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Optimization of osmotic dehydration process for Oyster mushrooms (*Pleurotus sajor-caju*) in sodium chloride solution using RSM

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Abstract: Sodium chloride (NaCl) and water transfer were quantitatively investigated during osmotic dehydration of Oyster mushrooms (*Pleurotus sajor-caju*) using response surface methodology with the NaCl concentration (10–20%, w/v), solution temperature (30–60° C) immersio n time (15–240 min) and solution to fruit ratio (4:1 to 8:1) were taken as independent process variables. Experiments were conducted in a thermostatically controlled agitating incubator. For each response, second order polynomial models were developed using multiple linear regression analysis. Analysis of variance (ANOVA) was performed to check the adequacy and accuracy of the fitted models. The response surfaces and contour maps showing the interaction of process variables were constructed. Applying desirability function method, the optimum operating conditions were found to be: solution temperature – 45° C, immersion time – 53.54 min, salt concentration – 14.09% and solution to fruit ratio 6.08:1. Corresponding to these optimum values water loss, solute gain and weight reduction were 38.13, 2.1 and 36.02 (g/100 g initial mass) respectively.

Keywords: Optimization, Osmotic dehydration, Oyster mushrooms, Response surface methodology

INTRODUCTION

Mushrooms have captivated human beings since ancient times. They are a product of transformation inedible waste into edible biomass, and are generally being accepted as food of high quality. Mushrooms are essentially saprophytes (plants without chlorophyll) which thrive by extracting nutrients from the dead and decaying plant and animal matters. They vary greatly in their colour, texture, shape and properties, bearing different names for different species. Mushrooms have been collected and consumed by people since centuries (Anonymous, 2012). Mushroom production and consumption have grown drastically in last two decades. Total commercial mushroom production worldwide has increased more than 21 times in 35 years from 3,00,000 tonnes in 1965 to about 7.5 million tonnes in 2000. Mushroom cultivation has great scope in China, India and in some of other developing countries (Bao, 2004; Royse, 2001). China has the lion share in the world in mushroom production (50,08,850 metric tonnes in 2011 and 26,69,841 tonnes in 2001) and is followed by United States and Canada (FAOSTAT, 2012).

Mushrooms have been appraised as sources of dietary nutrients and pharmacologically vital compounds useful in medicine since times immemorial. They are considered to be a source of many different nutraceuticals such as unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid and carote-noids. Thus, they are used directly in diet to promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present (Pereira *et al*., 2012; Vaz *et al*., 2010). *Pleurotus spp*. are commonly known as oyster fungus and are the world's third largest commercially important mushroom species produced and appreciated for their delicious taste, high vitamin, protein, carbohydrate, mineral but low fat content. They are known to degrade large insoluble components of lignocellulosic materials and hence play a significant role in their bioconversion to foods and dietary supplements (Bisaria *et al.,* 1987). Mushrooms are the most priced commodity among vegetables, not because of its nutritive value alone but also for its characteristic aroma and flavour which gives 'Umami' taste. Mushrooms even after harvesting continues to respire, mature and senesce resulting in weight loss, veil opening, browning, wilting and finally leads to the spoilage. So, soon after the harvest, fresh mushrooms need to be properly processed to retard post-harvest deterioration till its consumption. Therefore, osmotic dehydration being promising technology for preservation of vegetables and fruits can be used for post-harvest processing of mushrooms to enhance the mushroom product shelf-life (Zhang *et al.,* 2013).

Osmotic dehydration is one of the energy efficient means of dewatering process that entails the partial removal of water from food items. It works by soaking food in a higher osmotic pressure solution/hypertonic/ concentrated solution such as salts, alcohols, starch solutions and concentrated sugars (Anonymous, 2011).

Osmotic dehydration induces significant changes in the final dehydrated product such as volume reduction, membrane alteration and membrane separation from the cell wall. It also improves nutritional, sensorial and functional properties of food without changing its integrity (Torrengiani, 1993). Limited efforts have so far been made to process *P. sajor-caju* mushroom into dehydrated product. No attempt has been made to optimize the osmotic process parameters for osmo-cum -microwave dehydrated product of *P. sajor-caju* mushroom. The goal of the present study was to determine the effect of osmotic process parameters viz.solution temperature, salt concentration, duration of osmosis and solution to fruit ratio on water loss, solute gain and weight reduction and to optimize these parameters for developing higher quality finished dehydrated mushroom product.

MATERIALS AND METHODS

Experimental design and statistical analysis: The Response surface methodology (RSM) was applied to the experimental data using a commercial statistical package, Design-Expert trail version 8.0.7.1 (Statease Inc., Minneapolis, USA). RSM is an empirical statistical modeling technique employed for multiple regression analysis using quantitative data obtained from properly designed experiments to solve multivariate equations simultaneously (Prakash Maran *et al.*, 2013). Box-Behnken Design was used to design the experiments. The process parameters (independent variables) selected for the optimization were immersion time (β_1), osmotic solution concentration (β_2), osmotic solution temperature (β_3) and solution to fruit ratio (β_4). The range of each independent variable was; $\beta_{1:}$ 15-240min, $\beta_{2:}$ 10-20%, $\beta_{3:}$ 30-60° C and $\beta_{4:}$ 4:1-8:1. The effects of the variables were studied on water loss (WL), solute gain (SG) and weight reduction (WR) of the slices during osmotic process. The variables were standardized for ease in computation and to reduce their relative effect on the responses. The number of experiments (N) required for the development of Box-Behnken Design is defined as $N = 2k(k-1) + C_o$ (where k is number of factors and C_o is the number of central point). The design included 29 experiments with 5 central points. The following polynomial model was fitted to the data:

$$\mathbf{Y} = b_0 + b_1\beta_1 + b_2\beta_2 + b_3\beta_3 + b_4\beta_4 + b_{12}\beta_{12} + b_{13}\beta_{13} + b_{14}\beta_{14} + b_{23}\beta_{23} + b_{24}\beta_{24} + b_{34}\beta_{34} + b_{11}\beta_1^2 + b_{22}\beta_2^2 + b_{33}\beta_3^2 + b_{44}\beta_4^2 \quad \mathbf{(1)}$$

Where, b_n are constant regression coefficients; Y is the response (i.e. WL, SG and WR %); β_1, β_2, β_3 and β_4 are immersion time, salt concentration, temperature and STFR respectively. Statistical significance of the terms in the regression equations was examined. Response surface plots were generated with the same software.

Raw materials: Fully matured oyster mushrooms (*P. sajor-caju*) of commercial grade were procured from Mushroom Research Farm, Punjab Agricultural University, Ludhiana, India. The average moisture content of the mushrooms was found to be 90.3% on a wet basis initially. The mushrooms were cut into slices and were pre-treated using anti-microbial agent (Citric acid @ 40g/l) to avoid enzymatic browning (Brennan and Gormely, 2000). The commercial TATA salt was purchased from a local supermarket and was considered as an osmotic agent for being cheap and easily available.

Experimental procedure: The osmotic dehydration was conducted in 250 ml glass beakers, which was placed in a thermostatically controlled shaking incubator. For each experiment, known weights of mushroom (10 g) was taken in a glass beaker containing calculated volumes of osmotic solution (STFR) of different concentrations and were placed inside a temperature and agitation controlled incubator. At each sampling time 15–240 min, the mushroom slices were taken out and then gently blotted with soft adsorbent paper and weighed and the effect of temperature was investigated. In each of the experiments fresh osmotic solution was used. All the experiments were done in duplicates and the average value was taken for calculations. Agitation was necessary to improve the mass transfer, to maintain uniform concentration, temperature profile and to prevent the formation of a dilute solution film around the samples. For each experiment a constant agitation speed of 150 rpm was maintained. Analysis for each sample was carried out; from which WL, SG and WR data were obtained. Net loss of water and solute gain after osmotic dehydration was calculated using the relationship:

$$\mathbf{WL} = WR + SG \quad \mathbf{(2)}$$

$$\mathbf{SG} = (m - m_0)/M_0 \quad \mathbf{(3)}$$

$$\mathbf{WR} = (M_0 - M)/M_0 \quad \mathbf{(4)}$$

Where, M_0 - initial mass of sample (g), M - mass of sample after dehydration (g), m_0 - initial mass of the solids in sample (g), m - mass of the solids in sample after dehydration (g).

RESULTS AND DISCUSSION

RSM modeling and effect of process variables on responses: The experiments have found out the optimum combination of process parameters and their effects on WL, SG and WR for the osmotic dehydration of mushrooms as per Box-Behnken Design.

A model 'F-value' denotes 'F-statistic' and is the ratio of treatment mean sum of squares to the error mean sum of squares. Higher F-value represents the significance of the model. Lesser the value of F-value represents least significant/non-significant or more error in the model. F-value and p-value are inter-related.

In present study, from values of variables and sources of the Table 1, it can be noticed that as F-value increases p-value decreases, hence represents more significance. A Model F-value of 9.845, 29.708 and 7.65 for WL, SG and WR respectively implies that the

Table 1. ANOVA for water loss during osmotic dehydration of Oyster mushroom.

Source	R^2	Water loss		
		Sum of squares	F-val.	p-val.
Constant	38.667	381.826	9.845	< 0.0001
β1-Immersion time	1.724	35.677	12.879	0.003
β2- Salt	0.800	7.681	2.773	0.118
β3-Temp.	4.803	276.779	99.915	< 0.0001
β4-STFR	1.433	24.653	8.899	0.010
β12	0.448	0.802	0.290	0.599
β13	1.157	5.358	1.934	0.186
β14	0.608	1.480	0.534	0.477
β23	-0.471	0.888	0.321	0.580
β24	-0.452	0.817	0.295	0.596
β34	0.133	0.071	0.025	0.875
β12	-1.189	9.175	3.312	0.090
β22	-1.139	8.416	3.038	0.103
β32	-1.623	17.090	6.169	0.026
β42	-1.074	7.486	2.703	0.122
Lack of Fit		5.785		
R^2		0.908		
Adj. R^2		0.816		
Pred. R^2		0.798		
CV (%)		4.549		
Std.Dev		1.664		
Adeq Precision		11.452		

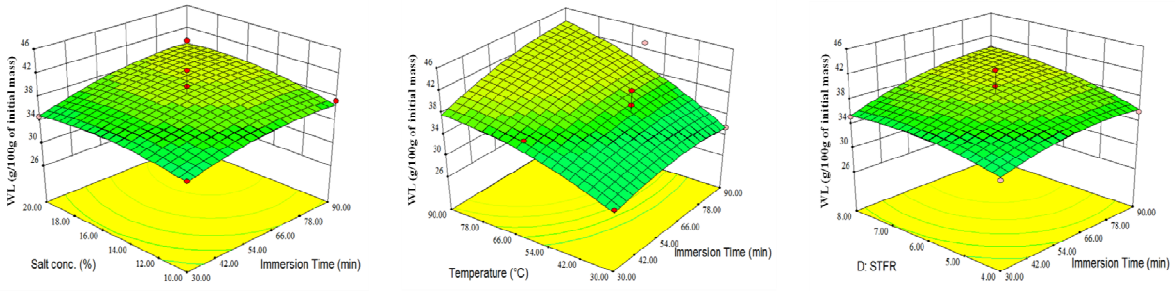
Table 2. ANOVA for solute gain during osmotic dehydration of Oyster mushroom.

Source	R^2	Solute gain		
		Sum of squares	F-val.	p-val.
Constant	2.237	15.720	29.708	< 0.0001
β1-Immersion time	0.467	2.613	69.131	< 0.0001
β2- Salt	0.216	0.558	14.770	0.002
β3-Temp.	0.870	9.073	240.051	< 0.0001
β4-STFR	0.284	0.966	25.555	0.0001
β12	0.018	0.001	0.033	0.858
β13	0.163	0.106	2.809	0.116
β14	-0.063	0.016	0.419	0.528
β23	-0.081	0.027	0.702	0.416
β24	0.098	0.039	1.020	0.330
β34	-0.019	0.001	0.038	0.848
β12	-0.035	0.008	0.208	0.656
β22	-0.067	0.029	0.765	0.396
β32	-0.577	2.156	57.041	< 0.0001
β42	0.038	0.009	0.247	0.627
Lack of Fit		0.404		
R^2		0.967		
Adj. R^2		0.935		
Pred. R^2		0.845		
CV (%)		9.860		
Std.Dev		0.194		
Adeq Precision		19.294		

model is significant ($P < 0.01$). The 'lack of Fit F-value' of 5.785, 0.404 and 3.903 for WL, SG and WR were not significant which indicates that the model was adequate for predicting the response. Moreover, the predicted R^2 values for WL, SG and WR of 0.798, 0.845 and 0.762 were in reasonable agreement with adjusted R^2 of 0.816, 0.935 and 0.769. The independent process parameters β_1, β_2, β_3 and β_4 were optimized for maximum water loss and minimum solute gain. An analysis of variance was conducted to determine the significant effects of process variables on each response. Tables 1, 2 and 3 showed that all the process variables were found to be statistically significant for WL, SG and WR at ($p < 0.05$). Coefficient of determination R^2 and adj-R^2 were calculated to check the adequacy and fitness of the model. The values of R^2 were calculated to be 0.908, 0.967 and 0.884 for WL, SG and WR respectively which signified the compatibility of the experimental data. The R^2 value was always between 0 and 1, and a value > 0.75 indicated aptness of the model. For a good statistical model, R^2 value should be close to 1.0. The adjusted R^2 value corrected the R^2 value for the sample size and for the number of terms in the model. These higher values of R^2 also signified the high significance of the model. If there are many terms in the model and the sample size is not very large, the adjusted R^2 may be noticeably smaller than the R^2. Here in this case also it can be noticed adjusted R^2 value were lesser than the R^2. The closer values of R^2 and adj. R^2 obtained in the study explicated that the predicted values are in good agreement with the experimental values. Higher values of coefficient of determination obtained for response variables indicated that the developed model for WL, SG and WR accounted for and adequately explained 79.8, 84.5 and 76.2 % of the total variation.

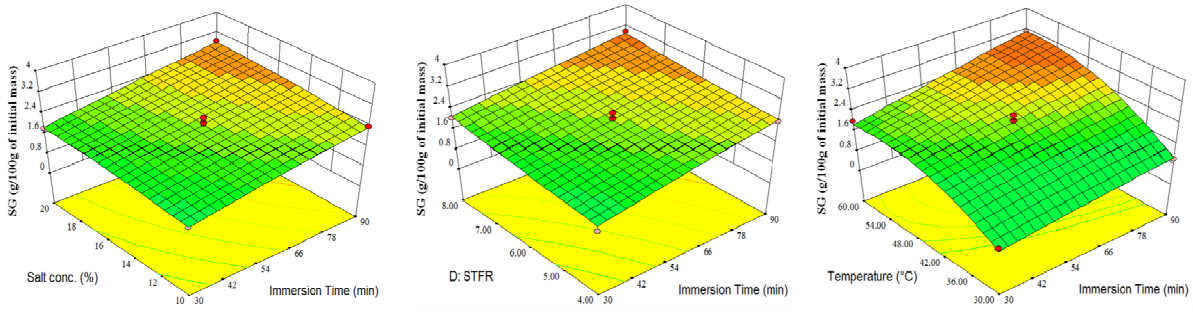
The coefficient of variation (CV %) indicated the relative dispersion of the experimental points from the predictions of the second-order polynomial models (Prakash Maran *et al.*, 2013). The values of CV are low as 4.549, 9.860 and 4.440 for WL, SG and WR, which indicated that the deviations between experimental and predicted values are low. The values of Adeq Precision are 11.452, 19.294 and 10.019 for WL, SG and WR. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In this work the ratio is found to be >10, which indicates an adequate signal. The comparative effect of each factor on WL, SG and WR were observed by the F-values in the ANOVA (Tables 1-3) and also by the magnitudes of coefficients of the coded variables. The F-values indicated that solution temperature and immersion time were the most influencing factors followed by STFR and salt concentration was least effective over WL, SG and WR.

The effect of β_1, β_2, β_3 and β_4 on the WL is given in fig.1. The WL increased rapidly in the early stages of



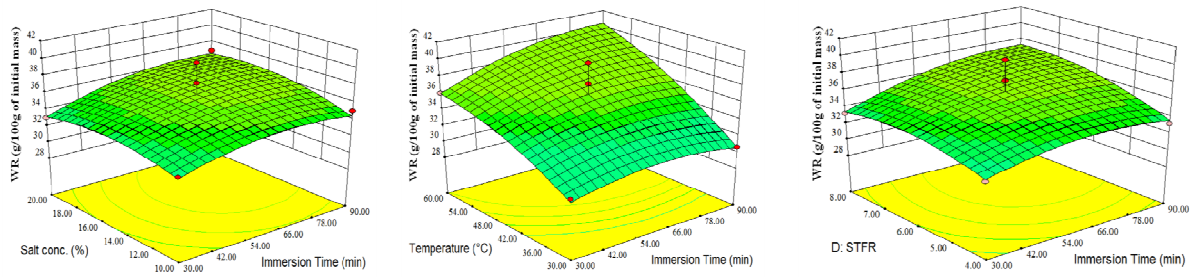
(a) **(b)** **(c)**

Fig. 1. *Water loss during osmotic dehydration of mushroom as a function of : (a) salt concentration and immersion time (b) solution temperature and immersion time (c) STFR and immersion time.*



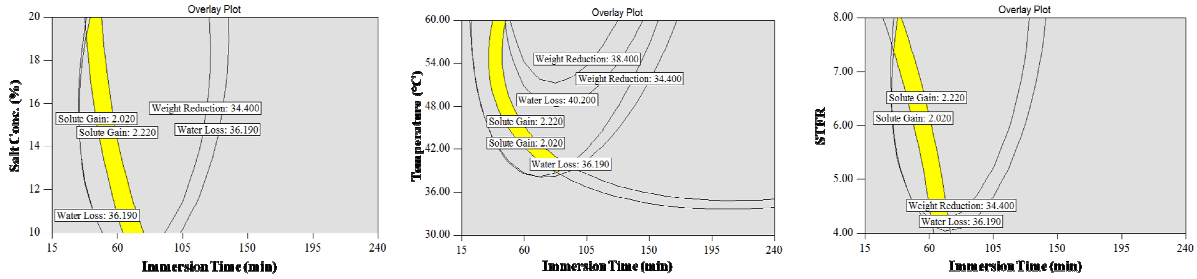
(a) **(b)** **(c)**

Fig. 2. *Solute Gain during osmotic dehydration of mushroom as a function of : (a) salt concentration and immersion time (b) solution temperature and immersion time (c) STFR and immersion time.*



(a) **(b)** **(c)**

Fig. 3. *Weight reduction (WR) during osmotic dehydration of mushroom as a function of : (a) salt concentration and immersion time (b) solution temperature and immersion time (c) STFR and immersion time.*



(a) **(b)** **(c)**

Fig. 4. *Superimposed contour plots of different responses for optimization of osmotic dehydration of mushroom as function of (a) salt concentration and immersion time (b) solution temperature and immersion time (c) STFR and immersion time.*

Table 3. ANOVA for weight reduction during osmotic dehydration of Oyster mushroom.

Source	R^2	Weight reduction		
		Sum of squares	F-val.	p-val.
Constant	36.430	252.925	7.650	< 0.0003
β1-Immersion time	1.258	18.980	8.037	0.013
β2- Salt	0.584	4.098	1.735	0.209
β3-Temp.	3.933	185.628	78.601	< 0.0001
β4-STFR	1.150	15.859	6.715	0.021
β12	0.430	0.740	0.313	0.584
β13	0.994	3.956	1.675	0.217
β14	0.671	1.803	0.763	0.397
β23	-0.390	0.608	0.257	0.620
β24	-0.550	1.210	0.512	0.486
β34	0.152	0.092	0.039	0.846
β12	-1.155	8.646	3.661	0.076
β22	-1.072	7.459	3.158	0.097
β32	-1.047	7.106	3.009	0.105
β42	-1.112	8.025	3.398	0.087
Lack of Fit		3.903		
R^2		0.884		
Adj. R^2		0.769		
Pred. R^2		0.762		
CV (%)		4.440		
Std.Dev		1.537		
Adeq Precision		10.019		

the immersion, after which the rate of water loss from mushrooms into the solution gradually slowed down with time towards equilibrium end point. On the other hand, the WL increased gradually with salt concentration over the entire osmotic dehydration process. WL increased with temperature especially in the early stages of the immersion. Higher temperatures seem to promote faster water loss through swelling and plasticising of cell membranes as well as the better water transfer characteristics on the product surface due to lower viscosity of the osmotic medium (Contreras and Smyral, 1981). Rapid removal of water in early stages with increasing temperatures of osmosis has been reported for mushrooms (Kar and Gupta, 2001; Murumkar *et al.*, 2007), green pumpkins (Chang *et al*., 2003), potatoes (Eren and Kaymak-Ertekin, 2006), litchi (Vishal *et al.*, 2009) and papaya (Jain *et al*., 2011).

So, initial stages of osmotic dehydration is the most important one, since the transport phenomena are faster and they have a dramatic impact on further evolution of the osmotic process (Biswal *et al*., 1991; Conway *et al*., 1983; Hawkes and Flink, 1978). The effect of β_1, β_2, β_3 and β_4 on the SG is given in fig. 2. The SG increases sharply with immersion time and temperature. The effect of β_1, β_2, β_3 and β_4 on the WR is given in fig.3. WR mainly depends upon WL and SG during osmotic dehydration process.

The experimental results are analyzed through RSM to obtain in empirical model for the best response. The mathematical expressions of relationship describing the effects of process variables to the response are shown below.

$$\mathbf{WL} = 38.67+1.72\,\beta_1+0.80\,\beta_2+4.80\,\beta_3+1.43\,\beta_4+0.45\,\beta_{12}+1.16\,\beta_{13}+0.61\,\beta_{14}-0.47\,\beta_{23}-0.45\,\beta_{24}+0.13\,\beta_{34}-1.19\,\beta_1^2-1.14\,\beta_2^2-1.62\,\beta_3^2-1.07\,\beta_4^2 \quad \mathbf{(5)}$$

$$\mathbf{SG} = 2.24+0.47\,\beta_1+0.22\,\beta_2+0.87\,\beta_3+0.28\,\beta_4+0.018\,\beta_{12}+0.16\,\beta_{13}-0.063\,\beta_{14}-0.081\,\beta_{23}+0.098\,\beta_{24}-0.019\,\beta_{34}-0.035\,\beta_1^2-0.067\,\beta_2^2-0.58\,\beta_3^2+0.038\,\beta_4^2 \quad \mathbf{(6)}$$

$$\mathbf{WR} = 36.43+1.26\,\beta_1+0.58\,\beta_2+3.93\,\beta_3+1.15\,\beta_4+0.43\,\beta_{12}+0.99\,\beta_{13}+0.67\,\beta_{14}-0.39\,\beta_{23}-0.55\,\beta_{24}+0.15\,\beta_{34}-1.15\,\beta_1^2-1.07\,\beta_2^2-1.05\,\beta_3^2-1.11\,\beta_4^2 \quad \mathbf{(7)}$$

Where, WL, SG, WR are water loss (%), solute gain (%) and weight reduction (%) respectively, and β_1, β_2, β_3 and β_4 are the coded values of the test variables, immersion time (min), salt concentration (%), solution temperature (^oC) and solution to fruit ratio respectively, as mentioned earlier.

The presence of positive interaction terms between β_1, β_2, β_3 and β_4 indicated that increase in their levels increased WL, SG and WR. The negative values of quadratic terms of process variables of osmosis indicated that higher values of these variables further reduced WL, SG and WR. The analysis of variance of quadratic regression model demonstrated that equation (5), (6) and (7) were highly significant models, as were

Table 4. Range of process parameters and their importance for optimization of osmotic dehydration and solution generated through the RSM Technique in salt solution.

Process parameters	Goal	Experimental range	Importance	Optimum values	Desirability
Time (min)	is in range	30 - 90	3	53.54	
Salt (%)	is in range	10 - 20	3	14.09	
Temp (°C)	target	45	3	45.00	
STFR	is in range	4:1 - 8:1	3	6.08:1	
Responses				Predicted values	0.626
WL (g/100g of initial mass)	maximize	29.19 - 43.19	3	38.1298	
SG (g/100g of initial mass)	minimize	0.448 - 3.10	3	2.1053	
WR (g/100g of initial mass)	maximize	28.744 - 40.09	3	36.0246	

evident from F-test with very low probability value (p model = < 0.0001, < 0.0001 and 0.0003) for WL, SG and WR respectively. This indicates that the linear terms of β_1, β_2, β_3 and β_4 of osmosis were highly significant at 5 per cent level.

Response surface and contour plots: Response surface plots as a function of two factors at a time, maintaining all other factors at fixed levels are more helpful in

understanding both the main and the interactive effects of these two factors. The response surface curves were plotted to understand the interaction of the variables and to determine the optimum level of each variable for maximum response. The 3D surface and contour plots for WL, SG and WR along with overlay plots are shown in Fig. 1-4 respectively. Figures show the effects of process variables such as β_1, β_2, β_3 and β_4 on WL, SG and WR during the osmotic dehydration of oyster mushrooms. The higher processing temperature and immersion time promote rapid water loss ad solute uptake along with increased STFR and salt concentration.

Temperature has an effect on the cell membrane permeability that could allow solute to enter by losing its selectivity. Decrease of solution viscosity at higher temperature may influence salt gain due to fact that lower viscosity decreases the resistance to diffusion of solutes into the sample tissue. Increased concentration of the salt solution also led to increase in salt gain. This is probably due to an increase of osmotic pressure gradient and consequent loss of functionality of cell plasmatic membrane that allows solute to enter. Rapid loss of water and uptake of solute near the surface in the beginning may result in structural changes leading to crust formation/compaction of surface layers and which results in increased mass transfer resistance for water and solutes (Alam *et al.,* 2010). During osmotic dehydration, water removal from the product is always accompanied by the simultaneous counter diffusion of solutes from the osmotic solution into the tissue. In most common operating conditions, mass transfer mainly occurs during the first 2 h for water loss and first 50 min for solute gains (Rault *et al*., 1989). Further, studies also confessed that moisture loss occurs in the first hour of the osmotic process (Rezagah *et al*., 2010). The mass transfer rates thus become progressively lower with time and water loss stops, whereas solute gain goes on increasing regularly (Ertekin and Çakaloz, 1995). Rapid removal of water in the early stages of osmotic dehydration has been reported by several authors (Ertekin and Çakaloz, 1996; Lazarides *et al*., 1995; Shi and Le Maguer, 2002).

Optimization of osmotic dehydration of mushroom: Design-Expert trail version 8.0.7.1 (Statease Inc., Minneapolis, USA) was used for getting optimal values for multiple responses, as discussed in section 2.1. Graphical multi-response optimization technique was adopted to determine the workable optimum conditions for the osmotic dehydration of oyster mushrooms. The contour plots for all the responses were superimposed and regions that best satisfy all the constraints were selected as optimum conditions. The main criteria for constraints optimization were maximum possible water loss, weight reduction and minimum solute gain. These constraints resulted in feasible zone (yellow coloured area in the superimposed contour plots) of the optimum conditions. Superimposed contour plots having common superimposed area of all the responses for osmotic dehydration in sodium chloride solution are presented from Figs. 1-4. These above results clearly indicate the suitability of the developed models. The optimum ranges of process parameters obtained for osmotic dehydration of oyster mushrooms were: 30 -90 min immersion time; 10-20% salt concentration; 45° C and 4:1-8:1 solution to fruit ratio. Numerical multi response technique was carried out for optimization of process. Equal importance of '3' was given to all the four parameters. The constraints were set such that the selected variables would be minimum from economical point of view for the most important product attribute. Goal of the present study was to maximize the water loss and weight reduction values as high as possible and to minimize solute as low as possible.

The optimum conditions were found to be β_1=53.54 min, β_2=14.09 %, β_3=45° C and β_4=6.08:1. At these optimum conditions, WL, SG and WR were found to be 38.13, 2.10 and 36.02 (g/100 g of initial mass) with overall desirability value of 0.626. Table 4. shows the software generated optimum conditions i.e. range of process parameters and their importance for optimization of osmotic dehydration and solution generated through the RSM Technique in sodium chloride salt solution.

Conclusion

It was concluded that RSM generated optimum operating conditions yielded maximum water loss and weight reduction and minimum solute gain in osmotic dehydration of Oyster mushroom. Analysis of variance has shown that the effects of all the process variables including solution temperature, immersion time, salt concentration and solution to fruit ratio were statistically significant. Second order polynomial models were obtained for predicting water loss, solute gain and weight reduction. The optimum conditions were found to be: solution temperature – 45° C, immersion time – 53.54 min, salt concentration – 14.09 % and solution to fruit ratio 6.08:1. At these optimum values, water loss, solute gain and weight reduction were found to be 38.13 (g/100 g initial mass), 2.1 (g/100 g initial mass) and 36.02 (g/100 g initial mass) respectively.

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