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Modelling and Optimization of Gamma Irradiation Parameters in Mushroom Storage Using Central Composite Design (CCD)

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Abstract

The present study aimed to optimize post-harvest treatment parameters for microbial reduction and shelf-life extension of *Agaricus bisporus* using Central Composite Design (CCD). Fresh mushroom samples were treated with varying levels of gamma irradiation, storage time, and temperature, each tested at five coded levels. Unlike traditional one-factor-at-a-time (OFAT) methods, CCD allowed evaluation of interaction and quadratic effects, providing a more robust statistical framework for identifying optimal preservation conditions. Analysis of variance (ANOVA) indicated that storage time and temperature significantly affected microbial reduction ($p < 0.05$), while gamma irradiation exhibited interactive effects with these variables. The optimized conditions identified were moderate gamma irradiation (1.5–2.0 kGy), storage at 4–6 °C, and controlled storage duration to minimize microbial load while maintaining product quality. The model demonstrated acceptable fit ($R^2 = 0.7999$), though a negative predicted R^2 suggested limited extrapolation capacity. Findings support that integrating CCD with OFAT effectively enhances parameter optimization, contributing to safer and extended post-harvest storage of *Agaricus bisporus*. This study offers a scientifically validated approach for commercial mushroom preservation through statistically optimized post-harvest treatments.

Keywords:

Agaricus bisporus, Central Composite Design, Gamma Irradiation, Shelf Life Extension, Microbial Reduction, Post-Harvest Optimization

Introduction:

Mushrooms are valuable dietary components due to their bioactive and nutritional profile. As button mushrooms (*Agaricus bisporus*) are frequently consumed fresh, their short shelf life becomes a significant commercial limitation [Devi S et al., 2020; Martine B, 2000]. While methods like drying, refrigeration, and irradiation have been adopted, fresh mushrooms continue to dominate consumer demand [Reyes et al., 2021; Kibar H, Kibar B, 2025].

Gamma irradiation, utilizing isotopes such as Cobalt-60 or Cesium-137, offers deep penetration and effective microbial reduction without significant quality degradation. These gamma rays are released from sealed radioactive sources during controlled exposure in shielded chambers [Mami et al., 2013; Park and Vestal, 2002; Gbedemah et al., 1998].

Traditionally, post-harvest treatments relied on one-factor-at-a-time (OFAT) experiments, which fail to account for variable interactions and are inefficient [Wadikar DD et al., 2008]. To address this, Response Surface Methodology (RSM) has emerged as a robust alternative. RSM combines experimental design, regression modeling, and optimization to investigate complex processes involving multiple factors.

After initial screening using Box-Behnken Design (BBD), Central Composite Design (CCD) is employed for more precise optimization. CCD extends the factorial design by incorporating axial and center points to allow the estimation of curvature in the response surface. It provides comprehensive modeling of the interaction between key variables and is suitable for response prediction and process optimization [Box G.E.P, and Draper NR, 1987].

In this study, CCD is applied to fine-tune the optimal levels of gamma irradiation, packaging method, and storage temperature in order to maximize the shelf life of mushrooms while minimizing microbial load. The use of CCD allows a detailed understanding of both linear and quadratic effects of each variable and their interactions. The findings can be applied to scale up safe and effective mushroom preservation protocols for commercial use.

2. Materials and Methods

2.1Sample Collection and Preparation for Experimental Design:

Fresh Agaricus bisporus mushrooms were procured from a local market, ensuring that only undamaged specimens free from bruising or visible microbial contamination were selected to preserve sample integrity. The mushrooms were carefully cleaned to remove debris and surface contaminants, followed by thorough washing with distilled water to eliminate any residual soil particles. After washing, the mushrooms were arranged evenly on a sterile tray and allowed to air-dry at ambient room temperature (~25 °C) until completely dry.

2.2 Optimization of Microbial Content Reduction in *Agaricus bisporus* Using RSM:

To optimize the postharvest treatment conditions for microbial reduction in *Agaricus bisporus* mushrooms, a five-factor central composite design (CCD) was applied. The independent variables included gamma irradiation (A), storage time (B), and time (C). These variables were studied across five levels (−α, −1, 0, +1, +α). The experimental analysis was conducted the triplicate. The CCD model contains a total of 20 runs for experiments. (8 factorial, 6 central, and 6 axial points) The experiments were taken to determine the mean values (CFU/ml) of microbial content by a full factorial design were based on the following first-order polynomial model considered in table 1.

$$Y = \alpha_0 + \sum_i \alpha_i x_i + \sum_{ij} \alpha_{ij} x_i x_j + \sum_{ijk} \alpha_{ijk} x_i x_j x_k \qquad (3)$$

Were y is the response (microbial growth CFU/ml) and where the ij^{th} and jk^{th} interaction coefficient; was the i^{th} and jk^{th} interaction coefficient and α_o was an intercept (Hussain et al., 2017).

Table 1: Range of values of independent variables

Independent variables	Factors	Units	-α	-1	+1	+α
Gamma irradiation	A	kGy	0.79	1.1	2	2.3
Storage time	B	days	1.5	5	15	18.4
Temperature	C	°c	-1.36	0	4	5.3

The growth of microbial growth was carried out in triplicate, and the average values were noted. Experimental values were taken as the response y. ANOVA was used to estimate the statistical parameters, and the values of

"Prob > f" than 0.05 indicated that y is the microbial growth CFU/ml. A is the value of gamma irradiation, and B is the value of time. C is the value of temperature term and are the linear coefficient; of term, and $\alpha_{1,}\ \alpha_{2,\ and}\ \alpha_3$ are the linear coefficients; and $\alpha_{1,}\ \alpha_1\ \alpha_2\ \alpha_{2\ and}\ \alpha_3\ \alpha_3$ are quadratic coefficients (Stavropoulou NA et al., 2022).

The experimental design matrix was generated and analysed using Design Expert software (13.0.3.1). The results were fitted to a second-order polynomial model to predict the response surface and identify optimal conditions for microbial content reduction. The resulting regression equation in coded terms is

$$Y=\beta_{0+}\beta_1A+\beta_2B+\beta_3C+\beta_4D+\beta_5E+\beta_{12}AB+\beta_{13}AC+\beta_{14}AD+\cdots+\beta_{11}A_2+\beta_{22}B_2+\cdots+\beta_{55}E_2$$

Eq (4)

2.2 Validation of the experimental model:-

To validate the model equation. Experiments were conducted in triplicates to determine the optimum value. To fit the polynomial equation was expressed as 3D dimensional (3D) surface plots to visualize the relation between responses and the experimental factors of each factor used in the design.

3. Results & Discussion:

3.1 Optimization by Central Composite Designs (CCD) and Statistical Analysis:

CCD was used for optimization of three variables: gamma irradiation (A), Time (B), and Temperature (C), each were studied at five coded levels, that is, ($-\alpha$, −1, 0, +1, $+\alpha$) as shown by Table 2 and the models were explained with twenty runs (Table 3).

Limitations and drawbacks of the single-factor optimization can be eliminated by employing RSM (Hussain et al., 2017), which was used to explain the combined effects of all the factors in a fermentation process. The commonly used response surface design was CCD (Martin M. Kasina et al., 2020), involving five levels, for each factor needed for quadratic terms to be estimable in the second-order model. In this study the responses of the CCD were well fitted with a second-order polynomial equation.

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Run	A Gamma irradiation kgy	B: Time mins	C: Temperature °c	Microbial growth Cfu
1	1.1	0.5	4	3.94
2	1.1	3	9	4.06
3	3	1.75	6.5	4.87
4	-0.364	1.75	6.5	2.92
5	-0.364	1.75	6.5	2.61
6	1	1.75	6.5	3.3
7	3	3	9	3.32
8	3	-0.3525	6.5	3.31
9	3	1.75	6.5	3.84
10	3	1.75	10.705	4.43
11	3	3	4	1.58
12	1	1.75	6.5	3.42
13	3	0.5	4	4.56
14	3	1.75	6.5	3.21
15	1	0.5	9	4.16
16	3	1.75	6.5	4.73
17	1	0.5	9	4.23
18	1	3	4	1.78
19	3	3.8525	6.5	1.93
20	3	1.75	2.295	2.16

3.2 Analysis of variance:

The analysis of variance (ANOVA) for the experiment assessing the effect of gamma irradiation, time, and temperature on microbial growth revealed that the overall model is statistically significant ($p = 0.0145$), indicating that at least one of the factors or their interactions significantly affects microbial growth. Among the individual factors, time (Factor B) and temperature (Factor C) were found to have statistically significant effects on microbial growth with p-values of 0.0118 and 0.0195, respectively. This suggests that variations in exposure time and temperature substantially influence microbial survival or reduction during treatment. In contrast, gamma irradiation (Factor A) alone did not show a significant effect ($p = 0.7760$).

The interaction terms AB (gamma irradiation × Time) and AC (gamma irradiation × Temperature) were also not significant, with high p-values of 0.9764 and 0.6785, respectively, suggesting that the combined effects of gamma irradiation with either time or temperature did not contribute notably to variations in microbial growth. However, the interaction between time and temperature (BC) approached significance ($p = 0.0522$), indicating a potential synergistic effect that might be relevant under certain conditions and may warrant further investigation. Among

the quadratic terms, only B^2 (time squared) was significant ($p = 0.0328$), implying a non-linear relationship between time and microbial growth, whereas A^2 and C^2 were not significant.

The residual and lack-of-fit analyses confirm that the model fits the data reasonably well. The lack-of-fit p-value of 0.3063 indicates that the model's error is not significantly different from the pure error, confirming that the chosen model is adequate for the data. The relatively low residual sum of squares (3.81) compared to the total sum of squares (19.07) further supports the model's reliability. Overall, time and temperature are key determinants in microbial reduction, with time showing a potential nonlinear impact, while UV irradiation, though included in the model, did not significantly contribute to microbial growth inhibition under the tested conditions.

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	15.25	9	1.69	4.44	0.0145	significant
A-gamma irradiation	0.0326	1	0.0326	0.0855	0.7760	
B-Time	3.60	1	3.60	9.45	0.0118	
C-temperature	2.94	1	2.94	7.72	0.0195	
AB	0.0004	1	0.0004	0.0009	0.9764	
AC	0.0695	1	0.0695	0.1822	0.6785	
BC	1.85	1	1.85	4.85	0.0522	
A^2	1.03	1	1.03	2.70	0.1316	
B^2	2.34	1	2.34	6.12	0.0328	
C^2	0.4423	1	0.4423	1.16	0.3069	
Residual	3.81	10	0.3815			
Lack of Fit	1.92	4	0.4808	1.53	0.3063	not significant
Pure Error	1.89	6	0.3153			
Cor Total	19.07	19				

3.3 Model Fitting and Statistical Analysis

The polynomial regression equation for microbial content reduction, based on the five variables Gamma irradiation (A), storage time (B), temperature (C), is represented as follows:

$$Y \text{ (microbial content)} = -26.13 + 0.1651A - 28.40B + 3.72C - 0.0102AB\ 0.0286AC + 1.62BC0.0453A^2 - 6.76\ B^2 - 0.1176C^2 \quad \text{Eq (2)}$$

Where Y, is the microbial content (log CFU/g or % reduction), and A, B, and C represent the independent variables as mentioned above.

The model analysis based on coded factor levels and Type III sum of squares shows that the model is statistically significant, with an F-value of 4.44 and a low probability (1.45%) of this occurring due to random noise. Significant model terms ($p < 0.0500$) include time (B), temperature (C), and the squared term of time (B^2), indicating their meaningful contribution to microbial growth reduction. Terms with p-values greater than 0.1000 are not significant, and eliminating them-unless needed to preserve model hierarchy-may improve model performance. The lack of fit is not significant ($F = 1.53$, $p = 0.3063$), which is desirable and suggests the model fits the data well. Fit statistics reveal a standard deviation of 0.6176, R^2 of 0.7999, adjusted R^2 of 0.6199, and a coefficient of variation (CV) of 18.07%. However, the negative predicted R^2 (-0.2246) suggests that the model may not predict new responses effectively, and a higher-order model or different transformations might yield better predictive power. Despite this, the adequate precision value of 6.576-above the recommended threshold of 4-indicates a sufficient signal-to-noise ratio, supporting the model's utility in exploring the design space.

A significant test showed that there could be contributions in the regression response relationship that were accounted for by the fitted models. Therefore, the non-significant values are desirable (Mohd Yusof et al., 2021).

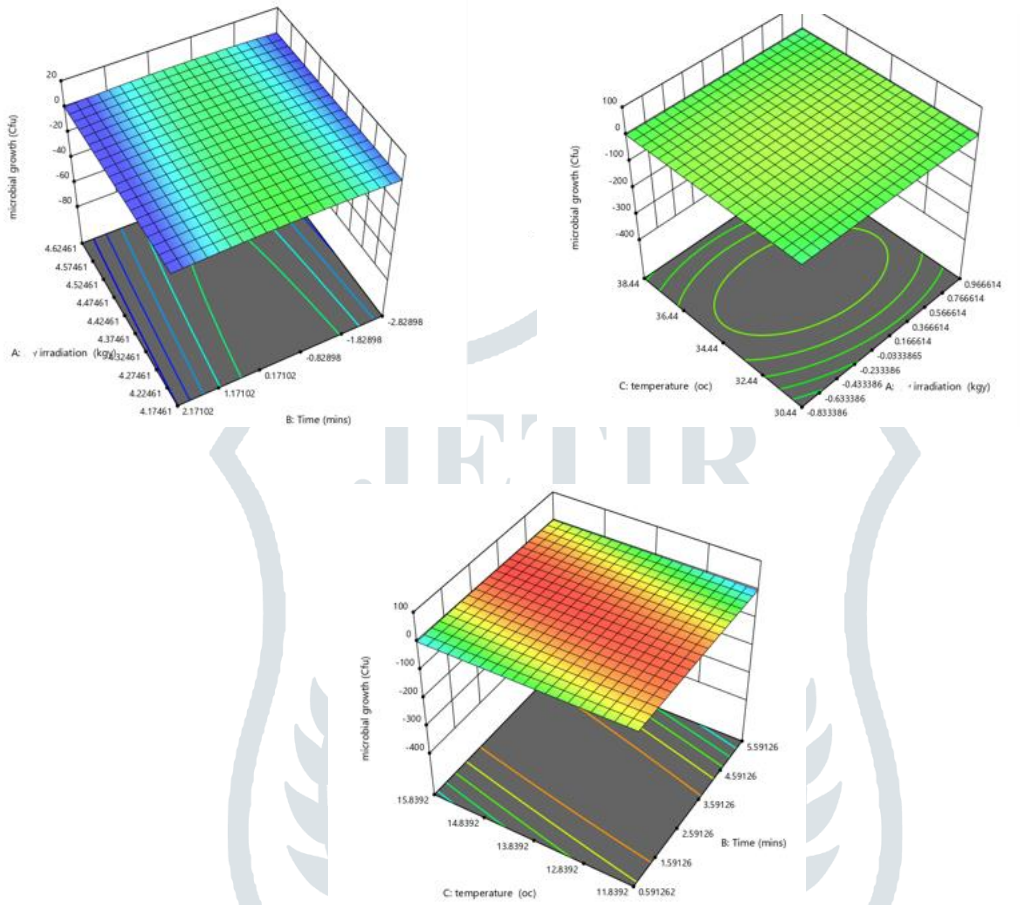

Fig 1: RSM by CCD method

3.4 Validation of the experimental model:

The 3D response surface plots in Fig 1: RSM by CCD method validate the model by visually illustrating the effects of UV irradiation, time, and temperature on microbial growth. The plots confirm that time and temperature significantly influence microbial reduction, as indicated by the curved surfaces and gradients along the respective axes, while UV irradiation shows minimal effect, consistent with its high p-value in the statistical analysis. Notably, the plot of time versus temperature exhibits a clear interactive effect, supporting the near-significant BC interaction and the significant quadratic effect of time (B^2). These visualizations reinforce the model's reliability and highlight that optimizing time and temperature is crucial for effective microbial control.

The Central Composite Design (CCD) was employed to statistically refine the optimization of three critical post-harvest parameters-gamma irradiation dose, storage time, and temperature-on the microbial inhibition in *Agaricus bisporus*. Unlike the One-Factor-At-a-Time (OFAT) approach, CCD enabled the evaluation of interaction effects and second-order (quadratic) relationships between the variables, thereby allowing a more precise understanding of their combined influence on microbial reduction and shelf-life extension.

The statistical output from CCD revealed significant interactions, particularly between irradiation dose and storage temperature, highlighting that moderate levels of irradiation (e.g., 1.5–2.0 kGy) at lower storage temperatures (~4–6 °C) were most effective in minimizing microbial load while preserving mushroom quality. The quadratic effect of time suggested that microbial reduction followed a non-linear trend, with extended storage beyond the optimum window contributing to recontamination or spoilage, even at low temperatures.

This supports the findings of Biswajith et al. (2024), who emphasized the dynamic ecological balance between microbial activity and fungal health. Disrupting harmful microbial populations while maintaining environmental conditions favorable for mushroom viability is critical for post-harvest preservation.

4. Conclusion

The CCD model provided a comprehensive and efficient optimization framework for evaluating and fine-tuning the post-harvest treatment conditions in *Agaricus bisporus*. By identifying the optimal ranges of gamma irradiation, storage time, and temperature, the model helped establish statistically validated conditions that significantly reduce microbial spoilage. The integration of CCD with OFAT collectively enhanced the predictive capacity of the experimental design and offered a scientifically sound basis for developing scalable preservation protocols for commercial mushroom storage and distribution.

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