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Enhancement in physiological and sensory attributes of button mushroom (*Agaricus bisporus*) as influenced by chemical and modified atmospheric packaging (MAP) treatments at low temperature storage

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Abstract

This study investigated the effects of modified atmosphere packaging (MAP) with different chemical treatments on the storage life and fruit quality of button mushroom. *Agaricus bisporus* fruiting bodies were chemically treated with CaCl_2 (2.5% w/v), citric acid (3% w/v) and sorbitol (0.1% w/v), then packed under three MAP air composition (MAPI: 6% O_2 , 12% CO_2 ; MAPII: 12% O_2 , 6% CO_2 ; MAPIII: 20.95% O_2 , 0.04% CO_2). After modified atmosphere packaging, the samples were stored at 8°C for 16 days and different observations were recorded at interval of 4 days. Button mushroom which were chemically treated followed by modified atmosphere packaging with air composition 6% O_2 and 12% CO_2 showed better water retention qualities, higher sensory ratings which lead to storage life of about 16 days. Samples with this air composition have very less changes in TSS, pH, browning index and electrolyte leakage rate. Samples without chemical treatments and higher oxygen concentration undergo very fast degradation and spoiled after 16 days.

Keywords: Modified atmosphere packaging, CaCl_2 , sorbitol, button mushroom

Introduction

Worldwide, demand for fresh, healthy and delicious food is continuously increasing as there is shift in people's lifestyle and due to breakthrough of retail marketing. Button mushroom is such food product which accomplishes all demands of the community. But due to very short shelf-life button mushroom cannot be stored too long and also faces trouble in its transportation. So, adoption of new technologies can boost its shelf life (Belay *et al.*, 2016) [4]. A number of experimental researches have been conducted for improving shelf life of mushrooms including modified atmosphere packaging and different chemical treatments. Modified atmosphere packaging mainly relies on changing gas composition which surrounds the edible food products depending upon packaging material thickness, its CO_2 and O_2 permeability and respiration rate of stored food products (Li & Ishikawa, 2014) [16]. It was reported that overwrapping the mushrooms by polyvinyl chloride film also enhances shelf life of button mushroom (Licciardello *et al.*, 2018) [14]. It was reported by Roy *et al.* (1995) [23] that 6% O_2 is the perfect oxygen concentration that can be used for packaging of button mushroom. Low oxygen concentration can effectively reduce cap development, aerobic respiration rates and enzymatic browning. In low concentration of oxygen, tyrosinase activity can be reduced which further decreases browning of button mushroom.

Different chemical treatments can effectively help in decreasing senescence of button mushroom when used with an appropriate gas composition packaging. Citric acid which is regularly used in chemical treatments reduces pH of the button mushroom, act as chelating agent and also increases the activity of the antioxidants (Brennan *et al.*, 2000) [5]. Sorbitol is the chemical which is used in enhancing post-harvest life of the food products. Sorbitol act as water holding agent and maintain the firmness of the button mushroom (Anantheswaran *et al.* 1996) [1]. Chemicals like potassium metabisulphite (KMS), citric acid, calcium chloride and sodium EDTA (Ethylene diamine tetra acetic acid) are used as ash guard and pays very

important role in long term preservation of food products. Sodium EDTA, citric acid, sorbitol and calcium chloride were used by Khan *et al.* (2014) ^[11] for improving shelf life of button mushroom. Jafri *et al.* (2013) ^[9] used sorbitol, citric acid and calcium chloride as chemical treatments for enhancing shelf life of mushrooms.

Materials and methods

Button mushroom (*Agaricus bisporus*) were grown in Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi and were harvested in the morning hours when their caps measured 3.0 to 4.0 cm in diameter. Button mushrooms which were free from any physical injury were selected for the experiment purpose. Fresh button mushroom were washed properly and then air dried on the filter paper followed by different chemical and MAP treatments provided at the Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Methodology

Fresh button mushroom free from injury were selected after washing and drying and followed by different chemical treatments. The three chemical treatments were 2.5% CaCl₂, 2.5% CaCl₂ + 3% citric acid + 0.1% sorbitol and Hot water treatment (blanched at 50°C). The button mushrooms were dipped in chemicals for 20 minutes followed by packaging of mushroom. After different chemical treatments button mushrooms were packed in polyethylene packets of polyamide 20µm-polyethylene 70µm with EVA (Ethylene-vinyl acetate sealant layer). Sample size of button mushroom taken was 40g. Packaging of mushrooms was done by modified atmosphere packaging (MAP) unit. The three MAP treatments taken were MAP₁ (12% CO₂; 6% O₂), MAP₂ (6% CO₂; 12% O₂) and MAP₃ (control, normal air composition). The button mushroom after chemical and MAP treatments were stored at 8°C temperature. All the observations i.e. biochemical and textural were recorded at interval of 4 days and carried out upto 16 days.

Head space gas analysis

Head space gas analysis was done for CO₂ (%) and O₂ (%) in samples. The head space gas composition of samples was measured by gas analyzer (MAP Mix 9001 ME, PBI Dansensor, Ringsted, Denmark). For measuring the air composition, a needle was inserted in samples of button mushrooms through septum to ensure hole remained closed and gas concentration were expressed in percentage.

pH of *Agaricus bisporus*

Fresh fruiting bodies of button mushroom (10g) were homogenized in a mixer grinder followed by centrifugation process for 20 min at 5000 rpm. The supernatant of the centrifuge tube was collected. The pH of button mushroom was determined from centrifuged extract by using digital pH meter (Labtronics).

Total soluble solids

Centrifuged extracts of button mushroom fruiting bodies were analysed for total soluble solids with the help of refractrometer (Model RFM 970, Bellingham and Stanley, UK) (Eissa, 2008) ^[7].

Relative electrolyte leakage

With the help of electrolyte leakage we can assess cell membrane permeability (Kaya *et al.*, 2002) ^[10]. Fruiting bodies of button mushroom were sliced into discs of 3mm thick, 3mm diameter and of around 5 grams. These discs were then immersed in 50 ml of distilled water for 1 hour for removing all the dirt and surface contamination. Then they were removed and further immersed in 50 ml distilled water and followed by incubation at ambient temperature of 20 ± 3°C. Electrolyte conductivity was measured after 3 hour and after boiling for 30 minutes with the help of conductivity meter (DDB-303A, Leici Instrument Co., Shanghai, China). Electrolyte leakage percentage was calculated by multiplying ratio of electrolyte leakage at 3 hours to the total value by 100.

Weight loss

Weight loss of button mushroom was calculated by subtracting weight of mushrooms after storage from initial weight of the button mushroom (Singh *et al.*, 2016) ^[26]. The weight loss was expressed as percentage loss in weight and it is given by:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

Instrumentation colour and browning index

Colour of the product is the very important parameter for deciding its market value and consumer acceptance. Whenever an object is visualized there are three physical factors i.e. source of light, the object and the light receptor mechanism (Kortei *et al.*, 2015) ^[12]. Instrumentally colour of the products is measured by the Hunterlab Colorimeter (Hunterslab Technical Manual, 2008) ^[8]. The colorimeter generates three parameters i.e. L*, a* and b*. In Hunterlab colorimeter these are three colour coordinates. L* denotes the lightness coordinate, a* denotes the red/green coordinate in which +a* indicates red colour and -a* indicates green coordinate. In case of b* which indicates yellow/blue coordinates +b* indicates yellow coordinate and -b* indicates blue coordinates. The L*, a*, and b* coordinate axis confirms the three-dimensional colour space. If the value of the L*, a*, and b* are known to us then we can easily describe colour of the product as well as its quadrant (Schnell *et al.*, 2005) ^[25]. The colour of the button mushroom was measured at 0 day and after storage period of 4 days. The colorimeter has a beam diameter of 8mm and the responses detectors were set at 0 viewing angle. The colorimeter was calibrated with reference white porcelain tile (L₀ = 96.6, a₀ = .48 and b₀ = 5.3) before placing samples. The value of lightness coordinate (L*) ranges from 0 (black) to 100 (white), a* ranges from +60 (red) to -60 (green) and b* ranges from +60 (yellow) to -60 (blue) were measured in triplets. Chroma (C) is the parameter which denotes colour saturation and hue angle (H₀) denotes relative amount of redness and yellowness where 0°/360° is defined for red/magenta, 90° denotes yellow, 180° for green and 270° for blue or purple colour.

Browning index (BI) represents the purity of brown colour. Browning index is considered as an important parameter related to browning of the product (Lopez-Malo *et al.*, 1998) ^[17]. The browning index can be calculated by formula (Mohammadi *et al.*, 2008) ^[20]: Browning Index (B.I) = [100 (x - 0.31)]/0.17, where x = (a* + 1.75 L*)/(5.645 L* + a* - 3.012 b*).

Statistical Design

All the experiments were completed in triplicates and data were analysed to two-way ANNOVA which considers MAP and chemical treatments.

Results and discussion

Head space gas composition

Decreasing trends in O₂ concentration and increasing trends in CO₂ concentration were observed during storage period of button mushroom (*Ares et al.*, 2006; Antmann *et al.*, 2008) [3, 2]. After 4 days O₂ concentration was around 15% in chemically treated samples and 12% in blanched samples,

which clearly indicates faster respiration rates in case of blanched samples (Fig 1). After 12 days, O₂ concentration was recorded negligible in MAP packed samples and below 3% in normal air packed samples. There is considerable rise in CO₂ concentration in all the samples which indicates sharp rise in respiration rates (Fig 2). Oxygen permeability rate of polyethylene film (50cm³/m².day.bar) prevented the complete depletion of oxygen from the packed samples. Drastic changes in atmosphere around button mushrooms were primarily due to consumption of O₂ and production of CO₂ as by-product of respiration which were much higher than permeability rates of packaging film (Jafri *et al.*, 2013) [9].

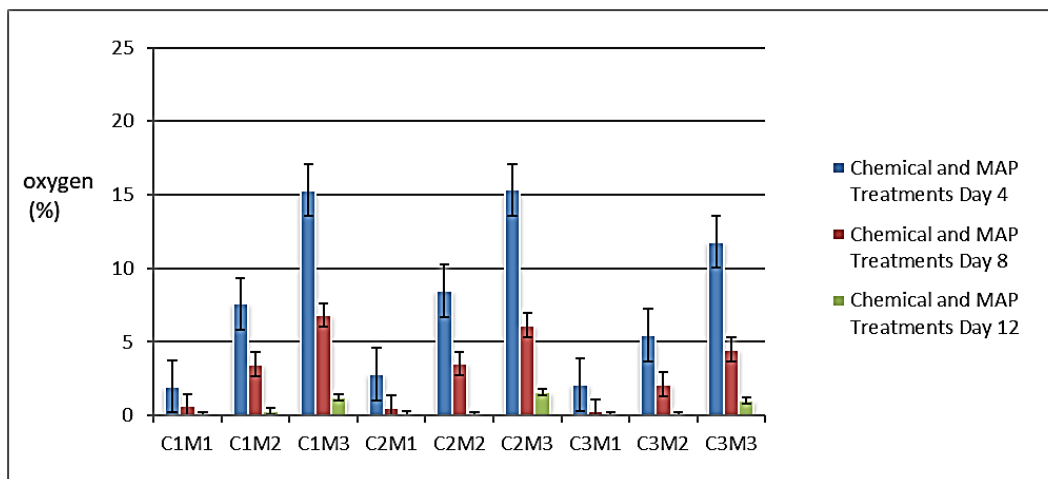


Fig 1: Effect of different chemical and MAP treatments on head space gas composition (percentage oxygen concentration) of button mushroom (*Agaricusbisporus*) at 8°C

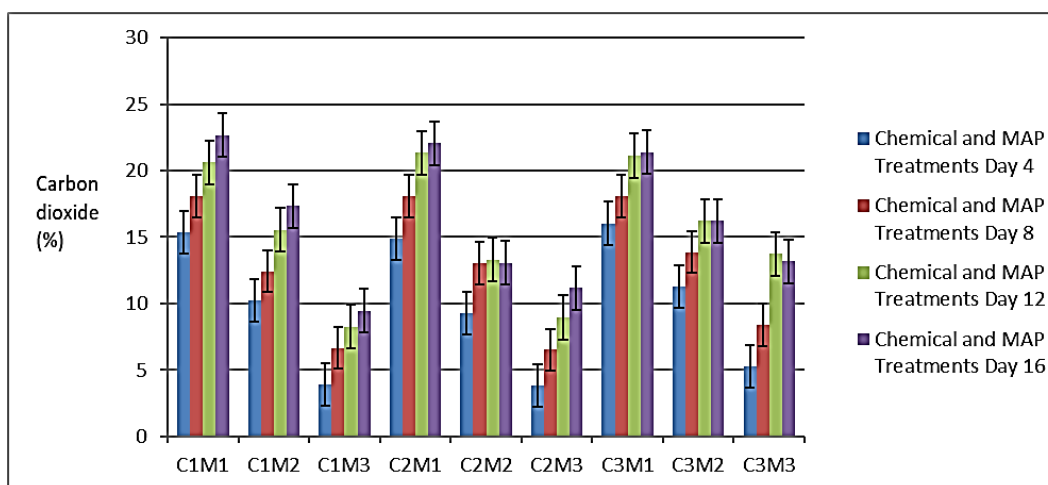


Fig 2: Effect of different chemical and MAP treatments on head space gas composition (percentage carbon dioxide concentration) of button mushroom (*Agaricusbisporus*) at 8°C

pH of *Agaricus bisporus*

pH of fresh button mushrooms was recorded as 6.68 and pH of fresh button mushroom after chemical treatment was recorded as 3.26. Lowering of pH of button mushrooms after chemical treatment was mainly due to acidic effect of citric acid and calcium chloride. These chemicals might have adsorbed on the surface of chemically treated button mushrooms and had been accumulated in supernatant after centrifugation of ground button mushroom samples. Decreasing trend in pH of samples was observed with the storage period (Table 1). With the storage time, growth of bacteria was observed and consequent formation of organic acid might have been reason for lowering of pH (Singh *et al.*, 2018) [27]. Sample C₁M₁ recorded best results as there was

very less decrement in pH value from initial value. Chemically treated and MAP packed samples showed significantly different results as compared to control samples. Decrement in pH was recorded in all samples either with low O₂ concentration or with high O₂ concentration but rate of decrement was comparatively higher in highO₂ concentration. Higher O₂ concentrations trigger aerobic bacteria and other microorganisms to proliferate on the edible food products (Parentelli *et al.*, 2007) [21]. In very low O₂ concentration anaerobic respiration and proliferation of bacteria like *Clostridium botulinum* might occur. Lowering of pH will occur in both the cases as recorded in the investigation and also reported by Jafri *et al.*, 2013 [9].

Total soluble solids

Total soluble solids increases with the storage time indicating higher respiration rates, senescence and ripening (Manurakchinakorn *et al.*, 2010) [19]. Chemically treated and MAP packed samples showed better results as compared to control samples (Magaraj *et al.*, 2011) [18]. Rate of increment was lesser and more gradual in case of C₁M₁ and C₂M₁ samples. Total soluble solids after 16 days reached to 6.80% in case of controlled samples, whereas, TSS for C₁M₁ and C₂M₁ were recorded as 5.63% and 5.30% respectively (Table 1). This investigation clearly shows that rate of senescence were lowest in case of C₂M₁ followed by C₁M₁ samples (Díaz-Mula *et al.*, 2011) [6].

Relative electrolyte leakage

Stability of cell membrane permeability was determined by relative electrolyte leakage. Cell membrane permeability to ions increases with storage period which indicate vulnerability of cell membrane to leakage (Tao *et al.*, 2006) [28]. There had been significant difference in cell membrane permeability for chemically treated and untreated samples. Samples with 6% oxygen i.e. C₁M₁, C₂M₁ and C₃M₃ were detected with little traces of ethanol gas indicating anaerobic respiration in packed samples (Table 1). MAP packed samples recorded less electrolyte leakage as low O₂ concentration decreases respiration rates, cap formation, and weight loss. Tyrosinase enzyme activity also gets reduced in low O₂ concentration which further prevents enzymatic browning (Li and Zhang, 2013) [15]. Chemically untreated and normal air packed sample C₃M₃ showed highest electrolyte leakage of about 17.73%.

Weight Loss during Storage

Button mushrooms are 90% water so major problem during postharvest is dehydration and rapid loss of moisture from button mushrooms which leads to excessive loss in weight. Dehydration is very common in button mushrooms as they are only covered by very thin layer of epidermal tissue (Singh *et al.*, 2018) [27]. Transpiration and respiration are the two factors which contribute to weight loss of horticultural products. Transpiration leads to loss of water and respiration leads to loss of dry matter of product (Wei *et al.*, 2017) [29]. Weight loss of button mushrooms were significantly affected by both MAP and chemical treatment combinations. Control samples recorded major weight loss after 16 days as compared to chemically treated and MAP packed samples. The main reason for weight loss in control samples was tissue fluid exudation which was accumulated in polyethylene bags. After 16 days, weight loss for C₃M₃ samples was recorded as 9.58%, whereas, chemically treated samples showed very less tissue exudation which might be due to insoluble calcium pectate layer in the tissue formed by calcium chloride (Table 1). After 16 days, minimum weight losses for chemically treated and MAP packed samples were recorded as 5.47% and 6.47% for C₁M₁ and C₂M₁ samples respectively. This investigation results demonstrated that different chemical treatments in collaboration with modified atmosphere packaging can effectively reduce moisture losses which contributes to major loss in weight of button mushrooms (Pereda *et al.*, 2011) [22].

Table 1: Effect of different chemical and MAP treatment combinations on physiological attributes of button mushroom (*Agaricus bisporus*) at 8°C

Parameters Days Treatments	pH				Total soluble solids (%)				Relative electrolyte leakage (%)				Weight loss (%)			
	4	8	12	16	4	8	12	16	4	8	12	16	4	8	12	16
C ₁ M ₁	3.33	3.09	3.00	2.85	4.47	4.23	5.17	5.63	4.48	6.63	9.90	15.50	2.42	3.42	4.58	5.47
C ₁ M ₂	3.27	3.00	3.00	2.72	4.23	5.12	4.87	6.13	5.03	6.97	10.83	16.27	3.08	5.08	6.00	6.75
C ₁ M ₃	3.22	3.07	2.50	2.22	4.40	5.43	6.23	6.23	5.03	7.38	11.80	16.47	3.25	5.42	7.33	7.75
C ₂ M ₁	3.42	3.24	3.07	2.46	4.35	4.77	4.23	6.30	3.87	6.60	9.13	14.37	2.77	4.00	4.73	6.47
C ₂ M ₂	3.20	3.10	2.10	2.15	4.52	5.48	5.47	6.70	4.17	7.90	10.33	14.20	3.30	5.67	7.33	7.92
C ₂ M ₃	3.13	3.07	2.72	2.63	3.83	5.47	6.57	6.60	4.20	8.37	11.10	17.20	3.47	6.87	6.92	8.55
C ₃ M ₁	6.59	6.50	5.37	4.87	5.50	4.88	5.67	6.33	4.57	7.20	11.93	15.83	3.33	5.42	6.25	6.83
C ₃ M ₂	6.18	6.15	5.52	4.87	5.07	5.71	6.30	6.47	5.10	8.22	16.25	17.33	3.67	6.50	6.67	7.88
C ₃ M ₃	6.24	6.32	5.68	4.54	6.13	6.16	7.43	6.83	5.60	9.20	17.50	17.73	5.07	6.67	8.25	9.58
Mean	4.29	4.17	3.66	3.26	4.72	5.25	5.77	6.36	4.67	7.61	12.09	16.10	3.37	5.45	6.45	7.47
S.E.m±	0.06	0.07	0.09	0.07	0.09	0.11	0.09	0.01	0.16	0.26	0.18	0.17	0.14	0.17	0.20	0.13
CD at 5%	0.18	0.21	0.26	0.20	0.27	0.31	0.25	0.28	0.47	0.70	0.52	0.48	0.41	0.48	0.58	0.36

C₁: 2.5% CaCl₂; C₂: 2.5% CaCl₂, 3% citric acid, 0.1% sorbitol; C₃: Blanched at 50°C

M₁: 12% CO₂, 6% O₂; M₂: 6% CO₂, 12% O₂; M₃: 0.04% CO₂, 20.95% O₂

Instrumentation colour and Browning index

Initially luminosity (L*-values) for fresh button mushroom was recorded as 89.46 which decreases with the storage period. After 16 days, luminosity (L*-values) ranged from 70.61- 66.58 which were significantly different (P<0.05) from luminosity after 4 days. L* values denotes lightness index which continuously decreases during storage. MAP packed and chemically treated button mushrooms showed better luminosity (L*-values) as compared to controlled samples. Maximum luminosity (L*-values) were recorded for C₃M₁(70.94) and minimum was recorded for C₃M₃ (69.86) after 16 days (Table 2). The red/green(a*-values) increases

with storage period and ranged from 1.47- 2.42 after 16 days. The yellow/blue(b*-values) also decreases with the storage period and ranged from 11.05-14.73 after 16 days. Decreasing L* values and increasing b* values indicates towards non enzymatic browning (Sasnauska *et al.*, 2011) [24]. Browning of button mushroom was comparatively lower in case of MAP packed and chemically treated button mushroom (C₁M₁ and C₂M₁) as compared to control samples (Kortei *et al.*, 2015) [13]. After 16 days maximum and minimum browning index recorded was 26.71 and 18.46 for the sample C₃M₃ and C₁M₁ respectively.

Table 2: Effect of different chemical and MAP treatment combinations on instrumentation colour and browning index of button mushroom (*Agaricus bisporus*) at 8°C.

Parameters	(L*)				(a*)				(b*)				Browning index			
Days Treatments	4	8	12	16	4	8	12	16	4	8	12	16	4	8	12	16
C ₁ M ₁	81.36	78.77	73.62	70.61	0.91	1.00	1.41	1.89	8.78	8.99	9.50	11.05	12.30	12.64	13.79	18.46
C ₁ M ₂	79.37	78.50	76.60	70.57	0.93	1.04	1.47	1.68	10.18	10.75	11.71	12.25	14.18	15.37	18.08	20.04
C ₁ M ₃	76.27	73.91	72.72	68.90	1.04	1.24	1.38	1.58	10.68	11.54	12.42	13.22	15.82	17.80	19.69	22.54
C ₂ M ₁	82.29	79.11	74.91	71.24	0.62	0.72	0.98	1.47	9.40	9.98	10.34	13.40	12.63	13.90	15.65	21.90
C ₂ M ₂	81.07	78.80	74.91	70.48	0.87	1.03	1.29	1.47	10.02	10.36	11.86	12.75	13.32	14.77	18.22	20.61
C ₂ M ₃	80.00	74.91	70.24	69.24	0.96	1.12	1.28	1.57	10.42	11.39	8.62	13.33	14.70	17.20	20.14	22.59
C ₃ M ₁	79.61	77.24	73.28	70.94	1.21	1.34	1.69	1.73	10.90	12.00	12.97	14.73	15.50	17.54	21.04	23.12
C ₃ M ₂	78.31	76.58	73.65	70.11	1.26	1.21	1.54	1.94	11.73	11.60	12.90	14.42	17.11	16.78	20.38	23.62
C ₃ M ₃	78.39	73.91	69.94	66.58	1.14	1.77	2.03	2.42	10.47	12.07	12.85	14.48	16.08	19.35	21.74	26.71
Mean	79.63	76.86	73.32	69.85	0.99	1.16	1.45	1.75	10.29	10.96	11.46	13.29	14.62	16.15	18.75	22.18
S.E.m±	0.25	1.70	1.15	1.40	0.07	0.12	0.11	0.08	0.15	0.24	0.48	0.16	0.32	0.33	0.34	0.40
CD at 5%	0.72	4.78	3.23	3.92	0.21	0.28	0.39	0.24	0.42	0.68	1.35	0.45	0.90	0.93	0.97	1.14

Conclusion

The present investigation suggested that there is quality enhancement of button mushroom by combined effect of both chemical and MAP treatment. MAP packed button mushroom samples depicted higher membrane stability and noticed very less weight loss during storage as compared to controlled samples. Browning of chemically treated MAP packed button mushroom samples was significantly lower as compared to controlled samples. MAP composition with 12% CO₂; 6% O₂ gas composition were best suited for maintaining post-harvest quality of button mushroom with low respiratory rates, low leakage of electrolyte, better sensory and colour parameters. Combined effect of integrated MAP and chemical treatments serves as effective approach for delaying senescence of button mushroom during post-harvest storage.

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