (R^2 = 0.88) obtained for response variable indicated that the developed model for color accounted for and adequately explained 88.9 per cent of the total variation. ANOVA indicated that the linear terms of aloe vera, citric acid and drying hours were highly significant at 5 per cent level (Table 3). The quadratic terms of aloe vera and drying duration were also significant at 5 per cent level. The comparative effect of each factor on lightness was observed by the F values in the ANOVA and also by the magnitudes of coefficients of the coded variables. The F values indicated that concentration of citric acid was the most influencing factor followed by duration of drying and aloe vera was least effective over whiteness loss.

High value of coefficient of determination (R^2=0.97) obtained for moisture content indicated that the developed model and adequately explained 97.08 per cent of the total variation. Linear terms of aloe vera, citric acid and drying time were highly

Table 3. Analysis of variance for colour value (L) during mushroom browning inhibition.

Source	Sum of squares	df	Mean sum of squares	F- value	P value (Prob > F)
Model	452.88	9	50.32*	6.23*	0.0124
X_1	322.71	1	322.71	39.94	0.0004
X_2	3.75	1	3.75	0.46	0.5174
X_3	4.22	1	4.22	0.52	0.4933
$X_1 X_2$	11.02	1	11.02	1.36	0.2810
$X_1 X_3$	0.013	1	0.013	1.637E-003	0.9689
$X_2 X_3$	4.24	1	4.24	0.53	0.4921
X_1^2	65.42	1	65.42	8.10	0.0248
X_2^2	10.32	1	10.32	1.28	0.2956
X_3^2	36.72	1	36.72	4.55	0.0705
Residual	56.56	7	8.08		
Lack of Fit	55.97	3	18.66	NS	
Pure Error	0.59	4	0.15		
Cor Total	509.44	16			
R^2	0.8890				
Adj R^2	0.7462				
Pred R^2	-0.7596				
CV%	3.49				

*Significant at 5% Level, **Significant at 1% Level and NS: Non-significant.

significant at 5% level of significance (Table 4). The quadratic terms of aloe vera and drying duration were also highly significant at 5 per cent level. The model F value of 25.82 implies that the model is significant ($P < 0.01$). The linear terms (X_1, X_2 and X_3) are significant ($P < 0.01$). The "lack of fit F value" was not significant which indicates that the developed model was adequate for predicting the response. The comparative effect of each factor on moisture loss was observed by the F values in the ANOVA (Table 4) and also by the magnitude of coefficients of the coded variables. The F values indicated that drying hour was the most influencing factor over moisture loss. The effect process variables on moisture content is graphically presented in Fig. 2.

Numerical optimization of the experimental data was done using design expert software by assigning the optimization targets to variables and responses (Table 5). Optimum values of process variables for adequate browning inhibition of mushroom was, aloe vera (60%), citric acid (1.5%) and drying time

Table 4. Analysis of variance for moisture content during mushroom browning inhibition.

Source	Sum of squares	df	Mean sum of squares	F- value	P value (Prob > F)
Model	7.96	9	0.88	25.82*	0.0001
X_1	0.085	1	0.085	2.47*	0.1599
X_2	0.062	1	0.062	1.82*	0.2195
X_3	6.70	1	6.70	195.45	< 0.0001
$X_1 X_2$	0.038	1	0.038	1.10*	0.3294
$X_1 X_3$	0.22	1	0.22	6.46*	0.0386
$X_2 X_3$	1.600E-005	1	1.600E-005	4.670E-004	0.9834
X_1^2	2.940E-003	1	2.940E-003	0.086*	0.7781
X_2^2	0.09	1	0.086	2.52*	0.1565
X_3^2	0.80	1	0.80	23.32*	0.0019
Residual	0.24	7	0.034		
Lack of Fit	0.16	3	0.053	NS	
Pure Error	0.08	4	0.021		
Cor Total	8.20	16			
R^2	0.9708				
Adj R^2	0.9332				
Pred R^2	0.6768				
CV %	1.42				

*Significant at 5% Level, **Significant at 1% Level and NS: Non-significant

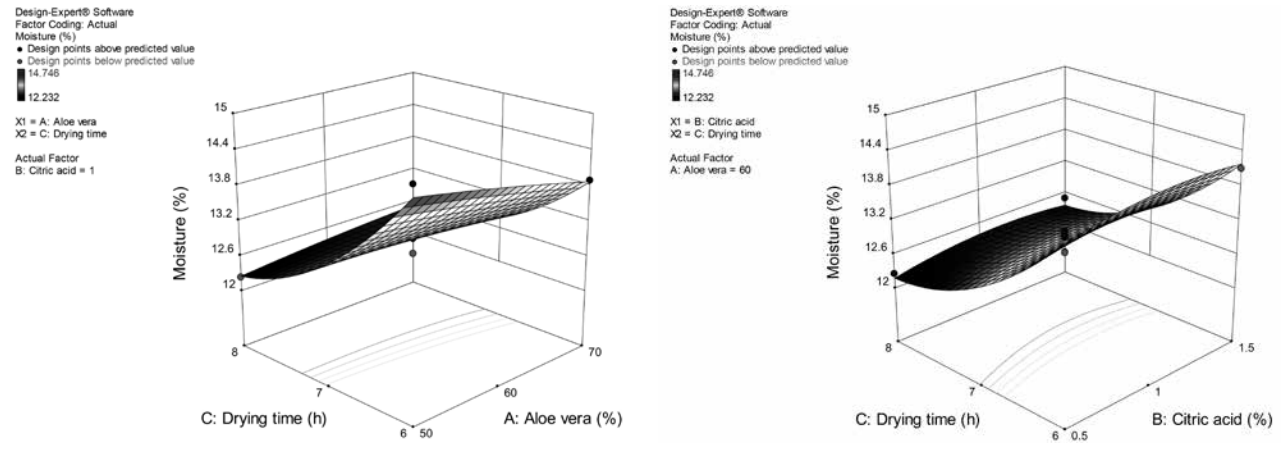

Fig. 2. Response surface plots showing the effect of process variables on moisture content of button mushroom.

Table 5. Range of process variables and responses for optimization of mushroom browning inhibition

Parameter	Goal	Lower limit	Upper limit	Importance
Aloe vera	Is in range	-	-	-
Citric acid	Is in range	-	-	-
Drying time	Is in range	-	-	-
Color *L*	Maximum	78.24	86.47	1
Color *a*	Minimum	36.65	45.34	4
Color *b*	Minimum	34.24	37.84	5
Moisture	Minimum	12.69	13.09	2
Ascorbic acid	Maximum	5.67	6.23	3

of 7 hour. This treatment was useful for inhibit the browning in mushroom during drying effectively. Model equation for the response variables predicted values under the identified optimum conditions, which were experimentally verified to be in general agreement in the model.

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