Evaluation of Modified Atmospheric Packaging (MAP), Chemical Treatments and Low Temperature on Biochemical and Textural Attributes of Button Mushroom (Agaricus bisporus)

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ABSTRACT

Background: The increasing awareness on the nutritional and medicinal value of button mushroom is the guiding light for the increased production and consumption of mushroom across the world. Due to its perishable nature it cannot be stored for long period. Different methods are used for increasing mushroom post-harvest shelf life which includes chemical treatments, low temperature storage conditions, modified atmosphere packaging (MAP) and use of thick packaging material.

Methods: Button mushroom were chemically treated with CaCl$_2$ (2.5% w/v), citric acid (3% w/v) and sorbitol (0.1% w/v) which was followed by packaging in polyethylene film under three different MAP compositions and stored at 8°C temperature for further studies. Physico-chemical and texture profile analysis were done for 16 days at interval of four days.

Result: Significant changes were obtained in three different MAP treatments. MAP composition with 6% O$_2$ and 12% CO$_2$ were found best for post-harvest storage of button mushroom. Blanched samples without chemical treatments and packed under ambient conditions were spoiled after 16 days as compared to chemically treated samples stored in MAP packaging.

Key words: Button mushroom, Modified atmosphere packaging, Sorbitol.

INTRODUCTION

Agaricus bisporus (Button mushroom) are very popular nutrient rich, edible fungi which are being widely consumed throughout the world. There has been an exponential increase in the mushroom cultivation due to its unique taste and high nutritive value. Fresh mushroom have very short post-harvest shelf life and can be stored for 3-4 days under ambient condition and 7-8 days under refrigerated condition (Jiang et al., 2012). Short life span of button mushroom may be due to the lack of cuticles which protect them from physical, microbial and water loss. Fresh button mushroom have bright white colour, firm texture, closed caps and can be easily chewed. But during storage these parameters undergo a fast and rapid change within a short period of time which leads to quality deterioration of button mushrooms (Kumar et al., 2014). Various techniques are used to enhance shelf life of mushroom and to maintain its market value. Modified atmosphere packaging when collaborated with different chemical treatments showed significant improvement in shelf life of button mushroom (Jafri et al., 2013). Modified atmosphere packaging (MAP) has proved to maintain quality of button mushroom by maintaining cell wall rigidity and delaying senescence. The modified atmosphere packaging (MAP) aims to change the gaseous composition that surrounds the respiring food commodity which in turn decreases respiratory rate. Decreased respiratory rate enhances the shelf life of the product (Khan et al., 2017). Modified atmosphere packaging along with chemical treatments and low temperature storage delayed post-harvest spoilage in button mushroom (Koushki et al., 2011). In modified atmosphere packaging usually concentration of oxygen is kept low (5-10%) but at extremely low concentration (<5%) anaerobic respiration can start which results in complete loss of quality of product. Carbon dioxide is the gas that has significant antimicrobial activity.

Major deterioration in button mushroom was reported mainly due to browning which causes spongy tissue and loss of turbidity. Chemicals like CaCl$_2$ and sorbitol helps to maintain cell wall turidity and reduces dehydration losses. Sorbitol has very good water retention capacity which holds water on the surface of the button mushroom. Citric acid is well known for its chelating action and act as antioxidants. Proper colour of mushroom can be maintained by soaking mushroom in various concentration of citric acid (0.1-1.0%) and calcium chloride (0.1-3.0%) (Pizzocaro et al., 1993).
Citric acid also helps in inhibiting polyphenol oxidase enzyme that is involved in browning of food. Packaging material also has equal importance in quality control of the mushroom. $O_2$ and $CO_2$ gas permeability rate and water vapour transmission rate are the most eligible parameters for selecting a film as a packaging material (Metha et al., 2011). Permeability of gases is the key elements in determining atmosphere surrounding product. Most commonly used films in MAP are low and high density polyethylene (PE), Polypropylene (PP), Polystyrene (PS) and Polyvinyl chloride. Oxygen and Carbon dioxide permeability rate are determined at 25°C while water vapour transmission rate are measured at 38°C. The permeability of these perforated films depends upon type of film, size and shape of pores and film thickness (Khan et al., 2017).

**Materials and Methods**

*Agaricus bisporus* i.e. button mushroom were obtained from Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi in the morning hours. Matured button mushroom which are physically free from any injury were sorted and selected for the experiment. Fresh button mushroom were washed properly and then air died 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.

**Chemical and MAP treatments**

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% $CaCl_2$, 2.5% $CaCl_2$ + 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-polylethylene 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% $CO_2$; 6% $O_2$), MAP2 (6% $CO_2$; 12% $O_2$) 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 carbon dioxide (%) and oxygen gas (%) in poly samples. The head space contents of packets were measured by gas analyzer (MAP Mix 9001 ME, PBI Dansensor, Ringsted, Denmark). A needle was inserted in the packets through septum to ensure hole remains closed and air was sucked by analyser and its composition was measured by analyser. Composition of air inside packets was expressed in percentage.

**Biochemical Observations**

The Vitamin C or ascorbic acid content in button mushroom was determined by volumetric method (Harris and Ray, 1935). Button mushrooms were dried in microwave oven, grinded into powder form in mixer grinder. 2g of sample was taken and dissolved in 3% $HPO_4$ and volume was made up to 100ml followed by centrifugation at 2000g for 15 minutes. Add 5 ml of supernatant into 10 ml of 3% $HPO_4$ and titrated against the dye ($V_2$ in ml). The initial and final volume of the dye solution was noted on appearance of the pink colour for each sample. The amount of Vitamin C or ascorbic acid in mg/g sample was calculated by formula:

$$\text{(Dye factor} \times V_2 \times 10000)/ (V_1 \times W)$$

Where

W is the weight of sample taken, $V_1$ is the sample of extract taken and $V_2$ is the required dye solution for titration.

The estimation of protein content was done by the method of Bradford, 1976. An amount of 1g of button mushroom samples were taken and were sliced into small pieces with blade and grinded in mortar and pestle with 5ml of phosphate buffer (pH 7.6). Then extract was taken for centrifugation which was at 8000 rpm for 20 minutes. Supernatant was collected in different test tubes and made equal by adding phosphate buffer. 40μl of the supernatant were taken out in different test tubes and mixed with 260μl of phosphate buffer separately, 3 ml of Coomassie Brilliant Blue solution was added and mixed properly. All the test tubes were incubated for 5 minutes at room temperature and absorbance was taken at 595nm with the help of spectrophotometer. A standard curve of absorbance (nm) against concentration (μg) of bovine serum albumin (BSA) protein was plotted. Protein content in the extracted mushroom sample was determined by standard curve and the amount of protein was expressed in mg/gram of button mushroom.

Total polyphenol content was measured by method described by Chirinang and Intarapichet, 2009. Button mushroom samples were air dried in oven at 65°C overnight and grinded into fine powder. Dried mushroom powder (1g) were mixed with 15ml of distilled water followed by keeping it on shaker incubator at room temperature (37°C) at 150 rpm for 24 hours. Samples were taken out from incubator, filtered and were centrifuged at 2000 rpm for 15 minutes in centrifuge tube. Total phenolic compounds of mushrooms extract was found by using Folín-Ciocalteu reagent. An aliquot of 100μL (1:5 dilution) was added to 100μl of Folín–Ciocalteu reagent (1:1 dilution with distilled water) and add 2ml of 10% sodium carbonate solution followed by incubating it for 30 minutes at room temperature. The absorbance was recorded in spectrophotometer at 750nm. The standard curve for total phenolic compounds was made by using 10-100μg of tannic acid. Total phenolic compounds were expressed in milligrams per gram of sample.

The radical scavenging activity of button mushroom was estimated by method of Chirinang and Intarapichet, 2009. Extract which was prepared for finding total phenolic compounds were used for finding the radical scavenging
Different concentration (0.05-1.0 ml) of button mushroom extract were mixed with 2.0 ml of 100 µM DPPH (2,2-diphenyl-1-picrylhydrazyl) in methanol in different test tubes. The test tubes were shaken for proper mixing and kept in dark for 30 minutes. The samples were taken for the centrifugation at 5000 rpm for 15 minutes. The absorbance was taken at 515 nm with the help of spectrophotometer. RSA of mushroom was determined by comparing it with standard curve drawn by using butylated hydroxyl anisole (BHA).

**Texture Profile Analysis (TPA Analysis)**

Texture profile analysis was done with the help of texture analyser (TA.XT Plus, Stable Micro Systems Ltd., UK). Texture profile analyser measures the hardness, cohesiveness, gumminess, springiness and adhesiveness of the food product. Load cell of 50 kg and diameter compression plate probe of 75 mm were used for measuring texture of button mushroom. Button mushroom fruiting bodies of 1.5 cm × 1.5 cm were sliced with the help of blade and placed between probes and were compressed to a depth of 4 mm. The speed of the probe was first standardized and was kept 2.0 mm/s during compression. Texture analysis was done at room temperature of 25°C. The textural properties like hardness, springiness, cohesiveness and chewiness were calculated and formula for the above parameters is given below: Hardness (kg.f) = \( F_1 \div t_2/t_1 \); Cohesiveness (dimensionless) = \( A_1/A_2 \); Chewiness (kg.f) = \( F_1 \times (t_2/t_1) \times (A_2/A_1) \) or Hardness × Springiness × Cohesiveness, where \( F_1 \) is the maximum force, which is the force in the first peak, \( A_1 \) and \( A_2 \) are the areas of the first and second peaks, respectively and \( t_1 \) and \( t_2 \) are the time intervals for the first and second peaks, respectively.

The data obtained was analysed using standard statistical procedures.

**RESULTS AND DISCUSSION**

**Head space gas composition**

It was recorded that there was a decreasing trend in \( O_2 \) concentration and increasing trend in \( CO_2 \) concentration with storage time (Fig 1 and Fig 2). Least concentration of \( O_2 \) was observed for chemically untreated samples after 12 days and highest concentration of \( CO_2 \) was observed for chemically untreated samples after 16 days. Changes in...
gas compositions might be due to higher respiration rate of button mushroom and gas permeability through packaging film at higher temperature when packed in MAP. There was significant difference among all chemically treated samples. Blanched samples packed in normal gas composition showed higher decrease in O$_2$ concentration and correspondingly higher increase in CO$_2$ concentration. After 12 days, O$_2$ concentration was negligible for all the chemically treated and untreated samples. Among, MAP treatments the least concentration of O$_2$ and highest concentration of CO$_2$ was observed in M$_3$, as compared to M$_2$, M$_1$ and at all stages of observation.

**Ascorbic acid content**

Ascorbic acid is considered as the most important antioxidant in citrus fruits, vegetables and mushrooms. Polyphenols are also reported to be strong antioxidants present in different horticultural crops (Tripathy et al., 2016). Ascorbic acid content of button mushroom was affected by both MAP composition and chemical treatments. It was observed that there is steadily decrease in ascorbic acid content with storage period. Lowest ascorbic acid content was observed after 16 days (6.17 mg/100g) of storage. Button mushrooms which are chemically treated and packed in MAP composition (C$_3$M$_3$) showed highest ascorbic acid content (7.64 mg/100g) after 16 days. Ascorbic acid content decreased from 8.01mg/100g to 4.55mg/100g in case of blanched samples packed in normal air composition (C$_3$M$_2$). Different oxidases present in button mushrooms especially ascorbic acid oxidase oxidises ascorbic acid to dehydroascorbic acid (DHA). DHA reductase further reduces dehydroascorbic acid (DHA) to ascorbic acid as this reaction is reversible. So formation of dehydroascorbic acid (DHA) does not indicate complete loss of ascorbic acid. But if DHA is further converted to diketogulonic acid (DKA) then it indicates complete loss of ascorbic acid as this reaction is irreversible in nature. Formation of DHA increases with time and temperature and higher availability of oxygen concentration (Arunuganathan et al., 2012) (Table 1).

**Soluble protein content**

Total soluble protein concentration decreases with the storage period as there was concomitant hike in protease activity. Protein degradation and denaturation might also be the reason for the decrement in soluble protein concentration (Rai and Saxena, 1989). It was observed that there was continuous decrease in protein content with storage time but no significant increase in total free amino acid content was observed. This clearly indicates that low molecular weight free amino acids were used by button mushroom as a source of energy (Murr and Morris, 1975). Chemically untreated and normal air composition packed samples showed the highest decrease in total soluble protein after 16 days (C$_3$M$_3$) (5.52mg/g fresh weight) as there was increase in protease activity which was further supported by higher concentration of oxygen. High concentration of CO$_2$ inhibits protease enzyme activity and hence very less change in protein content was observed.

**Table 1: Effect of different chemical and MAP treatment combinations on biochemical attributes and free radical scavenging activity of button mushroom (Agaricus bisporus) kept at 8°C.**

| Days | Treatments | Ascorbic Acid content (mg AA/100 g fresh weight) | Soluble Protein Content (mg GAE/g fresh weight) | Free Radical Scavenging Activity (mg AA/100 g fresh weight) |
|------|------------|-----------------------------------------------|-----------------------------------------------|---------------------------------------------------------|
|      | C$_1$ M$_1$ |                                |                                              |                                                         |
|      | C$_1$ M$_2$ |                                |                                              |                                                         |
|      | C$_1$ M$_3$ |                                |                                              |                                                         |
|      | C$_2$ M$_1$ |                                |                                              |                                                         |
|      | C$_2$ M$_2$ |                                |                                              |                                                         |
|      | C$_2$ M$_3$ |                                |                                              |                                                         |
|      | C$_3$ M$_1$ |                                |                                              |                                                         |
|      | C$_3$ M$_2$ |                                |                                              |                                                         |
|      | C$_3$ M$_3$ |                                |                                              |                                                         |

GAE: Gallic acid equivalent.
observed in MAP packed samples. The highest total protein content observed was in \((C_M)^2\) (8.52 mg/g fresh weight) at 4 days of storage (Table 1).

**Total polyphenol content**

Total phenolic compounds are the main constituents that are responsible for antioxidant activity of the mushrooms. Mushrooms with genus *Agaricus* have antioxidant properties mainly due to tocopherols (Elmastas et al., 2007). Phenolic compounds indicate antioxidant activity of the mushrooms and are found in methanolic extracts of wild edible mushrooms (Tsai et al., 2007). Antioxidants like Gallic acid and BHT (butylated hydroxyl toluene) are directly correlated with total polyphenols. Total phenolic compounds are directly linked with radical scavenging activity of the mushrooms. Total phenolic compound in fresh button mushroom was 5.98 mg GAE/g fresh weight which continuously decreased with storage period. But the rate of decrement was higher in case of chemically untreated and normal air packed samples. Lowest phenolic compounds was recorded (0.25 mg GAE/g fresh weight) in \(C_M^3\), whereas, highest concentration was recorded (2.10 mg GAE/g fresh weight) in \(C_M^1\) after 16 days. Chemically untreated and normal air packed button mushroom lost more than 70% of phenolic content after 16 days (Jiang et al., 2012) (Table 1).

**Radical scavenging activity**

Radical scavenging activity continuously declines during storage time. It was recorded that decrease in ascorbic acid content, total phenol content and radical scavenging activity follows same pattern with the storage time, indicates radical scavenging activity is dependent on both ascorbic acid content and phenol content (Utto et al., 2013). Jiang et al., 2012 also reported decrease in radical scavenging activity of fresh commodity with the storage time. After 16 days, maximum radical scavenging activity was recorded in \(C_M^1\) (5.43 mg AA/100 g fresh weight). Chemically untreated and normal air packed samples \((C_M)^2\) recorded minimum radical scavenging activity of 1.73 mg AA/100 g fresh weight. Samples with both MAP packed and chemically treated showed the better results as compared to MAP and chemical treatment alone (Jafri et al., 2012) (Table 1).

**Texture Profile Analysis (TPA Analysis)**

Major factor responsible for deterioration of button mushroom is its textural deformities. In present study, hardness of button mushroom decreases with the storage time. Both chemical and MAP treatment have significant effect on hardness of button mushroom. Button mushroom softening is mainly due to protein denaturation, polysaccharides degradation and disruption of vacuoles (Oliveira et al., 2012). Maximum hardness was recorded for \(C_M\), 5.4 kgf after 16 days. Rate of decrement in hardness was comparatively higher in chemically untreated and normal air packed samples as compared to treated and MAP packed samples (Table 2). Chemically untreated and normal air packed samples as compared to treated and MAP packed samples (Table 2).
samples demonstrated more fluid exudation which was accumulated in the polyethylene bags during storage period. Chemically treated samples showed very less fluid exudation as calcium chloride and sorbitol used in chemical treatment are efficient water holding and tissue firming agents. Springiness is the elasticity of the product and it showed decreasing trend with the storage period. Cohesiveness of button mushroom showed increasing pattern with storage period and increasing rate was comparatively higher in chemically untreated and normal air packed button mushroom samples. Chewiness is labour required to chew the food product and this also follows significant decreasing trend with storage time (Kortei et al., 2015). Major deterioration in button mushroom was due to loss of firmness and tissue softening. C$_1$M$_1$ and C$_2$M$_1$ showed minimum changes in their textural parameters followed by C$_1$M$_2$ and C$_2$M$_2$ during storage period. Chemical treatments are very effective method for maintaining firmness of edible products especially button mushroom (Antmann et al., 2008) (Fig 3 and Fig 4).

**CONCLUSION**

The present study showed post-harvest life of button mushroom can be enhanced by collaborative effect of both chemical treatment and modified atmosphere packaging results in firmness of tissue. Chemically treated and MAP packed samples showed higher radical scavenging activity and phenolic content as compared to controlled samples. Chemical treatment in combination with MAP has potential for maintaining button mushroom shelf life up to 16 days at 8°C i.e. low temperature storage. Chemical treatment effect for maintaining the quality of button mushroom was more pronounced than MAP alone. Chemically untreated samples showed browning, high weight loss and fast deterioration of textural parameters. It can be concluded that CaCl$_2$ (2.5% w/v), citric acid (3% w/v) and sorbitol (0.1% w/v) treatment with MAP composition of 6% O$_2$ and 12% CO$_2$ were found best for post-harvest storage of button mushroom. Chemical compounds other than sorbitol, citric acid and calcium chloride can be further explored with other MAP composition and different packaging material for enhancing post-harvest life of other highly perishable products.

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