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A Farmer Affordable Technique: Packaging with Nitrogen Gas (N_2) Enhanced Shelf-Life of Fresh Oyster Mushroom (*Pleurotus ostreatus*)

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ABSTRACT

The commercial viability of mushrooms in Bangladesh faces a challenge due to the mushrooms' short shelf life, arising from their high respiration rates, elevated moisture content, and the absence of a protective cuticular structure. To solve this problem, different ways of enhancing the packaging conditions have been explored. Our research focuses on the use of the inert gas, nitrogen, to extend the shelf life of *Pleurotus ostreatus* (oyster mushrooms). Nitrogen, being an inert gas, is assumed to ensure the preservation of the quality of fresh mushrooms. In our study, freshly harvested mushrooms were carefully packaged in polypropylene (PP) bags both with and without nitrogen gas. Which were then stored both at room temperature and in the refrigerator. Throughout the storage period, the changes in seven quality parameters - total soluble sugar (TSS), browning index (BI), weight loss, pH, moisture content, odor, and texture were assessed at two-day intervals. The results revealed no significant changes in TSS or pH levels compared to the controls. Notably, mushrooms stored in PP bags having nitrogen and stored in the refrigerator exhibited improved quality over eight days, showing better results in BI, moisture content, and texture.

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INTRODUCTION

Edible mushrooms are quite remarkable organisms due to their ability to derive nutrients from lignocellulosic materials as noted in several studies (Ahmed et.al, 2023). Among these fungal species, the oyster mushrooms stand out as excellent sources of vitamins, proteins, and healthy fats as highlighted by (Ofodile et al., 2020) and gaining popularity due to their therapeutic and medicinal uses (Chugh et al., 2022: Bell et al., 2022). However, the global mushroom industry faces a significant challenge due to the perishability of oyster mushrooms. Fresh oyster mushrooms have a smooth appealing texture with a less resilient surface (Myronycheva et al., 2017) that starts to degrade right after harvest and may completely deteriorate within the following three days (Wang et al., 2017; Olotu et al., 2015). This limited shelf life at ambient temperatures thus poses a challenge for the storage and distribution of oyster mushrooms.

Besides mushroom morphology, several chemical and environmental factors are responsible for such high perishability. One major contributor is the mushrooms' high respiration rates, surpassing that of other fruits and vegetables. This heightened respiration is attributed to the thin and porous structure of the mushroom epidermis (Wakchaure 2011), which ultimately releases a substantial amount of water vapor. Consequently, this release causes shrinkage, weight loss, and potential issues when moisture accumulates in the packaging, fostering bacterial growth that discolors the mushrooms (Castellanons et al., 2021). Browning, a crucial quality indicator for mushrooms, can arise from such microbial attacks, physical damage, or enzymatic reactions catalyzed by polyphenol oxidases (PPO). While breakage-induced browning can be addressed through air-filling and sealed packaging, combating browning caused by PPO activity is more challenging. PPO oxidizes phenolic compounds within the fungi in the presence of oxygen, posing difficulties in its preservation (Janusz et al., 2020). Fortunately, previous research has shown that both high respiration rates and PPO activity rely on atmospheric oxygen(Stolper, et al., 2010; Gholami et al., 2019). This suggests that controlling the oxygen concentration within the packaging could effectively mitigate these negative factors. While eliminating air from mushroom packets increases the risk of breakage, replacing the packaging air with inert gas nitrogen offers a plausible and convenient solution (Park et al., 2019). Thus, the objective of this study was to evaluate the effects of nitrogen as a packaging gas on the key quality parameters (moisture loss, weight loss, texture, browning index, pH, and total soluble sugar) of harvested oyster mushrooms. The findings from this research are expected to enhance our understanding of the post-harvest biology of mushrooms and provide valuable insights for producers and merchants seeking information on effective packaging techniques.

MATERIALS AND METHODS

In this study, freshly harvested oyster mushrooms were procured from the Mushroom Development Institute (MDI) at Savar, Dhaka, Bangladesh. The collected mushrooms underwent a meticulous sorting process based on size and appearance criteria. Specifically, mushrooms displaying a white, firm texture with a fresh and smooth appearance were selected as the experimental samples. Any damaged, extra-large, and small mushrooms were deliberately excluded from consideration. For packaging, we utilized polypropylene bags, with each bag containing a standardized amount of 200±5g of mushrooms. The mushroom packets were subjected to two treatment conditions: one group treated with N_2 gas and another without N_2 gas. After treatment, the bags were securely sealed and stored under two distinct temperature conditions, namely ambient and refrigeration. Each treatment condition consisted of five replications to enhance the robustness and reliability of the experimental design. The nitrogen gas treatment was conducted at the laboratory of the Department of Environment Sciences at Independent University, Bangladesh.

Quality attributes: The data were gathered according to the specified parameters.

Texture

The mushroom textures were analyzed using a Perten TVT 300 XP texture analyzer, with a focus on assessing the alterations in resilience and hardness of the fruiting bodies throughout the course of the investigation. Measurements were taken at a height of 40mm, employing a test speed of 1.5 mm/s, a trigger force of 25 g, and a compression depth of 5.0 mm, with the recorded data reflecting changes in the mushrooms' physical properties.

Moisture content (%)

The moisture level in the samples was analyzed using an AnD MX-50 Moisture Analyzer, equipped with both heating and weighing units. The mushrooms were subjected to constant heating at 110°C within the analyzer, and the resulting total moisture contents were recorded as a percentage. The moisture content was determined using the following expression:

$$\text{Moisture content } (\%) = \frac{\text{Weight of fresh sample} - \text{Weight of dried sample}}{\text{Weight of fresh sample}} \times 100$$

Browning Index

Browning index was measured using a color analyzer. From each treatment, three mushrooms were selected, and readings were taken in triplicate for each sample. The color parameter values, where L^* represents lightness and darkness, a* indicates redness or greenness and b^* stands for yellowness or blueness, were used to calculate the browning index using the following formula.

$BI = 100 \times \frac{X-0.31}{0.71}$ Where, $X = \frac{a+1.75L*}{5.645L*+a*-3.012b*}$----------(Bozkurt and Bayram, 2006).

Weight loss

For weight loss (W_L) of the samples, the initial weights (W_0) and the final weights (W_f) of the packaged mushrooms were weighed using an electronic balance (Xpart Weighing Scale). The readings were taken in grams and the following equation was used for the investigation.

($W_L = ((W_o - W_f)/W_o) \times 100$. ----------(Jafri et al., 2013).

Results were expressed as an average of three replicates.

Odor

The distinctive aroma of oyster mushroom is widely acknowledged, and alterations in smell are often associated with the extent of mushroom spoilage. To assess the odor of mushroom samples over varying storage durations, a panel of 10 evaluators was assembled. All the panel members possessed a high level of education (above secondary education) and were well-conversant in agricultural products. Most of the evaluators were experts in mushrooms, including scientists and technicians affiliated with the National Mushroom Development Institute. The olfactory profiles of the mushrooms were documented daily from the initial day through the last day of storage, with the intensity of the odor categorized accordingly (Table 1).

Table 1. Category of odor with score

Score	Odor
4	It has strong fragrance
3	Normal, No peculiar smell
2	Slightly unpleasant smell
1	Serious unpleasant smell

pH Measurement:

The pH is measured using a pH meter, which includes a sensing unit comprising a glass electrode and a reference electrode (commonly a calomel electrode) connected by KCl Bridge to the pH sensitive glass electrode. An indicating unit is also part of the system, providing the corresponding pH reading based on the electromotive force detected.

TSS Measurement

Total Soluble Solids (TSS) was measured by refractometer. The measurement was conducted by dripping the liquid extract of mushroom fruiting bodies onto the detector. The TSS value is expressed as % Brix, and the displayed value is derived from the ratio of the speed of light in a vacuum to the speed of light through the sample.

Statistical Analysis

Three independent replications of all experiments were conducted. Data were analyzed with the statistical analysis software MS Excel. All data were subjected to an analysis of variance and a least significant differences test to determine significant differences $P \leq 0.05$ among the treatments.

RESULTS AND DISCUSSION

Texture Analysis

From the perspective of consumer acceptance, texture stands out as a crucial quality parameter. Typically, as mushrooms are susceptible to softening during storage. Our investigation aimed to understand the impact of various storage conditions on the texture of mushrooms, with a focus on firmness, hardness, and resilience. As illustrated in Table 2, we observed a gradual decline in firmness over the storage period, indicating the susceptibility of mushrooms to softening during storage. The results indicate a relationship between texture and storage temperature, highlighting that lower temperature contributes to better-preserving mushroom texture. Additionally, a significant observation in our study was the effect of adding N_2 gas to the samples. This addition demonstrated a notable impact on preserving mushroom freshness, leading to prolonged firmness. The softening or loss of hardness in mushrooms during storage has been attributed to membrane changes (Anupama et al., 2022). As noted by Zivanovic et al., 2000, texture alternations are also linked to protein and polysaccharide degradation, hyphae shrinkage, disruption of the central vacuole, and expansion of the intercellular space at the piles surface.

Table 2. Analysis of the Texture of Oyster Mushrooms during Storage

Parameters	1st Day		2nd Day		4th Day		6th Day		8th Day	
	Hardness	Resilience	Hardness	Resilience	Hardness	Resilience	Hardness	Resilience	Hardness	Resilience
Ambient	172.00±3.5	0.24±0.05	75.66±2.08	0.55±0.02	-	-	-	-	-	-
N_2 + Ambient	176.67±3.05	0.32±0.04	154.33±4.16	0.56±0.02	84.66±6.65	0.58±0.07	-	-	-	-
Refrigeration	170.03±3.5	0.22±0.07	142±3.53	0.25±0.02	116.5±3.5	0.30±0.02	96 ±5.65	0.35±0.04	-	-
N_2 + Refrigerator	172.67±3.8	0.23±0.02	156.67±8.32	0.33±0.02	101.33±1.5	0.42±0.01	81.33±4.50	0.52±0.06	75.66±2.08	0.55±0.02

Moisture Content

The moisture content of food plays a pivotal role in influencing various attributes such as taste, texture, appearance, shape, and weight. Fresh oyster mushrooms typically have a moisture content ranging from 85% to 95% (Kumar et al., 2013). Upon analysis of the provided bar chart (Figure 1), a consistent decrease in moisture percentage is evident at two-day intervals. Notably, samples stored in ambient conditions exhibit more pronounced fluctuations in moisture percentage compared to their refrigerated counterparts. In contrast, samples subjected to refrigerated + N_2 gas show reduced moisture loss and display a tendency towards shrinkage. The prolonged shelf life and sustained freshness observed in refrigerated+N_2 samples can be attributed to the lower rates of moisture exchange and metabolic activities. The use of N_2 gas, being an inert gas, contributes to creating an environment that minimizes the impact of respiration on moisture content.

Browning Index

Consumers generally prefer white mushrooms such as Oyster, Milky white and Button mushroom varieties, due to their pristine white appearance. Post-harvest browning is a critical factor in determining mushroom pricing as it significantly impacts the quality of the mushrooms. This browning occurs mainly because phenolic substances convert into quinones through oxidation, primarily driven by the enzyme Polyphenol oxidase (PPO) (Huang et al., 2017) The browning

index gradually increases during the storage period, with mushrooms stored in all treated conditions ambient, refrigeration with N_2 or without N_2 (Table 3). Comparative analysis reveals that mushrooms stored in ambient conditions undergo noticeable browning and develop a slimy appearance by the second day of the experiment. In contrast, under refrigeration samples exhibited the least browning.

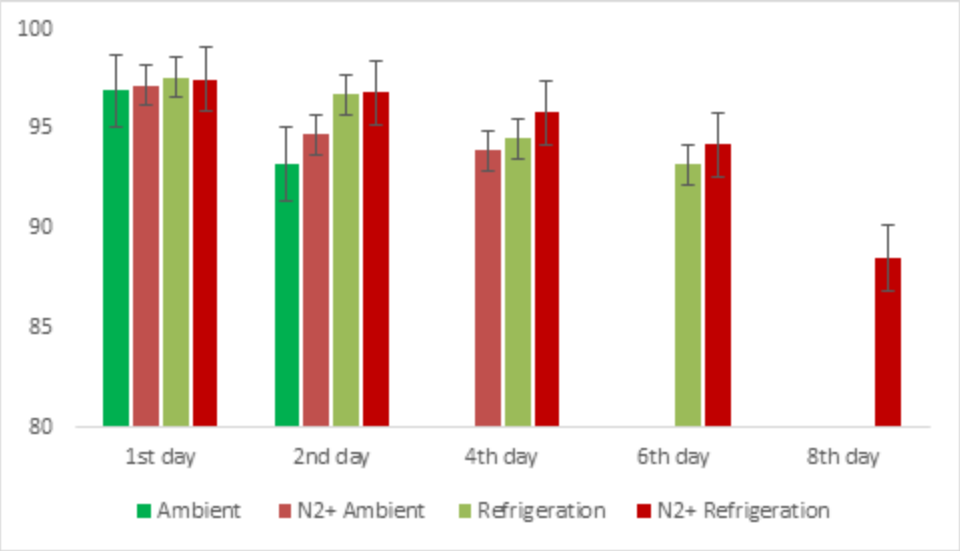

Figure 1. Moisture content (%) of mushroom during storage

Table 3. Browning Index of oyster mushroom during storage

Treatment	Total Soluble Sugar (TSS)				
	1st day	2nd day	4th day	6th day	8th day
N2 + Ambient	6.3±0.3	5.5±0.2	5.0±0.7	-	-
Refrigeration	5.9±0.8	5.2±0.6	5.1±0.3	4.6±0.8	-
N2 + Refrigerator	6.2± 0.6	5.7±0.6	4.9±0.4	4.9±0.5	4.8±0.4

Weight Loss and Microbial Study

Previous research, conducted by Xiao et al., 2011, has established weight loss as a prevalent factor in the post-harvest loss of mushrooms. However, in our study, no significant losses in the weight of mushrooms were observed. This discrepancy may be attributed to the relatively small quantity of mushrooms used in the experiment, with only 200g per packet. Similarly, throughout the experiment, no evidence of microbial contamination was identified.

Oduor:

The distinctive aroma of mushrooms is a result of the intricate interplay among various compounds, including alcohols, aldehydes, ketones, acids, hydrocarbons, esters, as well as groups such as heterocyclic, aromatic, and sulfur compounds. (Aisala et al., 2019). These compounds not only play a crucial role in determining the post-harvest quality of mushrooms, contributing to their distinctive aroma, but also serve important functions in the growth physiology and interactions of mycelium (Ditengou et al., 2015) Our sensory panel observed noticeable changes in the aroma of mushrooms under different storage conditions. At ambient temperature, the mushrooms exhibited signs of spoilage by the second day with a score of 1, indicating a seriously unpleasant smell. Similarly, under ambient temperature with N_2 gas, the smell had onset to change, registering a score of 2 for a slightly unpleasant smell within 4 days. In contrast, mushrooms stored under

refrigeration, both with and without N_2 remained unchanged, receiving a score of 3, indicating a normal, no peculiar smell up to day 6. Further assessment on day 6 focused solely on the refrigerated samples. The sample without N_2 had undergone a noticeable change in odor, while the sample with N_2 had also started to show alterations. These findings highlight the impact of storage conditions, particularly temperature and the presence of N_2 gas, on the aroma of mushrooms.

Changes in pH

The pH of foods is a critical factor influencing their appearance, texture, flavor, nutritional content, and safety. The pH value reflects the concentration of free hydrogen ions in a food, directly impacting its acidity or alkalinity. According to Table 4, the pH is gradually increasing in both ambient and refrigerated conditions, signaling a shift towards which indicates heading to alkaline conditions. To maintain the freshness of mushrooms post-harvest, it is recommended to keep the pH within the range of 6.0 to 7.0 (Fatih et al., 2020).

Table 4. Changes in pH among the Treatment

Treatment	**pH**				
	1st day	2nd day	4th day	6th day	8th day
N2 + Ambient	6.03±0.05	7.24±0.02	7.48±0.02		
Refrigeration	6.05±0.08	6.08±0.03	7.15±0.05	7.67±0.06	
N2 + Refrigerator	6.59±0.07	6.66±0.05	7.51±0.03	7.71±0.02	7.8±0.02

Changes in Total Soluble Sugar (TSS)

The concentration of total soluble sugar (TSS) was determined by assessing the cumulative content of sugar forms in the fruit, encompassing sucrose, glucose, fructose, and sorbitol. As depicted in Table 4, there is a gradual decrease in TSS level in both conditions, although no significant changes were observed. Particularly noteworthy is the observation that, after four days of storage, the TSS value remained constant in the refrigerator condition. Despite an overall decline in sugar levels in both storage conditions, it was noted that the samples subjected to refrigeration exhibited a slower rate of decrease in the sugar content, compared to those stored in ambient conditions.

Table 4. Changes in TSS among the Treatments

Treatment	**Total Soluble Sugar (TSS)**				
	1st day	2nd day	4th day	6th day	8th day
N2 + Ambient	6.3±0.3	5.5±0.2	5.0±0.7	-	-
Refrigeration	5.9±0.8	5.2±0.6	5.1±0.3	4.6±0.8	-
N2 + Refrigerator	6.2± 0.6	5.7±0.6	4.9±0.4	4.9±0.5	4.8±0.4

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CONFLICT OF INTEREST

The authors affirm that the research was carried out without any commercial or financial affiliations that might pose a conflict of interest.

REFERENCES

1. Ahmed R, Niloy MAHM, Islam MS, Reza MS, Yesmin S, Rasul SB and Khandakar J, 2024. Optimizing tea waste as a sustainable substrate for oyster mushroom (Pleurotus ostreatus) cultivation: a comprehensive study on biological efficiency and nutritional aspects. Frontiers in Sustainable Food Systems, 7: 1308053.
2. Aisala H, Sola J, Hopia A, Linderborg KM, Sandell M, 2019. Odor-contributing volatile compounds of wild edible Nordic mushrooms analyzed with HS-SPME-GC-MS and HS-SPME-GC-O/FID. Food Chemistry, 283: 566–578.
3. Anupama PP, Rathod MG, Gurunwad JM, and Kamble G, 2022. “A Comprehensive review on post-harvest progressing of mushroom. Publication, Distribution and Promotion by Bhumi Publishing, Nigave Khalasa, Kolhapur, ISBN: 978-93-91768-86-7
4. Bell V, Silva C R P G, Guina J, and Fernandes T H, 2022. Mushrooms as future generation healthy foods. Frontiers in Nutrition, 9: 1050099.
5. Bozkurt H, Bayram M, 2006. Colour and textural attributes of sucuk during ripening. Meat Science, 73(2): 344-350.
6. Castellanos-Reyes K, Villalobos-Carvajal R, Beldarrain-Iznaga T, 2021. Fresh Mushroom Preservation Techniques. Foods, 10(9): 2126.
7. Chugh RM, Mittal P, Arora T, Bhattacharya T, Chopra H, Cavalu S and Gautam RK, 2022. Fungal Mushrooms: A Natural Compound with Therapeutic Applications. Frontiers in Pharmacology, 13: 925387.
8. Ditengou FA, Müller A, Rosenkranz M, Felten J, Lasok H, van Doorn MM, Legué V, Palme K, Schnitzler JP, Polle A, 2015. Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. Nature Communications, 6(1): Article 1.
9. Fang, Y.; Wakisaka, M. 2021. A Review on the Modified Atmosphere Preservation of Fruits and Vegetables with Cutting-Edge Technologies. Agriculture, 11: 992.
10. Fatih T, Murat O, Mehmet M, 2020. The combined effect of exposure time to sodium chlorite (NaClO2) solution and packaging on postharvest quality of white button mushroom (Agaricus bisporus) stored at 4°C. Food Science and Technology, 40(4): 864-870.
11. Gholami R, Ahmadi E, Ahmadi S, 2019. Investigating the effect of chitosan, nanopackaging, and modified atmosphere packaging on physical, chemical, and mechanical properties of button mushroom during storage. Food Science and Nutrition, 8(1): 224-236.
12. Huang H, Ge, Z., Limwachiranon J, Li L, Li , Luo, Z, 2017. UV-C treatment affects browning and starch metabolism of minimally processed lily bulb. Postharvest Biology and Technology, 128: 105– 111.
13. Jafri M, Jha A, Bunkar DS, Ram RC, 2013. Quality retention of oyster mushrooms (Pleurotus florida) by acombination of chemical treatments and modified atmosphere packaging. Postharvest Biology and Technology, 76: 112–118.
14. Janusz G, Pawlik A, Świderska-Burek U, Polak J, Sulej J, Jarosz-Wilkołazka A, Paszczyński A. 2020. Laccase Properties, Physiological Functions, and Evolution. International Journal of Molecular Sciences, 21(3): 966.
15. Kumar A, Singh M, Singh G. 2013. Effect of different pretreatments on the quality of mushrooms during solar drying. Journal of Food Science and Technology, 50(1): 165-70.

16. Myronycheva, Olena, Bandura I, Bisko N, Andrii P. Gryganskyi, and Karlsson O, 2017. “Assessment of the Growth and Fruiting of 19 Oyster Mushroom Strains for Indoor Cultivation on Lignocellulosic Wastes.” BioResources 12(3): 4606–26.
17. Ofodile, Nwanneka, Nwachukwu V, Nicholas-Okpara VN, Emmanuel A, Ikegwu E, Saanu A, Peace C, Ezenwa, Osorinde R, 2020. Production and Nutritional Composition of Juice Powder from Oyster Mushroom Pleurotus Ostreatus (Jacq.) Kummer. Functional Foods in Health and Disease, 10: 482–92.
18. Olotu, Ifeoluwa O., Adewale O. Obadina, Olajide P. Sobukola, Mojisola Adegunwa, Abdulrasaq A. Adebowale, Esther Kajihausa, Lateef Oladimeji Sanni, Yemisi Asagbra, Bolanle Ashiru, and Tomlins Keith. 2015. Effect of Chemical Preservatives on Shelf Life of Mushroom (Pleurotus Ostreatus) Cultivated on Cassava Peels. International Journal of Food Science & Technology 50(6): 1477–83.
19. Park, Jong-Jin, Lee JJ, Olawuyi IF, and Lee W, 2019. Effect of Argon- and Nitrogen-Based Modified Atmosphere Packaging on Shiitake Mushroom Quality. Korean Journal of Food Preservation, 26(4): 391–98.
20. Stolper, Daniel A., Niels Peter Revsbech, and Donald E. Canfield. 2010. Aerobic Growth at Nanomolar Oxygen Concentrations. Proceedings of the National Academy of Sciences of the United States of America, 107(44): 18755–18760.
21. Wang, Qiong, Chu L, and Kou L, 2017. UV-C Treatment Maintains Quality and Delays Senescence of Oyster Mushroom (Pleurotus Ostreatus). Scientia Horticulturae, 225: 380–85.
22. Wakchaure, G C. 2011. Postharvest Handling of Fresh Mushrooms. In, 197–206.
23. Xiao G, Zhang M, Shan L, You Y, Salokhe VM, 2011. Extension of the shelf-life of fresh oyster mushrooms (*Pleurotus ostreatus*) by modified atmosphere packaging with chemical treatments. African Journal of Biotechnology, 10(46): Article 46.
24. Zivanovic S, Busher RW, Kim KS, 2000.Textural changes in mushrooms (*Agaricus bisporus*) associated with tissue ultrastructure and composition. Journal of Food Science, 65: 1404-1408.