Osmotic dehydration of mushroom

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Abstract
Response surface methodology with Central Composite Rotatable design was used to know the effect of three independent variables at five levels [Salt Concentration - 10, 15, 20, 25, 30%; Solution Temperature - 45, 50, 55, 60, 65 °C; Immersion Time - 60, 120, 180, 240, 300 min.] on dependent variables like Water Loss, Solute Gain and Weight Reduction of osmo-dehydrated button mushrooms.

The best combination was selected on the basis of optimization by Response Surface Methodology. Second order multiple regression equations were developed for all the dependent variables to know the effect of independent variables. Osmotic dehydration treatment facilitates better results with optimum solution of Salt Concentration - 10.21%, Solution Temperature - 50°C and Immersion Time - 120 minutes with an optimized yield as Water loss - 52.58%, Solute gain - 5.946% and Weight reduction of osmo-dehydrated sample - 47.023% with desirability 0.878.

Keywords: button mushroom, osmotic dehydration, central composite rotatable design, response surface methodology

Introduction
Mushroom is a macroscopic fungus with distinctive fruiting body and lacks in chlorophyll, hence it requires a substrate for absorption of nutrients. The real fungus which is a microscopic fine thread like structure is used to grow on the substratum or below the surface of soil, it comes together in very compact form and spread out like an umbrella structure, when it is matured. Mushroom constitutes both a nutritionally functional food and a source of physiologically beneficial components with all possible functional foods (Khan and Tania, 2012)\(^9\). Biological Compounds extracted from the mushrooms have anticancer, antimicrobial, antiviral, immune response-stimulating, and antioxidant properties. It has also been found effective against cancer, insomnia, cholesterol reduction, allergies, stress, asthma, and diabetes (Bahl, 1983)\(^3\). Mushrooms are good sources of essential minerals, especially selenium, potassium and copper. And also rich in vitamins include thiamine, riboflavin, niacin, biotin and ascorbic acid.

India stands at second place in the production of fruits and vegetables next to china. India has agro climatic diversity and it helps to grow mushroom. Button mushrooms are highly perishable in nature because of its high moisture content about (85-90%) hence begins deteriorating immediately within a day after harvest. Enzymatic action of polyphenol oxidase on phenolic substances causes deterioration of mushroom, which is identified by development of brown color, and it shortens the shelf life (Dunkwal et al. 2007)\(^5\). Because of their high perishable nature, it needs to be processed to preserve for the off-season use. Drying is an energy intensive process among the different methods of preservation. It reduces the water activity by decreasing moisture content. It is an easiest method to increase the shelf life of high moisture products (Shukla and Singh, 2007)\(^5\).

Osmotic dehydration is a cheapest and easiest technique, which is used to increase the shelf life of highly perishable products like fruits and vegetables. Most commonly used osmotic solution is NaCl for vegetables and sucrose, glucose and fructose. It is the process of removal of water which can be done by immersing the sample in hypertonic solution. The osmotic pressure difference between the solution and the food product causes the removal of water from the sample. Two major counter current flows occur in which water flows into the solution from the product and solute transported into the product from the solution.

The thermal damage on colour, flavour can be minimized by osmotic dehydration and it also causes reduction of enzymatic browning (Islam and Flink, 1982)\(^5\) which is the main critical factor on the quality of this kind of mushrooms. Although this method of dehydration may not enough to yield low moisture content to make the product stable, so it requires further processing like vacuum drying, air drying or freeze drying (Torringa et al. 2001; Vishal et al.,...
2009; Alam et al. 2010 and Dehkordi, 2010). Employing the osmotic dehydration as a pre-treatment of convective drying can improve the quality of final product. Hence, the hot air drying is to be done after osmotic dehydration of mushrooms (Shukla and Singh, 2007 and Dehkordi, 2010). Air drying causes physical and biochemical changes are caused by air drying in food stuffs. These changes may cause reduction of quality of end product and it can be avoided by optimization of drying process.

Materials and Methods
Sample preparation

The fresh and good quality button mushrooms were procured from the Mushroom Research Unit. Dr. RPCAU, Pusa. White button mushrooms of uniform size were washed thoroughly to remove adhering impurities and were graded properly to avoid variation in exposed surface area. The samples were vertically cut into uniform shape and size (approximately 5 mm thickness).

Determination of moisture content

The moisture content of the untreated, osmotically treated, blanched and osmo-air dried mushroom samples was determined using the standard hot air oven method. Samples (20 g) were dried for 24 hr. in the hot air oven at 102 ± 2 °C (AOAC, 1990).

Moisture content (MC\text{w}) was determined on wet basis as

\[
MC_w = \frac{W_m}{W_m + W_d} \times 100
\]

Where,

\( MC_w = \) Moisture content on wet basis, (percent)\n\( W_d = \) Bone dry weight, (g)\n\( W_m = \) Moisture evaporated, (g)

Experimental design and statistical analysis

The response surface methodology was applied using design expert (Triial version 12.0. STATEASE INC., Minneapolis, USA) to estimate the influence of process variables (solution concentration (X1), solution temperature (X2) and immersion time (X3)) on water loss (WL), solute gain (SG) and weight reduction (WR) during osmotic dehydration of mushroom. Central Composite Rotatable Design (CCRD) was employed for designing experimental data of three process variables at five levels i.e solution concentration (10, 15, 20, 25, 30), solution temperature (45, 50, 55, 60, 65) and immersion time (60, 120, 180, 240, 300) and generated 20 experiments.

Experimental procedure

The osmotic dehydration was carried out in glass beaker of 250 ml and the salt solutions were prepared by dissolving the required amount of salt in distilled water according to the different concentrations which were used in the process. 20 g of mushroom slices were placed into the beakers (250 ml) containing salt solution with solution to product ratio (SPR) 10:1 and those beakers were maintained at a particular constant temperature for a particular period of time using thermostatically controlled water bath. After particular time period the mushroom samples were taken out and gently blotted with absorbent paper and weighed.

The water loss (WL), solute gain (SG), weight reduction (WR) were evaluated based on the mass transfer between solid and liquid phase using the following equation which were suggested by the Nsonzi and Ramaswamy (1998).

\[
WL(\%) = \frac{w_0 s_0 - w_s x_t}{w_0} \times 100
\]

\[
SG(\%) = \frac{w_s s_t - w_0 s_0}{w_0} \times 100
\]

\[
WR(\%) = \frac{w_i w_s - w_0}{w_0} \times 100
\]

\( w_0 \) - Initial weight of sample at time 0 (g).\n\( w_s \) - Weight of dehydrated sample after time t (g).\n\( x_t, s_t \) - Moisture fraction of mushrooms at time 0, after dehydration for time t respectively.\n\( s_0, s_t \) - Solid fraction of mushroom at time 0 and after dehydration for time t respectively on dry basis.

Optimization of osmotic dehydration

The optimization of multiple responses was done by Numerical optimization technique using the design expert version 12.0 software. The desired goals were selected and applied for an each response. The possible goals are: maximize, minimize, and target, within range, none. All the independent factors (SC, ST and IT) kept within the range, water loss and weight reduction was kept maximized and salt gain was kept minimized.

Results and Discussion

The mushroom samples were subjected with different treatments like osmotic dehydration, blanching and followed by tray drying. As per the CCRD, 20 treatment combinations were used. Water loss (WL), salt gain (SG) and weight reduction (WR) of osmo-dehydrated sample were three major dependent quality attributes which were found to be dependent on three independent variables SC, ST and IT. Multiple regression equations were developed with the calculated data of different dependent variables by using computer programme (design expert 12). The coefficients were evaluated and analyzed statistically.

The sum of squares and errors were calculated and the regression sums of square were splitted into four parts, i.e., individual parameter, linear, interactive and square products of the terms appeared in equation. Response Surface Methodology (RSM) was applied to the experimental data by using statistical package Design expert, (Triail version 12) (statease, Minneapolis, USA). A polynomial equation was fitted to the data in order to obtain regression equation. Statistical significance of terms in regression equation has explained by Analysis of Variance (ANOVA). The same software has used to generate Response surface plots and then optimization of process variables was carried out. The significance of those variables was examined by calculating the F-value under a particular probability/P-value significant level. Based on the ANOVA table for each dependent variable, highly significant, the non-significant or the least significant independent variable was observed. The effect of simultaneous variation of two significant independent variables on a particular dependent variable, keeping the remaining two non-significant/ least significant independent variable constants was found out.
Impact of independent variables on water loss

It was observed from the Table 1 that the variation of water loss of Button mushroom samples was in the range from 44.26% to 54.79%. The minimum water loss was recorded at the combination of salt concentration (SC) of 20%, solution temperature (ST) of 55 °C and immersion time (IT) of 280.92 min, while the maximum water loss was recorded at the combination of salt concentration (SC) of 25%, solution temperature (ST) of 50 °C and immersion time (IT) of 120 min. The minimum value of weight reduction was observed at salt concentration (SC) of 15%, solution temperature (ST) of 60 °C and immersion time (IT) of 240 min. while the maximum solid gain was observed at the combination of salt concentration (SC) of 25%, solution temperature (ST) of 60 °C and immersion time (IT) of 120 min. The analysis of variance (ANOVA) for the impact of independent variable on solid gain was explained in a given Table 3 which shows that the effect of SC and IT were found to be statistically highly significant at 1% level. The interaction of SC and ST were also highly significant at 1% level (P≤ 0.01). The interaction of SC and ST were also highly significant at 1% level (P≤ 0.01). The second order polynomial multiple regression equations described the impact of independent variables on solute gain is as follows.

SG (%) = 10.09 + 1.525 SC + 0.354 ST - 0.68 IT + 0.75 SC x ST - 0.49 SC x IT - 0.412 ST xIT -0.29 SC² - 1.31 ST² - 1.17 IT² (R² =0.96)

The Fig. (b) reveals that the solute gain increases with increase in solution concentration at all solution temperatures, due to the increased concentration difference between sample and brine with increase in salt concentration. And also shows that solute gain increases with increase in solution temperatures at all solution concentrations. It may be due to the rupture of the cell membrane at higher temperatures. Similar trend have also been followed by Nsonzi and Ramaswamy (1998a) [11], Jain et al. (2011) [12], Pisalkar et al. (2011) [13].

Effect of independent variables on weight reduction

The overall variation of weight reduction of Button mushroom ranges from 38.59% to 46.91%, the maximum value of weight reduction was observed at salt concentration (SC) of 15%, solution temperature (ST) of 50°C and immersion time (IT) of 120 minutes. The minimum value of weight reduction was observed at solution concentration (SC) of 20%, solution temperature (ST) of 55 °C and immersion time (IT) of 280.92 minutes. The analysis of variance (ANOVA) for the impact of independent variable on the weight reduction was presented in given Table 4 it shows that the impact of ST and IT were

### Table 1: Central Composite Rotatable Design matrix with calculated values of response (dependent) variables

| Treatment | Independent Variables | Dependent Variables |
|-----------|-----------------------|---------------------|
|           | SC (%) | ST (°C) | IT (min.) | WL (%) | SG (%) | WR of sample (%) |
| 1.        | 15     | 50      | 120       | 52.3    | 5.98   | 46.91           |
| 2.        | 25     | 50      | 120       | 54.79   | 8.45   | 45.82           |
| 3.        | 15     | 60      | 120       | 47.82   | 6.1    | 41.72           |
| 4.        | 25     | 60      | 120       | 50.49   | 11.82  | 38.67           |
| 5.        | 15     | 50      | 240       | 46.76   | 6.23   | 40.53           |
| 6.        | 25     | 50      | 240       | 50.13   | 6.98   | 43.15           |
| 7.        | 15     | 60      | 240       | 46.02   | 4.95   | 41.07           |
| 8.        | 25     | 60      | 240       | 50.48   | 8.45   | 42.03           |
| 9.        | 11.59  | 55      | 180       | 49.93   | 6.67   | 43.26           |
| 10.       | 28.41  | 55      | 180       | 54.69   | 11.66  | 43.03           |
| 11.       | 20     | 46.59   | 180       | 51.89   | 5.96   | 45.93           |
| 12.       | 20     | 63.41   | 180       | 47.99   | 6.65   | 41.34           |
| 13.       | 10     | 55      | 79.08     | 50.19   | 7.7    | 42.49           |
| 14.       | 20     | 55      | 280.92    | 44.26   | 5.67   | 38.59           |
| 15.       | 20     | 55      | 180       | 50.06   | 11.12  | 38.94           |
| 16.       | 20     | 55      | 180       | 51.13   | 9.85   | 41.28           |
| 17.       | 20     | 55      | 180       | 50.5    | 9.7    | 40.8            |
| 18.       | 20     | 55      | 180       | 48.83   | 8.78   | 40.05           |
| 19.       | 20     | 55      | 180       | 51.93   | 10.88  | 41.05           |
| 20.       | 20     | 55      | 180       | 51.3    | 10.28  | 41.02           |
highly significant at 1% level of significance. The interaction of SC, IT and ST, IT was also highly significant at 1% level of significance. The quadratics of SC and ST were found as highly significant at 1% level of significance.

The second order polynomial multiple regression equations described the effect of independent variables on solute gain as follows.

\[ WR(\%) = 40.52 - 0.07 \text{SC} - 1.51 \text{ST} - 0.944 \text{IT} - 0.45 \text{SC} \times \text{ST} + 0.97 \text{SC} \times \text{IT} + 1.47 \text{ST} \times \text{IT} + 0.912 \text{SC}^2 + 1.08 \text{ST}^2 - 0.0086 \text{IT}^2 \ (R^2=0.95) \]

Fig. (c) shows the response surface plot of weight reduction of osmo-dehydrated Button mushroom samples varied with independent variable SC and ST. The figure reveals that the weight reduction decreases and then becomes increase with increase in solution temperature at all solution concentration. And weight reduction decreases and then increases with increase in solution concentration at all solution temperatures.

Statistical analysis

The effect of process variables on dependent variables was analyzed by the second order polynomial equation. Three modes of following form were developed to analyse the responses of water loss, solute gain and weight reduction.

\[ Y_i = a_0 + \sum_{i=1}^{n} a_i X_i + \sum_{j=1}^{n} \sum_{j=1}^{n} a_{ij} X_i X_j \]

Where \(a_0, a_i, \) and \(a_{ij}\) are regression coefficients, \(X_i\) and \(X_j\) are coded independent variables and \(Y\) is response variable.

The multiple linear regression equation analysis was used to evaluate the mathematical model for each response. Modelling was started with a quadratic mode including squared, linear and interaction terms. Analysis of Variance for an each response was used to determine the significant terms. If the probability levels that \(f\) -statistic determined from the data is \(<5\%\) then that term can be judged as statistically significant. \(R^2\), adjusted \(R^2\) predicted \(R^2\) and predicted error sum of squares (PRESS) were used to check the mode adequacy. A good model will have a large predicted \(R^2\), and a low PRESS.

**Optimization of osmotic dehydration of button mushrooms**

Response surface contour plots were generated using design expert version 12 (Statease Inc, Minneapolis, USA, trial version). The effect of interaction of any two process variables with holding the other variable constant can be explained by generating response plots. Those response plots are helpful in understanding both main and the interaction effects of these two factors. Such 3D response surface plot gives useful information about behavior of the system within the experimental design. The optimization process aimed to determine the optimum level of independent variable which could give maximum response.

To optimize the process conditions during osmotic dehydration of button mushrooms, the following considerations were taken: (1) maximization of water loss (WL) (2) minimization of solute gain (SG) and (3) maximization of weight reduction (WR) of osmo-dehydrated mushroom samples. Optimization was carried out with the help of commercial statistical package (Design Expert, Trial Version 12). The optimum solution from this package for osmo-dehydration of Button mushroom was emerged out as salt concentration (SC) -10.21% solution temperatures (ST) - 50°C and immersion time (IT) - 120 minutes in order to obtain the yield of WL- 52.585%, SG- 5.946% and WR of osmo-dehydrated sample- 47.023% with desirability 0.878.

**Table 2:** ANOVA for the impact of independent parameters on percent water loss

| Source | Sum of Squares | Degree of freedom | Mean Square | F-value |
|--------|----------------|------------------|-------------|---------|
| Model  | 124.15         | 9                | 13.79       | 21.35** |
| A-SC   | 32.28          | 1                | 32.28       | 49.94** |
| B-ST   | 18.12          | 1                | 18.12       | 28.03** |
| C-IT   | 35.39          | 1                | 35.39       | 54.75** |
| AB     | 0.2016         | 1                | 0.2016      | 0.3120NS|
| AC     | 0.8911         | 1                | 0.8911      | 1.38NS  |
| BC     | 8.80           | 1                | 8.80        | 13.62** |
| A²     | 5.38           | 1                | 5.38        | 8.32**  |
| B²     | 0.7422         | 1                | 0.7422      | 1.15NS  |
| C²     | 20.30          | 1                | 20.30       | 31.41** |
| Residual | 6.46          | 10               | 0.6463      |         |
| Lack of Fit | 0.4920 | 5                | 0.0984      | 0.0824NS|
| Pure Error | 5.97      | 5                | 1.19        |         |
| Cor Total | 130.61       | 19               |             |         |
| PRESS  | 12.49          | 1                |             |         |
| R²     | 0.9505         |                  |             |         |
| Adjusted R² | 0.9060       |                  |             |         |
| Predicted R² | 0.9044     |                  |             |         |

**Table 2:** ANOVA for the impact of independent parameters on percent water loss

**Table 3:** ANOVA for the impact of independent parameters on percent solute gain

| Source | Sum of Squares | Degree of freedom | Mean Square | F-value |
|--------|----------------|------------------|-------------|---------|
| Model  | 88.20          | 9                | 9.80        | 24.35** |
| A-SC   | 31.78          | 1                | 31.78       | 78.97** |
| B-ST   | 1.72           | 1                | 1.72        | 4.26NS  |
| C-IT   | 6.14           | 1                | 6.14        | 15.25** |
| AB     | 4.50           | 1                | 4.50        | 11.18** |
| AC     | 1.94           | 1                | 1.94        | 4.82NS  |
**Table 4:** ANOVA for the impact of independent parameters on percent weight reduction

| Source     | Sum of Squares | Degree of freedom | Mean Square | F-value  |
|------------|----------------|-------------------|-------------|----------|
| Model      | 96.75          | 9                 | 10.75       | 23.45**  |
| A-SC       | 0.0656         | 1                 | 0.0656      | 0.1432NS |
| B-ST       | 31.19          | 1                 | 31.19       | 68.04**  |
| C-IT       | 12.18          | 1                 | 12.18       | 26.57**  |
| AB         | 1.64           | 1                 | 1.64        | 3.57NS   |
| AC         | 7.45           | 1                 | 7.45        | 16.25**  |
| BC         | 17.29          | 1                 | 17.29       | 37.71**  |
| A²         | 12.00          | 1                 | 12.00       | 26.17**  |
| B²         | 16.99          | 1                 | 16.99       | 37.05**  |
| C²         | 0.0011         | 1                 | 0.0011      | 0.0023NS |
| Residual   | 4.58           | 10                | 0.4585      |          |
| Lack of Fit| 0.6804         | 5                 | 0.1361      | 0.1743NS |
| Pure Error | 3.90           | 5                 | 0.7808      |          |
| Cor Total  | 101.33         | 19                |             |          |
| PRESS      | 10.80          |                   |             |          |
| R²         | 0.9548         |                   |             |          |
| Adjusted R²| 0.9140         |                   |             |          |
| Predicted R²| 0.8934        |                   |             |          |

**highly significant at 1% level, *significant at 5% level, NS non significant**
Response surface showing the effect of SC and ST on (a) water loss (b) solid gain (c) Weight reduction

Conclusion
The effect of all the process variables on independent variables like Water Loss (WL), Solid Gain (SG) and Weight Reduction (WR) was studied with the help of analysis of variance and second order polynomial regression equations. Response surface methodology was used to determine the optimum conditions that can yield maximum water loss, minimum solid gain and maximum weight reduction. It can be concluded that osmotic dehydration should be carried out at an optimized conditions of 50°C solution temperatures in 10.21% salt concentration for 120 minutes immersion time in order to obtain the yield of maximum water loss - 52.585%, minimum salt gain - 5.946% and maximum weight reduction of osmo-dehydrated sample- 47.023%.

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