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Comparative Evaluation of Storage Temperatures on Physiological and Microbiological Parameters of Chemically Treated Button Mushroom (*Agaricus bisporus*) Stored under Modified Atmospheric Packaging (MAP)

Pallavi Srivastava[1], Nitish Kumar[1], Ruqaiya Bano[1], Pravin Prakash[2], Durga Shankar Bunkar[2], Garima Singh[1], Abhinay Shashank[2]

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ABSTRACT

Background: Button mushrooms have long been esteemed for their significant contribution to addressing various human ailments. Their delectable taste, rich flavor and enticing aroma render them a coveted centerpiece on every dining table. However, their inherent susceptibility to decay, owing to a rapid transpiration rate and thin epidermis, typically results in spoilage within a mere two days post-harvest.

Methods: The current experiments was done at Banaras Hindu University to investigate the effect of different temperature, chemical and MAP treatments on physico-chemical and microbiological parameters. Chemically treated button mushrooms with $CaCl_2$, citric acid and 0.1% sorbitol were packed in two MAP air compositions followed by storage at 8°C and 12°C for 16 days and observations were recorded at an interval of 4 days for 16 days.

Result: Chemically treated button mushrooms with the gas composition of 12% CO_2; 6% O_2 exhibit better quality retention parameters at 8°C, resulting in a shelf life of around 16 days. Blanched samples exhibit more tissue exudation and browning, responsible for fast tissue degradation and quality loss. Blanched samples without modified atmosphere packaging underwent spoilage and decay after eight days at 8°C.

Key words: $CaCl_2$, Modified atmosphere packaging, Temperature, Sorbitol.

INTRODUCTION

The growing demand for low-cholesterol, low-fat foods rich in nutrients, vitamins, and medicinal properties is expected to drive the global button mushroom market. However, button mushrooms have a short post-harvest lifespan, typically lasting only 1-4 days at ambient conditions and 4-6 days under refrigeration. This rapid deterioration is primarily attributed to high transpiration rates, metabolic activity, and moisture loss from their delicate epidermal structure (Ares *et al.,* 2007). The perishable nature of button mushrooms, coupled with significant post-harvest losses (30–35%), poses challenges for long-distance transportation and international trade. Common post-harvest issues such as browning, veil-opening, weight loss, and microbial degradation often result in substantial economic losses.

Modified atmosphere packaging (MAP), involving the alteration of ambient air composition to create an optimal environment for decreasing the mushroom's respiration rate and extending its shelf life, has shown promise in mitigating these challenges. Treatments such as chemical applications (*e.g.,* calcium chloride, sorbitol and citric acid), MAP and low-temperature storage have been demonstrated to effectively prolong the shelf life of button mushrooms to 12-16 days (Koushki *et al.,* 2011; Oliveiraa *et al.,* 2012).

[1]Deparatment of Agriculture, Integral Institute of Agricultural Science and Technology, Integral University, Lucknow-226 026, Uttar Pradesh, India.

[2]Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi- 221 005, Uttar Pradesh, India.

Corresponding Authors: Nitish Kumar, Pallavi Srivastava, Integral Institute of Agricultural Science and Technology, Integral University Lucknow-226 026, Uttar Pradesh, India.
Email: nitish10nduat@gmail.com; srivastavapallavi52@gmail.com

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The primary objective of this study is to determine the storage duration of button mushrooms packed under MAP while maintaining acceptable microbiological counts. Assessment of total plate count and yeast mold estimation will provide insights into the suitability of button mushrooms for consumption purposes (Farzana *et al.,* 2016).

MATERIALS AND METHODS

Button mushrooms (*Agaricus bisporus*) were obtained from the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, in the morning. Chemicals were supplied from Himedia (Mumbai, India). Button mushrooms were packed in polybags made of Polyamide 20µm-Polyethylene 70µm with EVA (Ethylene-vinyl acetate sealant layer). Oxygen permeability and water vapour transmission rate were 50 cm^3/m^2.day.bar (both at 23°C and 0% RH) and 2.8g/m^2.day (at 23°C and 85% RH), respectively.

Processing and packaging procedures

Fresh button mushrooms, free from wounds and blemishes, were selected and cleaned. Fresh button mushrooms free from physical injuries were dipped in a solution of $CaCl_2$ (2.5%, w/v), citric acid (3%, w/v) and sorbitol (0.1 %, w/v) for 4 min and were air-dried for 12 hours (at 25±5 °C and RH 85±5%). Test samples were prepared by placing (40±5 g) chemically treated button mushrooms in polyethylene packets of Polyamide 40 µm-Polyethylene 120 µm with EVA (Ethylene-vinyl acetate sealant layer). Packaging button mushrooms followed chemical treatment under different MAP treatments by modified atmospheric packaging unit (VAC-STAR* S 220 MP, Sugiez, Switzerland).

In case of MAP treatments we had taken three MAP treatments *i.e.*

I. MAP1 (12% CO_2; 6% O_2).
II. MAP2 (12% CO_2; 6% O_2).
III. MAP3 (Control).

The button mushrooms after chemical treatments and MAP packaging were stored at 8±1°C and 12±1°C for 16 days, and observations have recorded an interval of 4 days with the three replications.

After providing with different MAP treatments, the button mushroom air tight packets were stored at three different temperatures *i.e.*

I. Low temperature (8±1°C).
II. Normal temperature (12±1°C).

Headspace gas analysis

Percentage CO_2 and O_2 concentrations were determined with the help of a gas analyzer (MAP Mix 9001 ME, PBI Dansensor, Ringsted, Denmark) using a needle were inserted in samples through the septum to assure the closure of the hole, the gas composition was recorded.

Total soluble solids

Button mushroom extract was prepared and was analyzed with the help of a refractometer (Model RFM 970, Bellingham and Stanley, UK) (Eissa, 2008) for finding total soluble solids.

Ascorbic acid content

Ascorbic acid standard

100 mg of L-ascorbic acid was measured and volume was made up to 100 ml with 3% HPO_3 solution (Harris and Ray, 1935).

2, 6-di-chlorophenol indophenol dye solution

42 mg of sodium carbonate was dissolved in distilled water and 52 mg of 2, 6-di-chlorophenol indophenols was mixed into it followed by volume make up to 200 ml.

Standardization of dye

5 ml of the standard ascorbic acid solution was taken into a 100 ml flask, followed by 5 ml of the 3% HPO_3 solution. Microburette was filled with the dye solution. The ascorbic acid solution was titrated with the dye solution until the appearance of pink colour. Titrated value was recorded.

Dye factor: 0.5/Titrate value.

Oven-dried button mushrooms were ground into powder form in a mixer grinder and samples 2 g were taken and dissolved in 3 % HPO_3 and volume was made up to 100ml. The aliquot was centrifuged at 2000 g for 15 minutes. 5 ml of supernatant was taken into 10 ml of 3% of HPO_3 and titrated against the dye (V_2 in ml). The initial and final reading of the dye solution was recorded in the presence of pink colour. The concentration of ascorbic acid in mg/g sample was estimated by formula:

$$\frac{\text{Dye factor} \times V_2 \times 10000}{V_1 \times W}$$

Where,
W = Weight of sample taken.
V_1 = Sample of extract taken.
V_2 = Required dye solution for titration.

Total polyphenol content (TPC)

Oven-dried button mushrooms at 65°C were taken and ground into fine powder. Dried mushroom powder (1 g) was mixed with 15 ml of distilled water. The aliquot was kept on a shaker incubator at 150 rpm for 24 hours at room temperature. Button mushroom samples were taken followed by filtration and were centrifuged at 2000 rpm for 15 minutes. Total polyphenol compounds of mushrooms extract were calculated with the help of the Folin-Ciocalteu reagent. An aliquot of 100µL (1:5 dilution) was mixed with 100 µl of Folin–Ciocalteu reagent (1:1 dilution with distilled water) and 2 ml of 10% sodium carbonate solution was added.The absorbance was recorded in a spectrophotometer at 750 nm. The same extract of button mushroom prepared for total polyphenols was used for finding the radical scavenging activity. The absorbance of the samples was recorded at 515 nm using a spectrophotometer.

Total plate count (TPC)

One gram of *Agaricus bisporus* was homogenized in 9.0ml sterile 0.1% peptone water for 30 seconds to a homogenous suspension. 17.5 g of plate count agar was dissolved in 1000 ml distilled water followed by heating for complete dissolution. All the procedure was done in sterilized conditions in laminar flow (Omicron Sensing Inc. U.S.A, model: H2000-25mmWC). Plates were incubated at 37°C

for 24 hours in an incubator (Metrex Scientific Instruments (P) Ltd., New Delhi). The colonies were counted with the help of a digital colony counting meter, and colonies were expressed in a log colony-forming unit per gram (log cfu/gm). (Harrigan, 1998).

Yeast and moulds estimation

Yeast and molds were determined by the pour plate method. In aseptic condition, 25 g of test pieces were kept in a conical flask with a glass stopper which contains 225 ml of phosphate-buffered saline. Then conical flask contents were transferred into a sterile blender jar and blended at low speed for 1 minute. The 10^{-1} (1:10) dilution was obtained by adding 1 ml of aliquot to 99 ml of phosphate-buffered saline. Then again, we used a sterile pipette to transfer 1ml of 10^{-1} dilution to 99 ml of phosphate-buffered saline followed by cap placing shaking for 3 minutes (Teramura *et al.,* 2015):

Total moulds and yasts count per 1 g sample = Colonies count × dilution factor

Statistical analysis

All the observations were recorded in triplicates, and data were processed to two-way analysis of variance (ANOVA) considering different chemical, MAP treatment and storage time. S.Em± (Standard error of the mean) and critical difference at 5% was calculated (Dean and Voss, 1999).

RESULTS AND DISCUSSION

The blanched samples spoiled after 12 days, whereas chemically treated and MAP packed samples were intact even after 16 days. After 16 days of the storage period, CM1 had better retention in the quality parameter than other samples.

Headspace gas composition

A rapid decline in oxygen (O_2) [Fig 1(A) and Fig 1(B)] concentration and a simultaneous increase in carbon dioxide (CO_2) [Fig 2(A) and Fig 2(B)] concentration were observed at 8°C. Similar observations of decreased O_2 and increased CO_2 concentrations under modified atmosphere packaging (MAP) treatments during storage were documented in previous studies by researchers (Antmann *et al.,* 2008; Jafri *et al.,* 2013). The higher transpiration rates of button mushrooms at elevated temperatures, along with the permeability of polyethylene packaging bags, are likely contributing factors to the changes in gas concentration during storage.

Total soluble solids

An increase in TSS is possibly due to converting polysaccharides like cellulose, starch, pectin etc., into simple sugars like glucose and fructose. The rise in TSS of sample CM1 was slower at both 8°C and 12°C than other samples (Díaz-Mula *et al.,* 2011). The TSS of CM1 after 16 days of storage at 8°C and 12°C was 6.09% and 7.10%, respectivelyas shown in [Fig 3(A) and Fig. 3(B)]. Higher temperature increases the tissue temperature of button

Table 1: Effect of chemical, MAP treatments and storage time on total plate count and yeast and molds count of button mushroom (*Agaricus bisporus*).

Days	Unit	CM1		CM2		BM1		BM2		BM3		S.Em±	CD at 5%
		8°C	12°C	8°C	12°C	8°C	12°C	8°C	12°C	8°C	12°C		
Total plate count													
0	(cfu $g^{-1} \times 10^{-3}$)	60.35	60.35	60.35	60.35	60.35	60.35	60.35	60.35	60.35	60.35	-	-
4		70.33	81.00	85.00	90.00	126.67	137.67	122.00	141.67	178.33	195.33	2.955	8.27
8		106.00	110.00	104.33	112.20	125.33	143.33	134.67	154.00	158.67	176.67	3.49	9.78
12	(cfu $g^{-1} \times 10^{-4}$)	100.67	121.33	138.67	136.67	121.67	123.33	120.00	141.00	230.33	243.67	2.01	5.65
16		109.00	119.33	110.90	TNTC	179.33	TNTC	220.67	TNTC	224.00	TNTC	2.87	8.06
Yeast and molds count													
0	(cfu $g^{-1} \times 10^{-2}$)	30.23	30.23	30.23	30.23	30.23	30.23	30.23	30.23	30.23	30.23	-	-
4		37.00	52.67	48.00	54.67	93.33	101.00	64.67	70.33	109.00	117.00	2.34	6.58
8		72.00	81.67	84.00	90.67	125.00	139.67	120.67	144.33	174.33	197.00	5.68	NS
12		85.33	98.33	91.33	108.00	146.00	200.67	179.00	190.0	202.00	228.67	1.44	4.03
16		100.00	125.33	109.67	148.00	199.33	257.00	167.67	224.33	229.67	TNTC	1.25	3.51

(Means of three replications ± standard deviation).

mushrooms, which initiates the biochemical reactions responsible for the deterioration of mushroom quality (Gholamia *et al.,* 2017).

Ascorbic acid content

A sharp decline in ascorbic acid content was recorded with the storage period. Ascorbic acid is thermolabile in nature, and blanching in hot water can lead to appreciable loss of its concentration. Low-temperature storage is possibly the most suitable method for controlling ascorbic acid loss (Ishaq and Obirinakem, 2015). The rate of decrease of ascorbic acid content was higher at 12°C as compared to 8°C as shown in in [Fig 4(A) and Fig 4(B)]. A minimum decrease in ascorbic acid content was recorded for CM1 at 8°C.

Total polyphenol content

Polyphenol compounds with anti-oxidant activity are present in methanolic extracts button mushrooms (Tsai *et al.,* 2007).

Total phenolic content in fresh button mushrooms was recorded as 3.50 mg GAE/g fresh weight, continuously falling off with storage time. Sample CM2 (chemically treated, 6% CO_2; 12% O_2) observed a minimum (2.75 mg GAE/g fresh weight) decrease in total phenolic content at 8°C after 16 days .A significant reduction in total phenolic content ($P<0.05$) was recorded in all the samples. At the end of the storage period of 16 days, reduced phenolic content was found to be maximum at 12°C. Blanched samples lost more than 50% of phenolic content after 12 days of storage at 12°C as depicted in in [Fig 5(A) and Fig 5(B)] (Jiang *et al.,* 2012).

Free radical scavenging activity (RSA)

Radical scavenging activity of fresh button mushroom was found to be 10.25 mg AA/100 g fresh weight which follows the decreasing trend with the storage period reported by Alasalvar *et al.,* (2005) for modified air packed purple carrots

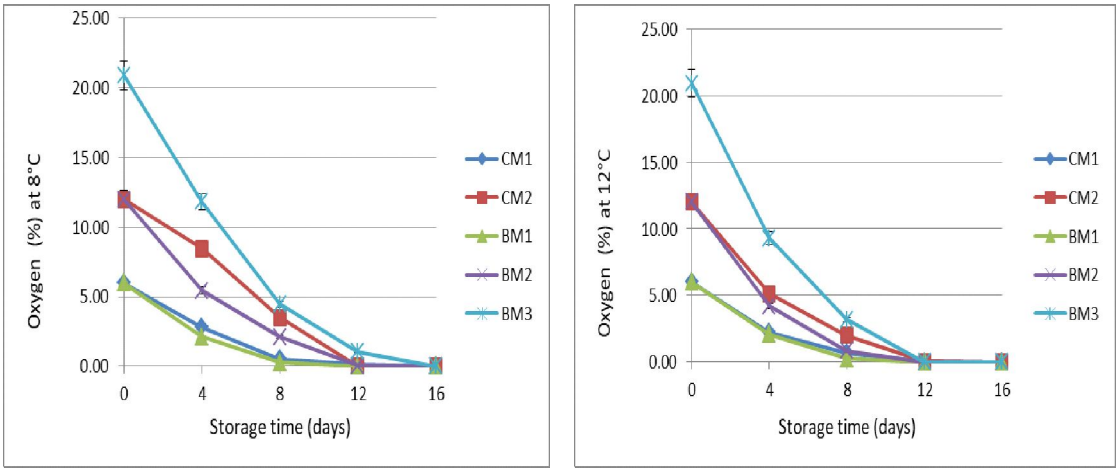

Fig 1: Effect of chemical, MAP treatments and storage time on oxygen (%) at 8°C 1(A), at 12°C 1(B) of button mushroom (*Agaricus bisporus*).

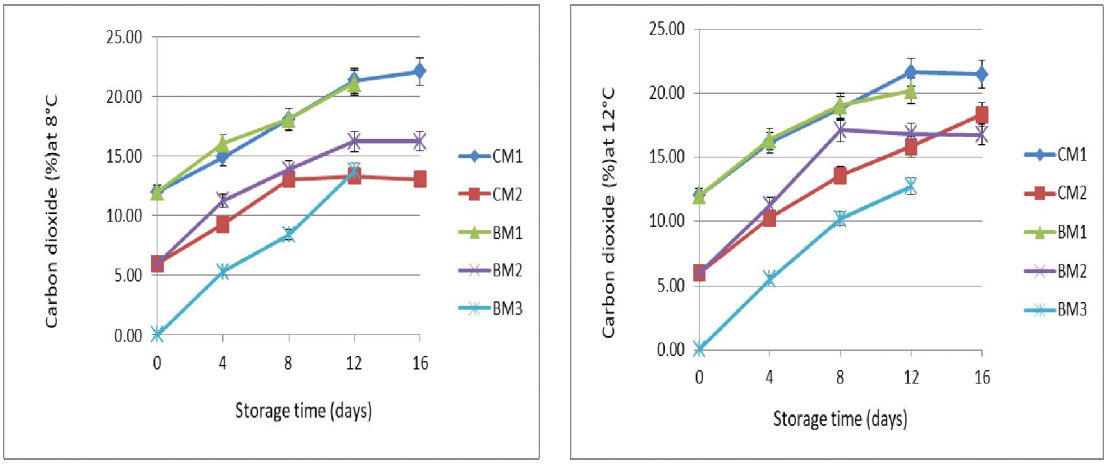

Fig 2: Effect of chemical, MAP treatments and storage time on carbon dioxide (%) 8°C 2(A), at 12°C 2(B) of button mushroom (*Agaricus bisporus*).

and Jiang *et al.* (2012) for chitosan-coated mushrooms. Studies showed fall-off in ascorbic acid content, total polyphenol content and free radical scavenging activity go along with the same pattern, indicating radical scavenging activity relies on both ascorbic acid content and phenol content (Utto *et al.,* 2013; Jafri *et al.,* 2013). An inference that can be drawn was that the combined effect of MAP, chemical and temperature treatments could effectively maintain the anti-oxidant activity of button mushroom more prominent than MAP treatment in maintaining anti-oxidant activity (Sami *et al.,* 2021). The trends are shown in Fig 6(A) and Fig 6(B).

Total plate count

TPC shows a steep shoot up with both temperature and storage period. Considering the general trend of temperature effect on TPC, it was evident that an increase in temperature increases the exponential growth phase of bacterial count with a short duration (Gaglio *et al.,* 2019). This investigation observed that 44% of chemically and MAP treated button mushrooms were in the maximum desirable count of the total. After 12 days of the storage period, bacterial colonies were too numerous to count (TNTC), so recorded data was taken up to the 4th dilution. Citric acid provides an acidic environment to the bacteria, which helps to reduce the growth of colonies (Rossouw and Korsten, 2016).

Yeast and molds count

Button mushroom surface content of yeast and molds increases sharply with the storage period (Eldin *et al.,* 2016). Initially, yeast and molds count recorded for fresh button mushroom was 30.23 cfu $g^{-1} \times 10^{-2}$, which reached 229.67 cfu $g^{-1} \times 10^{-2}$ at the end of the storage period. Samples treated with $CaCl_2$, citric acid and sorbitol and packed in 12% CO_2, 6% O_2 stored at 8°C were best in controlling yeast and mold counts for more extended periods.

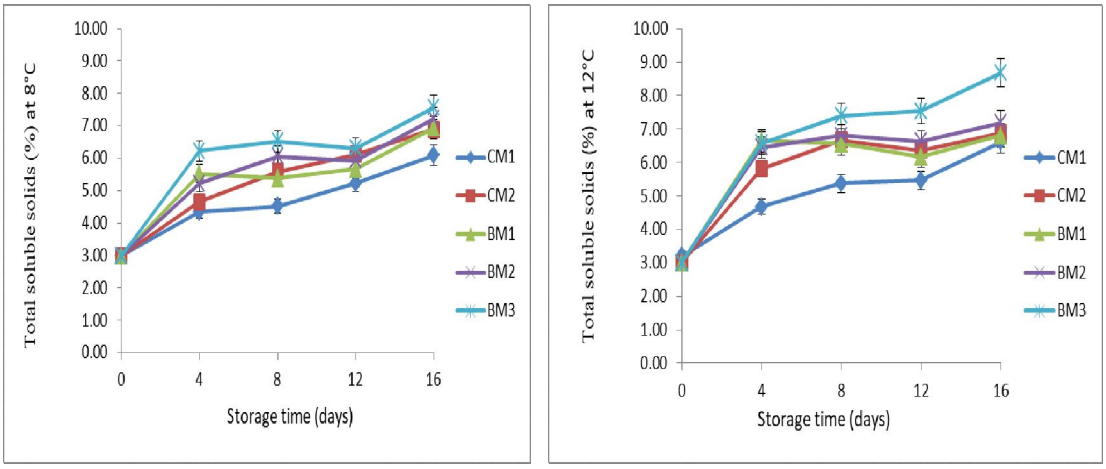

Fig 3: Effect of chemical, MAP treatments and storage time on total soluble solids (%) at 8°C 3(A), 12° 3(B) of button mushroom (*Agaricus bisporus*).

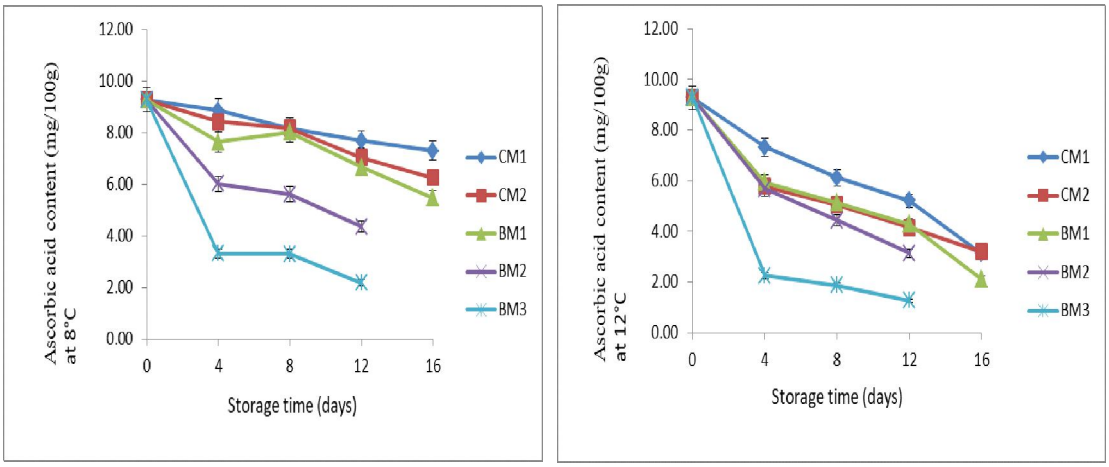

Fig 4: Effect of chemical, MAP treatments and storage time on ascorbic acid content (mg/100 g) at 8°C 4(A), 12°C 4(B) of button mushroom (*Agaricus bisporus*).

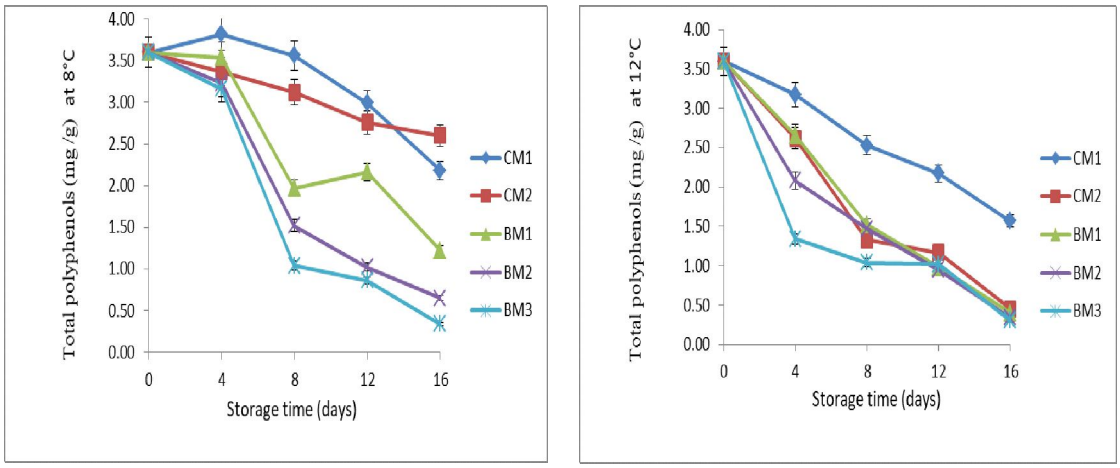

Fig 5: Effect of chemical, MAP treatments and storage time on total phenolic content (mg/g) at 8°C 5(A), 12°C 5(B) of button mushroom (*Agaricus bisporus*).

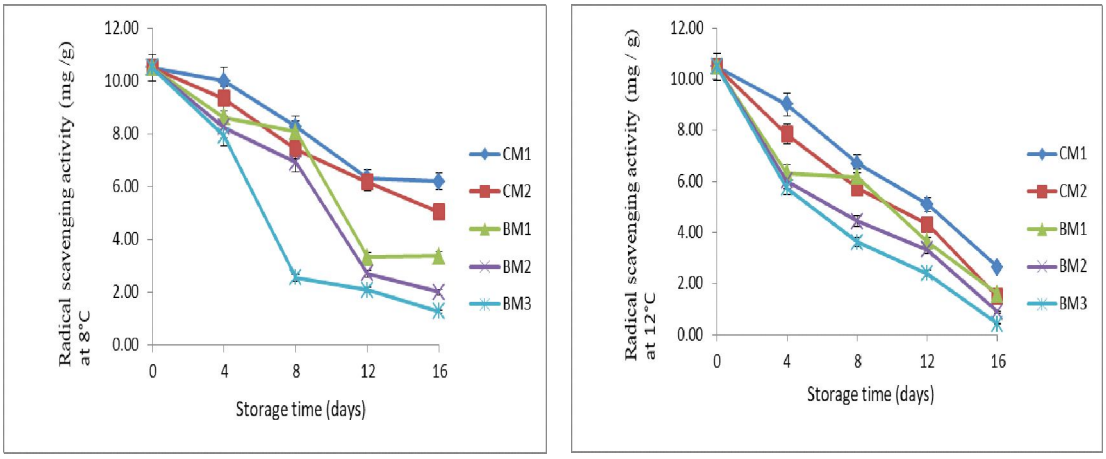

Fig 6: Effect of chemical, MAP treatments and storage time on radical scavenging activity (mg/g) at 8°C 6(A), 12°C 6(B) of button mushroom (*Agaricus bisporus*).

CONCLUSION

All chemical, MAP, and temperature treatments exhibited a general decline in quality parameters, with particularly rapid degradation observed at 12°C. However, button mushrooms packed under a modified atmosphere demonstrated superior performance even after extended storage periods of up to 12 days at 8°C (Singh *et al.,* 2018). The accelerated degradation at higher temperatures may be attributed to tissue softening, possibly resulting from protein and polysaccharide degradation, as well as vacuole disruption. Chemical treatments involving calcium chloride, citric acid, and sorbitol yielded the most favorable outcomes compared to blanched samples. Given the highly perishable nature of button mushrooms, distinct preservation methods such as treatment with calcium chloride, citric acid, sorbitol, or overwrapping with polyethylene films containing modified atmospheres are essential. Various alternative techniques, including refrigeration, calcium chloride cultivation, ionizing radiation, and coating, can also be explored to enhance the post-harvest shelf life of button mushrooms.

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Conflict of interest

The authors would hereby like to declare that there is no conflict of interests that could arise.

REFERENCES

Alasalvar, C.M., Al-Farsi, P.C., Quantick, F., Shahidi, R., Wiktorowicz, Z.(2005). Effect of chill storage and modified atmosphere packaging (MAP) on free radical scavenging activity anthocyanin, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots. Food Chemistry. 89: 69–76.

Ares, G., Lareo, C., Lema, P. (2007). Modified atmospheric packaging for the post-harvest storage of mushrooms: A review. Fresh Produce. 1: 32-40.

Dean, A., Voss, D. (1999). Design and Analysis of Experiments. Springer, New York. Antmann, G., Ares, G., Lema, P., Lareo, C., (2008). Influence of modified atmosphere packaging on sensory quality of shiitake mushrooms. Postharvest Biology and Technology. 49: 164-170.

Díaz-Mula, H.M., Martínez-Romero, D., Castillo, S., Serrano, M., Valero, D.(2011). Modified atmosphere packaging of yellow and purple plum cultivars. 1. Effect on organoleptic quality. Postharvest Biology and Technology. 61: 103-109.

Eldin, M.A.S. and Geosel, A.(2016) Efficacy of hydrogen peroxide on post-harvest quality of white button mushroom. Egyptian Journal of Horticulture. 42(1): 1-17.

Eissa HAA .(2008) Effect of chitosan coating on shelf-life and quality of fresh-cut mushroom. Polish Journal of Food and Nutrition Sciences. 58: 95-105.

Farzana, T., Mohajan, S., Saha, T., Hossain, MN. (2016), Development of a healthy soup powder using phytonutrient enriched mushroom-moringa leaf. DIU Journal of Allied Health Sciences. 3(1-2): 33-40.

Gaglio, R., Saitta, A., Cruciata, M., La Rosa, A., Barbaccia, P., Moschetti, G.and Settanni, L. (2019). Microbiological characteristics of wild edible mushrooms and effect of temperature during storage of *Morchella conica*. Journal of Food Quality and Hazards Control. 6: 2-7.

Gholamia, R., Ahmadia, E. and Farris, S.(2017). Shelf-life extension of white mushrooms (*Agaricus bisporus*) by low temperatures conditioning, modified atmosphere and nanocomposite packaging material. Food Packaging and Shelf Life. 14: 88-95.

Harrigan, W. F. (1998). Laboratory Methods in Food Microbiology 3rd Edition, 532.

Harris, L.J. and Ray, S.N. (1935). Diagnosis of Vitamin C sub-nutrition by urine analysis. Lancet 1: 71-77.

Ishaq, A. E. and Obirinakem (2015). Effect of temperature and storage on vitamin C content in fruits juice. International Journal of Chemical and Biomolecular Science.1(2): 17-21.

Jiang, T., Feng, L., Zheng, X. (2012) Effect of chitosan coating with thyme oil on post-harvest quality and shelf life of shiitake mushroom (*Lentius edodes*). Journal of Agricultural and Food Chemistry. 60: 188-196.

Jafri A, Jha, A, Bunkar, DS, Ram, RC. (2013). Quality retention of oyster mushrooms (*Pleurotus florida*) by a combination of chemical treatments and modified atmosphere packaging. Postharvest Biology and Technology. 76: 112-118.

Koushki, M., Abras, S.K., Mohammadi, M., Hadian, Z., Poorfallah, Nb., Sharayei, P.,Mortazavian, A.M.(2011). Physico-chemical properties of mushrooms as affected by modified atmosphere packaging and $CaCl_2$ dipping. African Journal of Agricultural Research. 6: 5414-5421.

Oliveiraa, F., Sousa-Gallaghera, M.J., Mahajana, P.V., Teixeirab, J.A. (2012). Evaluation of MAP engineering design parameters on quality of fresh sliced mushrooms. Journal of Food Engineering 108: 507-514.

Rossouw W, Korsten L.(2016) Cultivable microbiome of fresh white button mushrooms. Letters in Applied Microbiology. 64: 164-170.

Sami, R., Elhakem, A., Alharbi, M., Benajiba, N., Almatrafi, M., Abdelazez, A. and Helal, M.(2021). Evaluation of anti-oxidant activities, oxidation enzymes, and quality of nano-coated button mushrooms (*Agaricus Bisporus*) during storage. Coatinggs. 11: 149.

Singh, S., Gaikwad, K.K., Lee, M., Lee, Y.S. (2018). Thermally buffered corrugated packaging for preserving the post-harvest freshness of mushrooms (*Agaricus bispours*). Journal of Food Engineering. 216: 11-19.

Teramura, H. Ushiyama,M., Ogihara, H. (2015). Evaluation of a novel dry sheet culture method (Sanita-kunR) for rapid enumeration of yeasts and molds in foods. Journal of Microbiological Methods. 109: 16-19.

Tsai, S.Y., Wu, T.P., Huang, S.J. and Mau, J.L.(2007). Nonvolatile taste components of *Agaricus bisporus* harvested at different stages of maturity. Food Chemistry. 103: 1457–1464.

Utto, W., Junboon, T., Wongsaree, T. and Manoi, L.(2013) Influences of modified atmosphere packaging using active breathable films on anti-oxidant activity and quality of minimally processed Tea leaves (*Cratoxylum formosum.* Dyer). International Food Research Journal. 20(2): 661-671.