



RESEARCH ARTICLE

Multi-response optimisation of gas composition in modified atmosphere packaging for postharvest quality retention of button mushrooms (*Agaricus bisporus*)

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Abstract

Button mushrooms (*Agaricus bisporus*) are highly susceptible to deterioration due to their rapid respiration rate, enzymatic browning and microbial load, which limit their shelf life. This study focused on optimising the gas composition of modified atmosphere packaging (MAP) to delay the physiological deterioration of button mushrooms using a simple lattice mixture design. The effects of oxygen (O₂), carbon dioxide (CO₂) and nitrogen (N₂) at different combinations on colour (L*, a* and b*), firmness, physiological loss in weight (PLW), polyphenol oxidase (PPO) activity, antioxidant activity and microbial load were evaluated. Modified atmosphere packaging under optimised conditions significantly ($p < 0.0001$) maintained the post-harvest quality of button mushrooms compared with the control. The optimised condition (69 % O₂, 10 % CO₂ and 21 % N₂) improved lightness by 7.5 % and firmness by 14.3 %, while reducing browning (a* by 22.0 %, b* by 8.5 %) and physiological weight loss by 93.5 %. The PPO activity decreased by 15.2 % and antioxidant activity increased by 17.7 %, indicating delayed senescence and oxidative deterioration. The quadratic Scheffe model revealed that all responses exhibited a strong correlation, showing a high R² value (> 0.90) with a non-significant lack of fit. Numerical optimisation predicted an optimal gas composition of 69 % O₂, 10 % CO₂ and 21 % N₂, with a desirability value of 0.725. Validation experiments closely matched predicted values, confirming that optimised MAP is an effective and practical approach for enhancing postharvest quality and extending the shelf life of button mushrooms.

Keywords: enzymatic browning; mixture design; optimisation; physiology; postharvest quality

Introduction

The button mushroom (*Agaricus bisporus*) is one of the most commonly cultivated and consumed varieties, followed by shiitake and oyster mushrooms (1). Despite high production and consumption, its shelf life is only 3–4 days at room temperature and 8 days under refrigeration due to its high perishability (2). The intrinsic factors, such as high respiration rate, high enzyme activity, high moisture content and lack of cuticle layer, lead to rapid deterioration (3). Mushrooms are rich in nutrition and bioactive compounds such as lentinan, antioxidants, dietary fibre, ergosterol, vitamins B₁, B₂ and C, folates and essential minerals (4). Several traditional preservation technologies have extended the shelf life of mushrooms, such as refrigeration, chemical washing and edible coatings (4). In addition, non-thermal technologies including irradiation, ultrasound, cold plasma, pulsed electric field and plasma-activated water have been investigated for postharvest preservation of button mushrooms (5, 6). Although these methods can extend mushroom shelf life, they often cause quality loss, lower preservation effect, high capital costs and face low consumer acceptance. Therefore, alternative preservation approaches are

required, such as high-oxygen modified atmosphere packaging, which has shown promising results in shelf life extension of various food products.

Modified atmosphere packaging (MAP) is considered a simple, effective and cost-efficient packaging method (7). It functions by altering the gas composition inside the package, which has shown a promising effect in extending shelf life and reducing these postharvest losses (3). This approach helps slow down the products respiration and overall metabolic activity, which in turn delays enzymatic breakdown, oxidation and quality deterioration (1). The effectiveness of MAP mainly depends on maintaining a proper balance between O₂ and CO₂ levels, since an unsuitable gas composition can lead to anaerobic respiration, browning and off-flavour formation (8). Higher oxygen concentrations help retain mushroom whiteness and reduce enzymatic browning, whereas carbon dioxide slows respiration and inhibits microbial growth. However, too much CO₂ can damage the tissue and soften the texture (9). Therefore, identifying an appropriate gas combination is necessary to preserve both the quality and safety of fresh mushrooms.

Although several studies have investigated modified atmosphere packaging for button mushrooms, limited work has focused on the systematic optimisation of gas composition using response surface methodology. Most earlier studies have evaluated fixed gas combinations, whereas the present study employs a mixture design coupled with a multi-response desirability approach to optimise the levels of O₂, CO₂ and N₂. In addition, this study simultaneously considers physical, biochemical and microbiological quality parameters, providing a more comprehensive understanding of the influence of gas composition on the postharvest behaviour of button mushrooms. Therefore, the present work aims to optimise MAP conditions using response surface methodology to enhance the postharvest quality of button mushrooms.

Materials and Methods

Plant material, packaging condition and storage

Button mushrooms selected for the study were directly procured from the farm located in Ooty, Tamil Nadu, India. They were uniform in size (3 ± 0.5 cm), shape, colour and free from mechanical damage and insect infestation. The button mushrooms were identified based on morphological characteristics and confirmed as *Agaricus bisporus* with reference to standard taxonomic descriptions. The mushrooms were immediately packed in a punnet package covered with shrink wrap (0.2 ± 0.1 mm) and transported to the laboratory within 4 hr of harvest. All the experiments were conducted in the Department of Food Process Engineering, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The chemicals used for the study were of analytical grade.

Storage

Button mushrooms (70 ± 5 g) were packed in low-density polyethylene (LDPE) pouches of 6×8 inches with a thickness of 60 ± 1 μ m. The packed button mushrooms were stored in a refrigerator at 4 ± 2 °C. Surface dirt was gently wiped off with sterile tissue paper and the mushrooms were not washed to prevent excess surface moisture. Physicochemical analysis was evaluated at an interval of 2 days. All the experiments were performed in triplicate to ensure uniformity and consistency.

Modified atmospheric packaging (MAP) equipment setup

The MAP unit consists of gas cylinders, gas flow regulators, a gas mixer and a packaging unit. The gases such as O₂, CO₂ and N₂ were passed to the gas mixer through pressure regulators. The flow of each gas was controlled using individual flow-control valves to obtain the required gas composition. The mixer blended the gases in the predetermined proportions to form the modified atmosphere. Fresh button mushrooms were weighed and placed inside the packaging material. The headspace air inside the package was then flushed with the prepared gas mixture for a specified duration to replace the atmospheric air. After flushing, the packages were immediately hermetically sealed using the packaging unit to maintain the desired gas composition during refrigerated storage.

Experimental design and statistical analysis

Response surface methodology (RSM) with a simple lattice design was used for the selection of the best gas concentration for the shelf life extension of button mushrooms (10). Design Expert software (Version 13, Stat-Ease Inc.) was used for the optimisation of MAP process parameters. This design evaluates the effect of

independent variables such as gas concentrations at various levels such as O₂(10–80 %), CO₂(10–80 %) and N₂ (10–80 %) on the responses L*, a*, b*, firmness (N), physiological loss in weight (%), antioxidant activity (%), total plate count (log cfu g⁻¹) and yeast and mold count (log cfu g⁻¹).

Based on the simple lattice design, the total number of runs obtained was 16, including 4 vertex points, 6 interior points and 6 edge points. The experiments were conducted and the observations of physicochemical properties on the second day of storage were used for the RSM. The second day of storage was chosen for RSM analysis because it represents an early stage at which the effects of modified atmosphere conditions on physicochemical and microbial properties become noticeable, without significant deterioration of the control samples. This timing allows for clear differentiation between treatments and supports more reliable and accurate model development for optimisation. The quadratic Scheffe model was fitted to the responses to study the effect of the process variable on the responses. The model revealed the coefficient of determination (R²) value, probability value (*p*-value), *F* value and lack of fit.

The numerical optimisation of MAP conditions for button mushrooms was carried out using the desirability function approach integrated with response surface methodology (RSM). Individual responses were transformed into desirability values ranging from 0 (undesirable) to 1 (highly desirable). The optimisation objective was to maximise colour retention (L*), firmness and antioxidant activity, while minimising physiological loss in weight (PLW), polyphenol oxidase (PPO) activity and microbial load. Appropriate desirability functions were assigned based on the acceptability limits of quality parameters. An overall desirability index was computed as the geometric mean of individual desirability, with equal weight assigned to all responses. The optimal O₂, CO₂ and N₂ composition was obtained by maximising the overall desirability. Model adequacy was confirmed through experimental validation and the error percentage between predicted and experimental values was calculated, demonstrating good agreement and reliability of the developed model.

Physicochemical properties

Colour

The surface colour (L*, a* and b*) of the button mushroom caps was analysed using a tintometer (LC 100/ SV 100). L* represents lightness (0 = black, 100 = white), a* indicates redness (+) to greenness (-) and b* indicates yellowness (+) to blueness (-). The samples were focused through the lens of the tintometer under controlled conditions, without exposure to ambient light. A standard white tile was used for calibration before the measurement. The corresponding colour values were recorded (11).

Firmness

The firmness of button mushrooms was measured using a texture analyser (Shimadzu, EZ-S) (12). A penetration test was performed at a depth of 8 mm with a probe speed of 1 mm sec⁻¹ and pre- and post-tests were performed using a cylindrical probe of 3 mm. The results were expressed in Newton.

Physiological loss in weight (PLW)

The PLW of button mushrooms was determined by measuring the difference between the initial weight of mushrooms and the weight on the day of observation (2). The formula used for the measurement of PLW is presented in Eqn. 1.

$$\text{PLW (\%)} = [(\text{initial weight} - \text{final weight}) / \text{initial weight}] \times 100 \quad (\text{Eqn. 1})$$

Polyphenol oxidase

5 g of button mushroom tissue was homogenised in 10 mL of sodium phosphate buffer at pH 7.0. The mixture was centrifuged at 8000 rpm for 20 min. The supernatant (0.5 mL) was added with 2.0 mL of sodium phosphate buffer, followed by the addition of 1 mL catechol solution. Polyphenol oxidase activity was measured at an absorbance of 410 nm for 2 min at 30 sec intervals. The formula used for measuring PPO activity is given in Eqn. 2 (13).

$$\text{PPO activity (U mL}^{-1} \text{ min}^{-1})$$

$$= \text{Absorbance at 410 nm} / 0.001 \times \text{reaction time} \quad (\text{Eqn. 2})$$

Antioxidant activity

The antioxidant activity of button mushrooms was analysed using the DPPH radical scavenging assay (5). 2 g of mushrooms were extracted with 10 mL of methanol for 24 hr. The extract was centrifuged at 6000 rpm for 15 min, after which 0.1 mL of the supernatant was mixed with 3.9 mL of methanolic DPPH solution (25 mg L⁻¹). The absorbance at 515 nm was measured immediately after 30 min of incubation in the dark. The methanolic DPPH solution served as the control and methanol served as the blank. Antioxidant activity was expressed as DPPH radical scavenging activity and calculated according to Eqn. 3, using ascorbic acid as the reference standard.

$$\text{Antioxidant activity (\%)} = (1 - \text{absorbance of sample} / \text{absorbance of control}) \times 100 \quad (\text{Eqn. 3})$$

Microbial load (log cfu g⁻¹)

The total plate count method was used to measure the bacterial load and yeast and mould counts in button mushrooms (14). 1 g of the sample was homogenised in sterile conditions and the extract of 0.1 mL was serially diluted. For the total bacterial count, dilutions of 10⁻⁵ and 10⁻⁶ were plated on nutrient agar using the spread plate method. The plates were sealed and incubated 37 °C for 24–48 hr. Whereas the determination of yeast and mould count utilises potato dextrose agar and plating was performed at dilutions of 10⁻³ and 10⁻⁴. The plates were sealed with a film and incubated at 28 °C for 4 days. After incubation, colony counts were recorded and the results were expressed as log cfu g⁻¹.

Results and Discussion

Effect of gas concentration on the physicochemical properties of button mushrooms

Colour

The changes in L*, a* and b* values of button mushrooms in response to gas concentration are shown in Fig. 1a–c. The L*, a* and b* values for fresh button mushrooms were 91.3, 3.2 and 9.2, respectively. The L* value of the control sample decreased to 84.2. Whereas, the a* and b* values increased to 4.1 and 10.6 after the 2nd day of refrigerated storage (4 °C). The increase in browning and the reduction in lightness of mushrooms are due to enhanced PPO activity, leading to oxidation of phenolic compounds into brown pigments. Additionally, moisture loss and microbial activity during storage may have further contributed to colour deterioration (5). In contrast, mushrooms stored under modified atmosphere packaging (MAP) exhibited better retention of colour attributes. The L*, a* and b* values were in the range of 90.1–89.2, 3.9–3.4 and 9.3–10.3, respectively. Table 1 revealed that button mushrooms packed with high oxygen atmosphere (80–45 %) maintained the L*, a* and b* values between 89.2 and 90.9, 3.3–3.5 and 9.3–9.6, irrespective of the gas concentrations. Whereas, the optimised condition recorded an L*, a* and b* value of 90.1, 2.8 and 9.7, respectively, which were closely comparable to the fresh button mushrooms. It was observed from the results that the MAP maintained the colour of button mushrooms by lowering respiration rate and senescence, compared with the control.

The colour retention under MAP conditions has been reported for both button mushrooms and saffron milk cap mushrooms (5, 15). In contrast, studies on modified atmosphere packaging indicate that low oxygen packaging (LOP) systems generally offer better overall quality retention than atmospheres dominated by high CO₂ or nitrogen, although their effectiveness depends on the balance between O₂ and CO₂ (16). The improved colour retention under MAP is due to its ability to regulate the internal atmosphere, thereby reducing the respiration rate and delaying senescence. The lower oxygen level limits oxidative enzymatic reactions and the appropriate CO₂ level suppresses PPO activity and microbial growth, thereby minimising browning and maintaining tissue integrity (17). Furthermore, controlled oxygen helps to stabilise cell membranes and reduce oxidative stress, thereby limiting pigment oxidation and phenolic leakage. As a result, higher L* values and stable a* values are

Table 1. Simple lattice mixture design for optimisation of gas composition for modified atmosphere packaging of button mushrooms

Sl. No.	O ₂ (%)	CO ₂ (%)	N ₂ (%)	L*	a*	b*	Firmness (N)	PLW (%)	PPO activity (U mL ⁻¹ min ⁻¹)	Antioxidant activity (%)	Total plate count (log cfu g ⁻¹)	Yeast and mould count (log cfu g ⁻¹)
Control	-	-	-	84.2	4.1	10.6	2.1	4.92	112	62.9	5.2	4.9
1.	10	10	80	89.4	3.8	9.9	2.5	0.8	92.0	71.8	4.55	3.72
2.	30	10	60	90.1	3.6	9.7	2.6	0.6	93.5	72.9	4.53	3.7
3.	45	10	45	90.6	3.5	9.6	2.6	0.5	94.8	73.4	4.5	3.68
4.	45	10	45	90.4	3.5	9.6	2.6	0.5	95.2	73.2	4.48	3.66
5.	80	10	10	90.9	3.3	9.4	2.6	0.3	97.5	73.9	4.42	3.62
6.	80	10	10	90.8	3.3	9.3	2.5	0.3	98.2	73.6	4.4	3.6
7.	60	20	20	90.2	3.4	9.5	2.6	0.4	96.0	73.0	4.45	3.64
8.	10	30	60	88.9	3.9	10.1	2.4	1.0	94.0	70.9	4.52	3.69
9.	20	40	40	88.4	3.7	10.3	2.3	1.2	95.0	69.8	4.47	3.65
10.	40	40	20	89.3	3.5	9.9	2.5	0.7	96.5	72.2	4.44	3.63
11.	10	45	45	88.1	3.9	10.5	2.2	1.4	94.5	68.9	4.49	3.67
12.	45	45	10	89.6	3.5	9.8	2.5	0.8	97.0	72.5	4.43	3.61
13.	45	45	10	89.4	3.5	9.8	2.5	0.9	97.3	72.1	4.41	3.6
14.	45	45	10	89.2	3.6	9.9	2.4	1.0	97.8	71.8	4.39	3.58
15.	10	60	30	87.6	4.0	10.7	2.1	1.6	95.5	67.4	4.46	3.64
16.	10	80	10	87.0	4.0	10.9	2.0	1.8	96.0	66.2	4.36	3.56

*O₂: Oxygen; CO₂: Carbon dioxide; N₂: Nitrogen; PPO: Polyphenol oxidase.

maintained. However, excessive CO₂ may harm tissue physiology, highlighting the need for optimal gas composition (18). Statistically, the gas concentrations had a significant ($p < 0.0001$) effect on the colour of button mushrooms. The R² values for L*, a* and b* values were found to be 0.985, 0.975 and 0.988, indicating the highest correlation with the fitted quadratic Scheffe model as shown in Table 2.

Firmness

The firmness of fresh button mushrooms was 2.7 N and it decreased to 2.1 N on the 2nd day of refrigerated storage. The rapid reduction in firmness was mainly due to tissue softening associated with moisture loss from the epidermis and degradation of structural cell wall components during storage (13). As shown in Fig. 1d, the firmness of button mushrooms was maintained high between 2.5 and 2.6 at the O₂ concentration of 80–45 %. In contrast, low oxygen level treatments recorded comparatively lower firmness values of 2.2–2.4 N and the lowest firmness of 2.0 N was observed at the treatment (10 % O₂, 80 % CO₂ and 10 % N₂). The optimised condition maintained a firmness of 2.4 N. These findings are consistent with earlier reports, which demonstrate that MAP can effectively slow softening in button mushrooms, although the extent of firmness retention varies depending on gas composition (19, 20). A controlled atmosphere storage with 1–3 % O₂ and 15–17 % CO₂ has been reported to significantly delay quality deterioration and maintain overall texture by reducing metabolic activity and senescence in button mushrooms (8). The retention in firmness under controlled atmospheres has also been observed in other perishable commodities such as apricot (21).

The loss in firmness of button mushrooms is mainly due to degradation of cell wall polysaccharides and moisture accumulation, while excess CO₂ may further accelerate tissue softening. However, moderate levels of CO₂ are known to inhibit the activity of cell wall-degrading enzymes, thereby maintaining cell wall integrity and slowing the softening process. It has been reported that CO₂ levels around 10 % are beneficial for preserving texture, whereas excessively high concentrations may induce physiological injury and hasten softening (9). Simultaneously, adequate O₂ availability maintains normal metabolic activity and prevents anaerobic respiration, which can weaken tissue structure. Thus, the improved firmness under the optimised condition indicates that a balanced gas composition is more effective than extreme atmospheric conditions. The quadratic Scheffe model fitted to the experimental data showed the strongest correlation with an R² value of 0.962. A significant ($p < 0.0001$) effect of gas concentrations on the firmness of button mushrooms had been observed and presented in Table 2. The retention in firmness of apricot fruit during storage has been observed in the previous research (20).

Table 2. Coded equation obtained from the quadratic Scheffe model

Sl. No.	Response	Coded quadratic equation	R ²	CV	F value	p value	LOF
1.	L*	$Y = 91.07A + 85.94B + 89.65C + 3.44AB + 2.08AC - 1.39BC$	0.985	0.1927	131.52	< 0.0001	NS
2.	a*	$Y = 3.31A + 4.02B + 3.81C - 0.58AB - 0.30AC - 0.03BC$	0.975	1.24	78.68	< 0.0001	NS
3.	b*	$Y = 9.34A + 10.92B + 9.85C - 1.18AB + 0.06AC + 0.33BC$	0.988	0.618	167.60	< 0.0001	NS
4.	Firmness	$Y = 2.55A + 1.99B + 2.52C + 0.81AB + 0.28AC - 0.13BC$	0.962	1.84	51.25	< 0.0001	NS
5.	PLW	$Y = 0.28A + 1.84B + 0.78C - 0.83AB - 0.23AC + 0.14BC$	0.972	10.65	70.99	< 0.0001	NS
6.	PPO activity	$Y = 97.74A + 96.08B + 92.14C + 1.29AB - 0.6518AC + 2.22BC$	0.961	0.4249	50.54	< 0.0001	NS
7.	Antioxidant activity	$Y = 73.70A + 66.09B + 72.09C + 8.90AB + 1.34AC - 0.66BC$	0.981	0.5261	108.07	< 0.0001	NS
8.	Total plate count	$Y = 4.41A + 4.37B + 4.55C + 0.08AB + 0.05AC + 0.13BC$	0.957	0.3049	44.98	< 0.0001	NS
9.	Yeast & mould count	$Y = 3.61A + 3.56B + 3.72C + 0.0454AB + 0.0361AC + 0.10BC$	0.954	0.3214	41.97	< 0.0001	NS

PLW: Physiological loss in weight; PPO: Polyphenol oxidase.

Physiological loss in weight

The variation in physiological loss in weight of button mushrooms under different gas compositions is presented in Fig. 1e. The PLW of button mushrooms for fresh button mushrooms on the 2nd day of refrigerated storage was 4.92 %, indicating rapid respiration and moisture removal from the tissues (21). From Table 1, it was observed that at high oxygen levels, PLW remained low in the range of 0.3–0.4 %, whereas, at a moderate oxygen level, the physiological loss in weight was maintained around 0.5–0.7 %. In contrast, low oxygen treatments showed comparatively higher loss (1.0–1.8 %). The PLW of button mushrooms at the optimised condition was maintained at a low level in the range of 0.49–0.50 %, which was lower than the control. A low reduction in PLW was observed for button mushrooms packed with 80 % O₂ and stored at 4 °C (9). In addition, the strawberry fruits stored under high oxygen conditions have a low PLW as observed by (22). The lower physiological weight loss (PLW) observed under high-oxygen conditions is mainly due to reduced respiration and transpiration (23). High oxygen atmospheres suppress browning mainly by inhibiting polyphenol oxidase activity and maintaining tissue integrity rather than by reducing respiration rate. As a result, metabolic activity slows down, leading to reduced moisture loss and a delay in quality deterioration (22).

The statistical analysis showed that the model was highly significant ($p < 0.0001$) with an R² value of 0.972 and an F-value of 70.99. The fitted model revealed a non-significant lack of fit, confirming the adequacy of the quadratic model. The coded equation obtained by fitting the experimental data to the quadratic Scheffe model has been depicted in Table 2.

Polyphenol oxidase (PPO) activity

Polyphenol oxidase activity plays an important role in the browning of button mushrooms. The impact of gas concentrations on the polyphenol oxidase activity of button mushrooms was depicted in Fig. 1f. The PPO activity of fresh button mushrooms was 90 U mL⁻¹ min⁻¹, which increased to 112 U mL⁻¹ min⁻¹ on the 2nd day of storage, indicating rapid browning of button mushrooms. The increase in PPO activity is due to the copper-containing enzyme catalyzes the oxidation of phenolic compounds (such as GHB, GDHB and tyrosine) into quinones, which then further react to form brown melanin pigments (24).

It was observed from the results that MAP maintained the PPO activity in the range of 92–98.2 U mL⁻¹ min⁻¹. The PPO activity at moderate oxygen conditions was around 93–96 U mL⁻¹ min⁻¹. High oxygen treatments showed slightly higher activity (about 97–98 U mL⁻¹ min⁻¹), while low oxygen treatments also exhibited elevated values compared to the optimised treatment. The

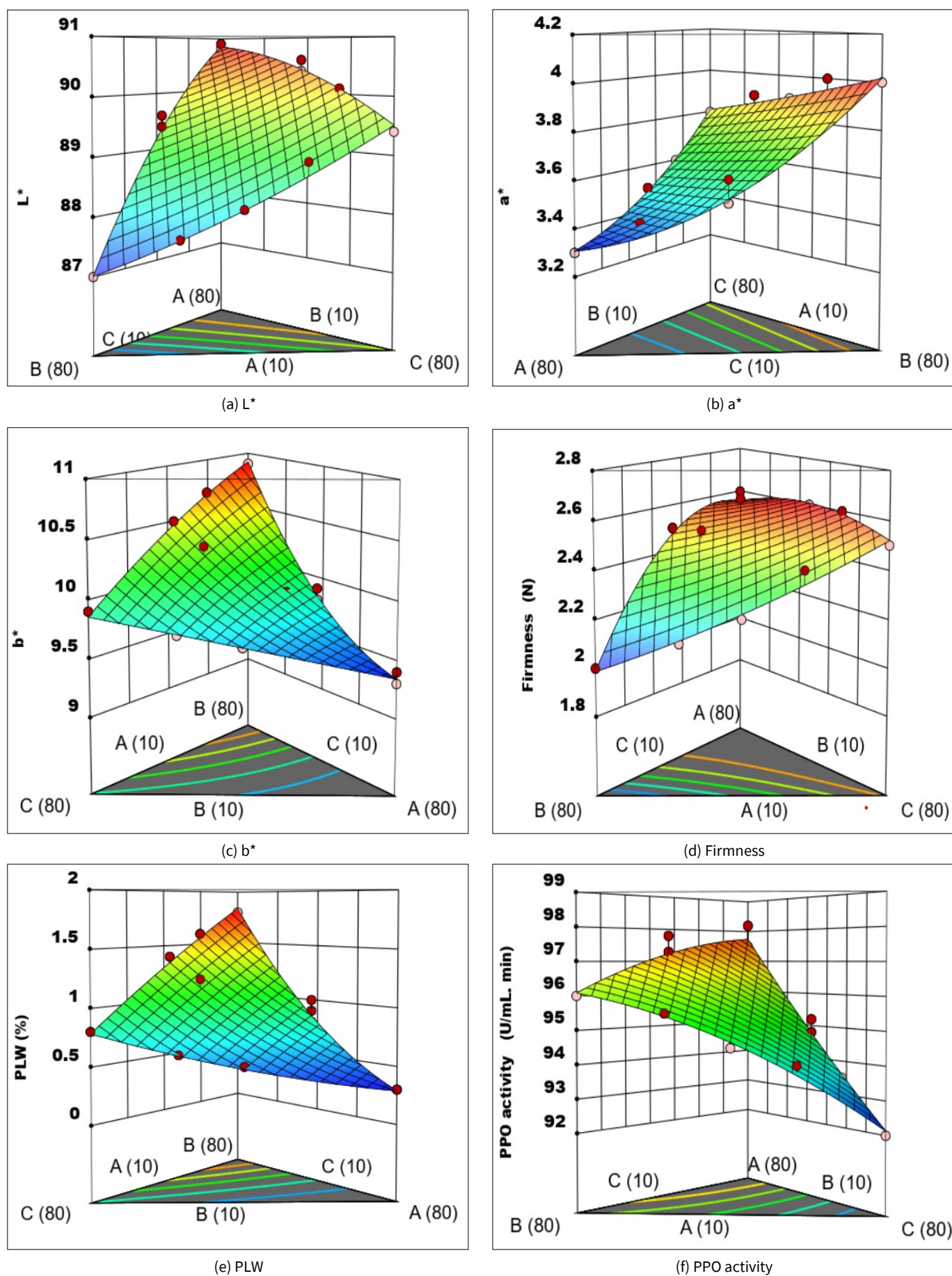


Fig. 1. Impact of different gas concentrations on the physicochemical properties of button mushroom. a: O_2 ; b: CO_2 ; c: N_2 ; d: Firmness; e: PLW; f: PPO activity.

optimised condition maintained PPO activity at approximately 92–95 U mL⁻¹ min⁻¹, indicating delayed enzymatic browning. The MAP helps to reduce browning mainly by limiting the amount of oxygen available and by keeping the cell membranes intact. When the membranes remain stable, the phenolic compounds stay separated from the PPO enzyme, so the browning reaction occurs much more slowly. However, high CO₂ concentrations may disrupt membrane stability and stimulate PPO activity (15). The observed increase in PPO activity under high-oxygen packaging conditions is consistent with earlier findings in button mushrooms (5, 25), suggesting that elevated oxygen levels promote enzymatic browning reactions. The effect of gas concentrations had a significant ($p < 0.0001$) effect on the PPO activity of button mushrooms. The fitted quadratic Scheffe model showed the highest coefficient of determination (0.961) with a non-significant lack of fit. The statistical results and the obtained coded equation were presented in Table 2.

Antioxidant activity

The antioxidant activity of fresh button mushrooms was 74.2 %, which declined to 62.9 % in the control sample on the 2nd day of storage, indicating progressive oxidative deterioration. It was observed from Fig. 2a that MAP maintained the antioxidant activity of button mushrooms, irrespective of gas concentration. High oxygen treatment maintained antioxidant activity around 73–74 %, while moderate oxygen conditions retained about 72–73 %. Low oxygen treatments showed comparatively lower values (approximately 66–69 %). The optimised condition preserved antioxidant activity close to 73–74 %, very similar to the fresh sample. The improved retention may be attributed to reduced oxidative reactions and slower degradation of phenolic compounds under the modified atmosphere. A retention in antioxidant activity was observed in button mushrooms stored under low modified atmosphere packaging (26). In contrast, there was an increase in antioxidant activity of button mushrooms with high oxygen MAP storage (27).

The statistical model was highly significant ($p < 0.0001$) with $R^2 = 0.981$ and F-value = 108.07. The CV was low (0.5261 %) and the lack-of-fit was non-significant, indicating a good fit of the model. The response of the dependent variable with respect to the changes in the independent variables predicted by a quadratic Scheffe model is presented in Table 2.

Microbial load

The changes in total bacterial count, yeast and mould count of button mushrooms stored under different gas compositions are presented in Fig. 2b and 2c. The control sample on the 2nd day of storage showed rapid microbial proliferation, where the total plate count increased to 5.2 log cfu g⁻¹ and the yeast and mould count reached 4.9 log cfu g⁻¹, indicating deterioration and spoilage of button mushrooms.

Under modified atmosphere packaging, microbial growth was suppressed during storage, as shown in Table 1. For total plate count, mushrooms stored under moderate oxygen conditions maintained microbial levels around 4.4–4.5 log cfu g⁻¹, whereas slightly higher counts (about 4.5–4.6 log cfu g⁻¹) were observed under low-oxygen treatments. High-oxygen atmospheres recorded comparatively lower counts close to 4.4 log cfu g⁻¹. The optimised gas composition also maintained the total plate count within 4.4–4.6 log cfu g⁻¹. Overall, high oxygen treatments recorded the lowest

yeast and mould count (about 3.56–3.62 log cfu g⁻¹), moderate oxygen maintained around 3.6–3.7 log cfu g⁻¹ and low oxygen treatments showed slightly higher counts. The optimised conditions maintained yeast and mould levels around 3.6–3.7 log cfu g⁻¹. These findings are consistent with earlier reports, where MAP significantly reduced microbial growth in mushrooms compared to ambient storage conditions. Previous studies have shown that CO₂ concentrations in the range of 10–20 % can effectively inhibit microbial proliferation by extending the lag phase and reducing the growth rates of spoilage microorganisms. Similarly, high-oxygen MAP has been reported to reduce total bacterial populations in fresh-cut produce such as broccoli, which aligns with the slightly lower microbial counts observed under higher O₂ conditions in the present study (28).

However, some contradictory findings have also been reported. Several studies on *Agaricus bisporus* suggest that low O₂ (2–5 %) combined with elevated CO₂ (10–15 %) is more effective in suppressing microbial growth due to reduced respiration and microbial metabolism (23, 24). In contrast, the present study indicates that higher O₂ levels (80–45 %) also contributed to microbial control, possibly by preventing anaerobic conditions that favour the growth of certain spoilage organisms. This observation is supported by earlier reports indicating that excessively low O₂ levels can induce anaerobic respiration, leading to the proliferation of facultative anaerobes and development of off-odours (29). Therefore, extremely low oxygen environments may not always be advantageous, as they can adversely affect product quality despite reducing overall metabolic activity. The reduced microbial growth in MAP is largely due to the inhibitory action of CO₂ on bacteria and fungi. When CO₂ dissolves in the tissue, it lowers the internal pH of microbial cells and disrupts their enzyme activity and membrane function, which slows down their growth. In contrast, fresh mushrooms spoil quickly in normal air because of their high moisture and rich nutrient content (28). The quadratic Scheffe model adequately described both responses with high coefficients of determination ($R^2 = 0.957$ for TPC and 0.954 for YMC), low coefficients of variation (0.3049 and 0.3214) and a non-significant lack of fit, confirming good model fitness (Table 2).

Validation of the predicted results for optimisation of gas concentrations for MAP

The adequacy of the quadratic Scheffe model used for the prediction of optimum gas composition was evaluated by conducting experiments at the predicted gas concentrations. Numerical optimisation was carried out by fixing goals to the independent and dependent variables. The dependent variables, such as L* value, antioxidant activity and firmness, were fixed at maximum. Whereas, the a*, b*, physiological loss in weight, polyphenol oxidase activity, total plate count and yeast and mould count were minimised. An importance level was assigned to be 3 for all the dependent variables. The fitted model predicted the optimal gas concentration was O₂ of 69 % and, CO₂ of 10 % and N₂ of 21 % with an overall desirability of 0.725, as shown in Table 3. The error percentage for the predicted and the experimental data was found to be in the range of 0.2–4.7 %, indicating the reliability of the model. The optimised MAP is a simple and cost-effective method that slows respiration, controls microbial growth and maintains the quality of button mushrooms. Therefore, this gas composition can be practically used to extend shelf life and reduce postharvest losses during refrigerated storage.

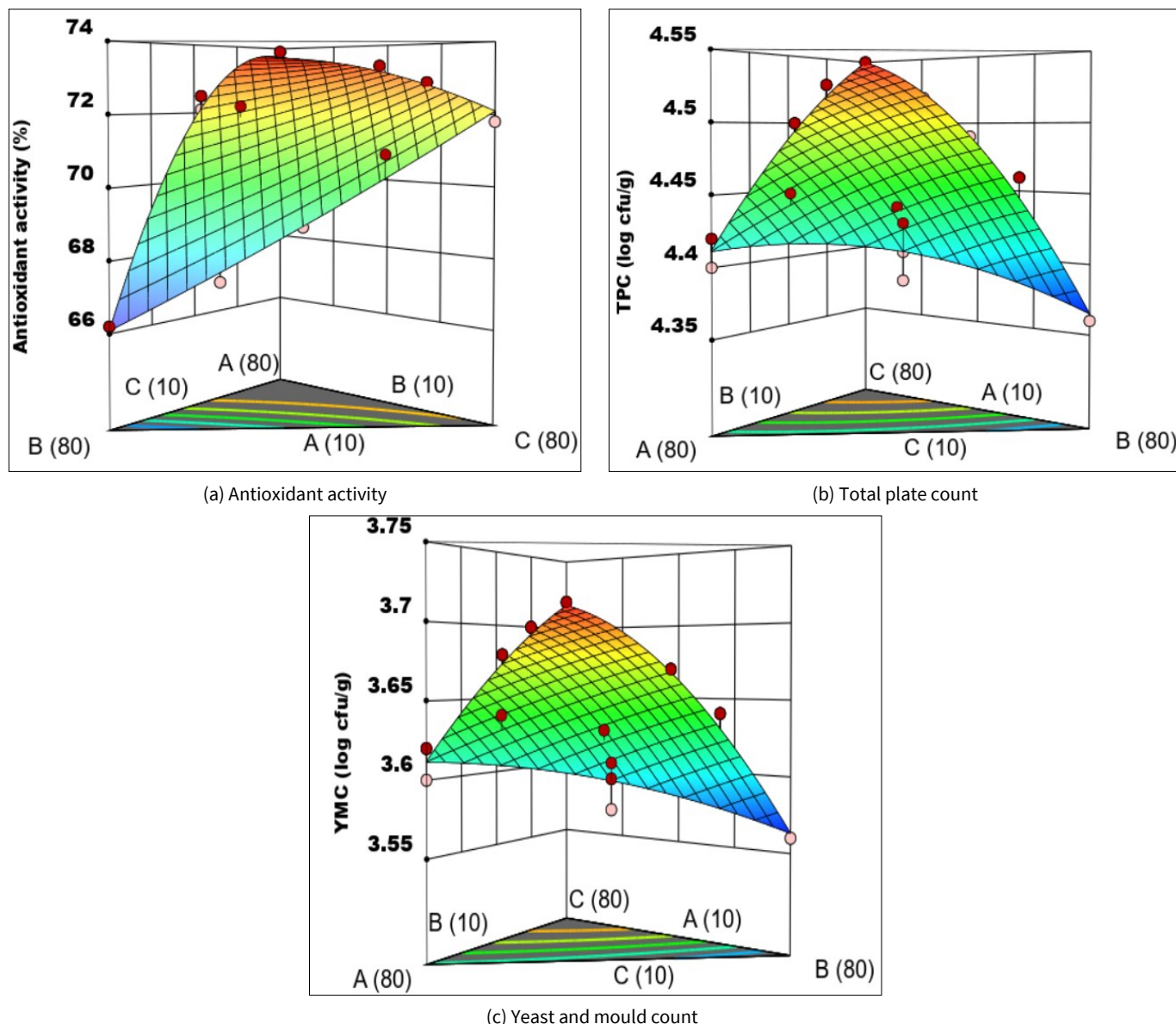


Fig. 2. Response surface plot for the changes in biochemical and microbial quality of button mushrooms. a: O₂; b: CO₂; c: N₂.

Table 3. Validation of the model using experimental and predicted data

Parameters	O ₂ (%)	CO ₂ (%)	N ₂ (%)	L*	a*	b*	Firmness (N)	PLW (%)	PPO activity (U mL ⁻¹ min ⁻¹)	Antioxidant activity (%)	Total plate count (log cfu g ⁻¹)	Yeast and mould count (log cfu g ⁻¹)	Desirability
Predicted	69	10	21	90.7	3.3	9.6	2.5	0.33	96	73	4.4	3.6	0.725
Actual	70	10	20	90.5	3.2	9.7	2.4	0.32	95	74	4.6	3.7	-
Error %	1.4	0	4.7	0.2	3.0	1.0	4.0	3.30	1.04	1.37	4.5	2.7	-

O₂: Oxygen; CO₂: Carbon dioxide; N₂: Nitrogen.

Conclusion

The present study showed that modified atmosphere packaging plays an important role in preserving the quality of button mushrooms after harvest. The optimised gas combination helped maintain colour and firmness, reduced weight loss and browning-related enzyme activity, preserved antioxidant content and limited the growth of bacteria, yeast and moulds compared with the control samples. Response surface methodology effectively identified the suitable atmosphere (about 69 % O₂, 10 % CO₂ and 20–21 % N₂) and the validation results closely matched the predicted values with very low error, confirming the reliability of the model. Overall, the optimised MAP condition provides a simple and practical method to maintain freshness and extend the refrigerated shelf life of button mushrooms, thereby helping to reduce

postharvest losses and improve their market quality. Future research may focus on evaluating the optimised MAP conditions at commercial scale, including storage, transportation and integration with other non-thermal treatments, along with sensory quality and consumer acceptance. It may also explore its applicability across different mushroom varieties and packaging materials to enhance industrial relevance.

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Authors' contributions

PN conducted the investigation, data analysis and prepared the initial draft. MB contributed to conceptualisation, provided resources and reviewed and edited the manuscript. SG supervised and validated the study. AR and AS assisted with the investigation and manuscript revisions. GT provided resources and overall supervision and contributed to manuscript revisions. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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