Research Article

Optimization of Freeze-Drying Process Parameters for Qualitative Evaluation of Button Mushroom (Agaricus bisporus) Using Response Surface Methodology

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Received 20 January 2017; Accepted 9 March 2017; Published 28 March 2017

Academic Editor: Elena González-Fandos

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Button mushroom cubes of constant cross-sectional area (0.75 cm × 1.5 cm) and varying thickness (2 mm, 5 mm, and 8 mm) were freeze-dried. Pressure (0.04, 0.07, and 0.10 mbar), primary drying temperature (−2°C, −5°C, and −8°C), and secondary drying temperature (25°C, 28°C, and 31°C) were taken as drying parameters. The protein, ascorbic acid, and antioxidant contents were taken as quality estimates for freeze-dried mushrooms. It was observed that the secondary drying temperature affected the protein (p < 0.05) and antioxidant content (p < 0.01) significantly, whereas all three freeze-drying parameters affected the ascorbic acid content with higher effect due to temperature parameters (p < 0.01) as compared to pressure (p < 0.05). The optimized values for protein, ascorbic acid, and antioxidant content obtained using response surface methodology were 7.28 ± 0.56 mg/g, 26.92 ± 0.87 mg/100 g, and 8.60 ± 0.44 mg/g, respectively, as compared to 8.43 ± 0.21 mg/g, 28.00 ± 0.53 mg/100 g, and 9.10 ± 0.10 mg/g, respectively, for fresh button mushrooms. The optimum values for process variables were obtained as 0.09 mbar, 0.36 cm, and −7.53°C and 25.03°C for pressure, sample thickness, and primary and secondary drying temperatures, respectively.

1. Introduction

Mushrooms are a good source of protein, minerals, vitamins, and antioxidants [1–3] along with numerous medicinal properties [4] but are susceptible to microbial attack due to high level of moisture content in the range of 85–92%. Drying of mushrooms by conventional methods is generally practiced to reduce water activity but results in nutritional losses [5–7]. Freeze-drying or lyophilization has been identified as an effective method for drying bioproducts with minimal deterioration to available food nutrients [8, 9] as compared to methods involving heat treatment. It works on the principle of sublimation, whereby the free water present in the material under process is frozen and directly converted into vapour state without entering the liquid phase [10, 11]. This helps prevent enzymatic browning and microbial degradation in food products. Although few articles have been cited which deal with the drying of mushrooms by lyophilization, the effect of freeze-drying parameters such as pressure and primary and secondary drying temperatures on the quality attributes of button mushroom remains unexplored and requires proper attention.

Freeze-drying is an expensive process which is mostly utilized in the pharmaceutical industry [12] and requires parametric optimization as far as food is concerned due to an increased cost of production [13]. Therefore, it becomes even more necessary to reduce process times and simultaneously identify better process parameters which could lead to better quality produce. This work, therefore, aims to study the effect of some unusual levels of freeze-drying process parameters on the protein, ascorbic acid, and antioxidant characteristics of button mushroom (Agaricus bisporus).

Response surface methodology (RSM) is a collection of statistical tools which uses multiple regressions to determine second-order equation coefficients. It is widely used and often recommended for biochemical processes [14].
2. Materials and Methods

2.1. Raw Material. Fresh button mushrooms were obtained from the Mushroom Research Center (MRC) of G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The mushrooms were grown in a controlled environment and were procured on the day of harvest upon which they were brought to the laboratory within 30 minutes and kept under 4°C until further use. Mushrooms with head size diameter of 35–40 mm were used for conducting the experiments. The initial moisture content of the mushrooms was recorded as 614.3 ± 0.1% to 1328.5 ± 0.1% on dry basis (d.b.).

2.2. Experimental Design. Box-Behnken design (BBD) was chosen for the multiobjective optimization problem as recommended by Ferreira et al. (2007) [15]. The number of experiments was determined using the following equation:

\[ N = 2k(k-1) + C_0, \]

where \( N \) is the number of experiments, \( k \) is the number of factors, and \( C_0 \) is the number of central points. For a four-variable problem, \( N \) computes to 29 experiments including 5 central point experiments at \( k = 4 \). The experimental design matrix has been shown in Table 1. A short range study was carried out based on preliminary experimentation and due to the unavailability of research data within this range. Design Expert 9.0 was used for the experimental design and optimization of process parameters.

2.3. Sample Preparation. Button mushrooms were cut manually into cuboids of dimensions of 0.75 cm × 1.5 cm. The thickness, being a variable, was adjusted as per the decided levels. The pieces obtained were weighed and prefrozen at −22°C for 24 h before lyophilization.

2.4. Protocol Followed. Frozen mushroom samples were lyophilized in standard test tubes. The weight and sample temperature of the sample were measured at 30 min intervals. The product temperature as well as the weight has been considered to provide an indicative primary drying endpoint. The termination criteria for primary drying were set as follows: temperature of the product (\( T_p \)) reaching beyond the triple point temperature of water (0.1°C) as well as the weight of the product achieving a constant value. Due to lower residual ice content with drying time [16], the product temperature increases beyond the triple point temperature of water. This would mean that sublimation can no longer take place, marking the onset of secondary drying.

The temperature was raised during the secondary drying cycle as per the experimental levels. A constant weight criterion was considered for the end of the secondary drying period. The freeze-dried samples were kept in a hermetically sealed high density polyethylene bag for further analysis.

2.5. Measurement of Protein Content. Analysis of protein content was carried out by Lowry's method as described by Pomory (2008) [17]. The sample preparation was done by adding 10 mL of 0.1M sodium phosphate buffer (pH 8.0) to known amount of ground freeze-dried mushroom samples. The mixture was then centrifuged at 9000 rpm for 20 min. Five milliliters of reagent A was then added to 1 mL of the supernatant collected from the centrifuged sample. This solution was then allowed to stand for 10 min after which 0.5 mL of 2 N Folin-Ciocalteu reagent was added to it. The mixture was shaken vigorously and kept in dark for 2-3 hrs as recommended by Pomory (2008) [17] for stable spectrophotometric reading. The blank was prepared using the same procedure without addition of the sample. The absorbance was measured at 660 nm.

The standard curve for protein was generated at 660 nm using different concentrations (0.2, 0.4, 0.6, 0.8, and

| Test number | Coded value of variables | Actual values of variables |
|-------------|---------------------------|---------------------------|
| X1 | X2 | X3 | X4 | PDT (°C) | SDT (°C) | P (mbar) | ST (cm) |
| 1 | 1 | −1 | 0 | 0 | −8 | 25 | 0.07 | 0.5 |
| 2 | 1 | −1 | 0 | 0 | −2 | 25 | 0.07 | 0.5 |
| 3 | 1 | 0 | 0 | −2 | 31 | 0.07 | 0.5 |
| 4 | 1 | 0 | 0 | −2 | 31 | 0.07 | 0.5 |
| 5 | 0 | 0 | −1 | −1 | −5 | 28 | 0.04 | 0.2 |
| 6 | 1 | 0 | 1 | −1 | −5 | 28 | 0.1 | 0.2 |
| 7 | 0 | 0 | −1 | 1 | −5 | 28 | 0.04 | 0.8 |
| 8 | 0 | 0 | 1 | 1 | −5 | 28 | 0.1 | 0.8 |
| 9 | −1 | 0 | 0 | −1 | −8 | 28 | 0.07 | 0.2 |
| 10 | 1 | 0 | 0 | −1 | −2 | 28 | 0.07 | 0.2 |
| 11 | −1 | 0 | 0 | 1 | −8 | 28 | 0.07 | 0.8 |
| 12 | 1 | 0 | 0 | −2 | 28 | 0.07 | 0.8 |
| 13 | 0 | −1 | −1 | 0 | −5 | 25 | 0.04 | 0.5 |
| 14 | 0 | 1 | −1 | 0 | −5 | 31 | 0.04 | 0.5 |
| 15 | 0 | −1 | 1 | 0 | −5 | 25 | 0.1 | 0.5 |
| 16 | 0 | 1 | 1 | 0 | −5 | 31 | 0.1 | 0.5 |
| 17 | −1 | 0 | −1 | 0 | −8 | 28 | 0.04 | 0.5 |
| 18 | 1 | 0 | −1 | 0 | −2 | 28 | 0.04 | 0.5 |
| 19 | −1 | 0 | 1 | 0 | −8 | 28 | 0.1 | 0.5 |
| 20 | 1 | 0 | 1 | 0 | −2 | 28 | 0.1 | 0.5 |
| 21 | 0 | −1 | 0 | −1 | −5 | 25 | 0.07 | 0.2 |
| 22 | 0 | 1 | 0 | −1 | −5 | 31 | 0.07 | 0.2 |
| 23 | 0 | −1 | 0 | 1 | −5 | 25 | 0.07 | 0.8 |
| 24 | 0 | 1 | 0 | 1 | −5 | 31 | 0.07 | 0.8 |
| 25 | 0 | 0 | 0 | 0 | −5 | 28 | 0.07 | 0.7 |
| 26 | 0 | 0 | 0 | 0 | −5 | 28 | 0.07 | 0.7 |
| 27 | 0 | 0 | 0 | 0 | −5 | 28 | 0.07 | 0.7 |
| 28 | 0 | 0 | 0 | 0 | −5 | 28 | 0.07 | 0.7 |
| 29 | 0 | 0 | 0 | 0 | −5 | 28 | 0.07 | 0.7 |

X1-PDT: primary drying temperature. X2-SDT: secondary drying temperature. X3-P: Vacuum pressure. X4-ST: Sample thickness.
1.0 mg/mL) of Bovine Serum Albumin in sodium phosphate buffer. The protein content in the sample was estimated by tracing the absorbance against the concentration from the standard curve.

2.6. Measurement of Ascorbic Acid. Standard indophenol method was used as described by Ranganna (1986) [18]. The dye acts as an indicator and reduces to a colorless solution on addition of ascorbic acid. The end-point of titration is indicated by a rose-pink color due to excess unreduced dye. Ascorbic acid standard was titrated against 2,6-dichlorophenolindophenol.

Sample was prepared by taking a known quantity of freeze-dried mushroom sample which was ground to form a powder. 10 mL of 3% metaphosphoric acid was then added to it and kept in an incubator shaker for 30 min at 120 rpm and 30°C. The solution was filtered and 5 mL of the aliquot was taken for titration against the standardized dye.

The total ascorbic acid content is determined using the following equation:

\[
\text{mg ascorbic acid/100 g} = \frac{\text{titre} \times \text{dye factor} \times \text{volume make up} \times 100}{\text{aliquot of extract taken} \times \text{weight taken}}
\] (2)

2.7. Measurement of Antioxidant Content. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was followed for determining the antioxidant content. DPPH gives a deep violet color at a wavelength (\(\lambda_{\text{max}}\)) of 520 nm [19] due to electron delocalization. When DPPH is added to a substance which acts as a hydrogen donor, a stable nonradical form of DPPH is generated with subsequent change of violet color to pale yellow color.

The phenolic components of the freeze-dried mushroom samples were extracted with 50 mL methanol in an incubator shaker at a prior optimized temperature-speed combination of 30°C and 120 rpm. The mixture was filtered using Whatman I filter paper and the extract was collected. Four milliliters of different concentrations (0.5, 1.5, 2.5, 3.5, and 4.5 mg/mL) of the sample in methanol was made in separate test tubes and 1 mL of 0.1 mM DPPH was added to each. The test tubes were then shaken vigorously and allowed to stand under dark conditions for the reaction to proceed. Control was prepared using similar procedure in the absence of the sample solution. Methanol was taken as blank. The absorbance for the samples was measured at 517 nm. Antioxidant content was expressed as the concentration in mg/mL corresponding to 50% DPPH inhibition or IC\text{50}. The percentage of Free Radical Scavenging Activity (FRSA) was determined by (3) as proposed by Barros et al. (2007) [20]:

\[
\% \text{ FRSA} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100.
\] (3)

All measurements were carried out at 27°C.

2.8. Statistical Analysis. The experiment had a completely randomized design with five replicates. The mean values for all parameters were examined for significance by Analysis of Variance. The statistical data was generated using Design Expert software v.9.0 (Stat-Ease).

3. Results and Discussion

The final moisture content of dried mushrooms was measured as 2.7 ± 0.3% to 9.6 ± 0.2% (d.b.). Menlik et al. (2009) [13] reported similar results while freeze-drying strawberries where they obtained a final moisture content of 9% (d.b.). The decreasing trend in moisture content data indicated the drying process to be in the falling rate period. It was observed that a careful selection of primary and secondary drying temperatures could lead to a further reduction in drying time. This effect was particularly prominent when the primary drying temperature was increased at a pressure less than 0.07 mbar. However, further decrease in pressure leads to higher structural collapse.

Second-order models for prediction of protein, antioxidant, and ascorbic acid were developed in RSM. The model coefficients (in coded form) have been shown in Table 2. The effect of individual parameters for the quality estimates of freeze-dried mushrooms and model characteristics (linear, quadratic, and interaction terms) have been estimated using Analysis of Variance (ANOVA) and represented in Table 3.

3.1. Protein Content. Protein content was measured by modified Lowry’s method. The maximum value of protein content observed in dried mushrooms was 7.28 ± 0.56 mg/g as compared to 8.43 ± 0.21 mg/g in fresh mushroom sample. The protein content was found to be in accordance with that reported by Braaksma and Schaap (1996) [21]. The interactive effect of process parameters showed no statistical significance (\(p > 0.05\)) which indicates that the protein content remained mostly unaffected with variation in process parameters. A significant but slight effect (Table 3), however, was observed with variation of secondary drying temperature (\(p < 0.05\)). This was expected considering the heat sensitivity of the protein structure.

3.2. Ascorbic Acid Content. The ascorbic acid content was calculated using (2). Fresh samples exhibited 28 ± 0.53 mg/100g ascorbic acid content, while for dried samples ascorbic acid ranged between 17.8 and 26.9 mg/100g as obtained by indophenol method. The percentage of retention of ascorbic acid was in the range of 63–96% which was in accordance with the findings of Giri and Prasad (2009) [5] who reported an 85% retention. Ascorbic acid remained constant for a short PDT range (−5°C to −6°C) but a slow and gradual decrease was observed as it approached a higher temperature. Increase in ascorbic acid content was observed up to 0.06 mbar beyond which there was a steep decline (Figure 1). This could mean that, at lower pressures, the drying cycle time will be increased, thereby oxidizing vitamin C over time [22].

SDT and PDT affected the ascorbic acid of mushroom at 1% level of significance, while sample thickness and
Table 2: Model coefficients generated by RSM.

| Model terms | Ascorbic acid | Antioxidant content | Protein content |
|-------------|---------------|---------------------|-----------------|
| A-PDT       | 0.76          | -0.46               | 0.09            |
| B-SDT       | -1.95***      | 0.09                | 0.27            |
| C-pressure  | -0.40         | -0.07               | -0.27           |
| D-sample thickness | 0.12       | 0.82*** | -0.53       |
| AB          | -1.04         | -0.51               | -0.48           |
| AC          | 2.17***       | -0.25               | 0.19            |
| AD          | 0.38          | -0.09               | 0.76            |
| BC          | -0.09         | 8.50E-03            | -0.07           |
| BD          | 0.67          | -1.42***            | -0.55           |
| CD          | 0.80          | -0.09               | 0.77            |
| A squared   | -1.60***      | 0.40                | 1.66***         |
| B squared   | -1.10         | 1.20***             | 2.17***         |
| C squared   | -1.02         | -0.44               | 1.23**          |
| D squared   | 2.32***       | 1.06***             | -1.26**         |
| Error       | 1.84          | 0.67                | 1.47            |
| Model adj-$R^2$ | 0.86        | 0.806              | 0.801           |

* *** and ** denote 1% and 5% levels of significance, respectively.
adj: adjusted.

3.3. Antioxidant Content. The antioxidant content was expressed in terms of the concentration (mg/g sample) corresponding to 50% DPPH inhibition. The variation of antioxidant was recorded as 2.153–8.604 mg/g for freeze-dried mushrooms as compared to 9.105 mg/g for fresh mushrooms. No significant difference in the antioxidant content of fresh and optimized freeze dried mushroom samples was observed which was in accordance with the findings of Shofian et al. (2011) [24]. The interactive effect of secondary drying temperature and sample thickness (Figure 3) was found to be significant ($p < 0.01$). Independently increasing the interactive parameters resulted in an increase in antioxidant content which was in contradiction to the studies conducted by Rˇeblovˇa (2012) [25] who reported a decrease in antioxidants with increasing temperatures. It was also observed that easily oxidizing phenolics showed a slower decrease in antioxidant activity. Based on this observation, it can be concluded that there may be more quantities of phenolic acids in button mushrooms such as gallic and caffeic acids which are not readily deteriorated. However, this would require a deeper analysis of the antioxidant composition and the reaction kinetics involved during freeze-drying of mushroom. Another important theory by Shofian et al. (2011) [24] suggests the liberation of phenolic content from the mushroom matrix due to freeze-drying, thereby increasing pressure showed an effect at 5% level of significance. Levels of ascorbic acid decreased with increase in secondary drying temperatures (Figure 2). This was expected for the fact that vitamin C is heat-sensitive and might get degraded at higher temperatures [23].

Figure 1: Effect of pressure and primary drying temperature (PDT) on ascorbic acid content.

Figure 2: Effect of secondary drying temperature (SDT) on ascorbic acid content.
TABLE 3: Model characteristics and effect of individual parameters on the freeze-drying of mushrooms.

| Source              | df | SS     | MS     | F-value |
|---------------------|----|--------|--------|---------|
| **Ascorbic acid**   |    |        |        |         |
| Model               | 14 | 162.41 | 11.60  | 6.31*** |
| Linear              | 4  | 54.83  | 13.71  | 7.45*** |
| Quadratic           | 4  | 65.96  | 16.49  | 8.96*** |
| Interactive         | 4  | 28.09  | 7.02   | 3.82**  |
| PDT                 | 5  | 47.28  | 9.456  | 5.14*** |
| SDT                 | 5  | 59.63  | 11.93  | 6.48*** |
| Pressure            | 5  | 30.12  | 6.02   | 3.27**  |
| Sample thickness    | 5  | 39.96  | 7.99   | 4.34**  |
| Error               | 14 | 25.75  | 1.84   |         |
| **Antioxidant content** |    |        |        |         |
| Model               | 14 | 38.97  | 2.78   | 4.17*** |
| Linear              | 4  | 10.88  | 2.72   | 4.06**  |
| Quadratic           | 4  | 19.00  | 4.75   | 7.09*** |
| Interactive         | 4  | 9.44   | 2.36   | 3.52**  |
| PDT                 | 5  | 4.96   | 0.99   | 1.48    |
| SDT                 | 5  | 18.62  | 3.72   | 5.56*** |
| Pressure            | 5  | 1.59   | 0.32   | 0.47    |
| Sample thickness    | 5  | 23.59  | 4.72   | 7.04*** |
| Error               | 14 | 9.35   | 0.67   |         |
| **Protein**         |    |        |        |         |
| Model               | 14 | 82.84  | 5.92   | 4.03*** |
| Linear              | 4  | 5.16   | 1.29   | 0.88    |
| Quadratic           | 4  | 68.61  | 17.15  | 11.67***|
| Interactive         | 4  | 6.98   | 1.74   | 1.19    |
| PDT                 | 5  | 21.32  | 4.26   | 2.90    |
| SDT                 | 5  | 33.66  | 6.73   | 4.58**  |
| Pressure            | 5  | 13.22  | 2.64   | 1.80    |
| Sample thickness    | 5  | 19.35  | 3.87   | 2.63    |
| Error               | 14 | 20.54  | 1.47   |         |

* * * and * * denote 1% and 5% levels of significance, respectively.

df: degree of freedom.
SS: sum of squares.
MS: mean sum of squares.

the antioxidant content which could be a possible explanation for the phenomena taking place in the current study.

4. Conclusions

Quality characteristics of freeze-dried mushrooms in terms of protein, ascorbic acid, and antioxidant content were measured in this research work. The underlying objective was to understand the effects of freeze-drying process variables on the quality parameters. Primary and secondary drying temperatures showed higher effects as compared to pressure and sample thickness. On the basis of the study, the optimized values for primary and secondary drying temperatures are \(-7.53^\circ C\) and \(25.03^\circ C\), respectively. Further, the optimum values for pressure and sample thickness were obtained as 0.09 mbar and 0.36 cm. It is recommended that studies

![Figure 3: Effect of sample thickness and secondary drying temperature (SDT) on antioxidant content.](image-url)
involving freeze-drying of food materials should consider independent analysis of primary and secondary drying cycles to reduce drying times and better nutritional retention.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Acknowledgments
The authors wish to thank Er. Anurag Kushwaha, Department of Post-Harvest Process and Food Engineering, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, for his valuable assistance and suggestions for carrying out this study. Also, the authors would like to extend their thanks to M. R. C., G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, for growing and providing fresh button mushrooms for conducting the research work.

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