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Optimization of moisture absorber with packaging material to increase the shelf-life and quality of white button mushroom (*Agaricus bisporus*) at ambient condition

[*1] Neha Singh, [2] Vigya Mishra, [3] Devina Vaidya

[1] Warner School of Food and Dairy Technology, SHIATS, Allahabad, Uttar Pradesh, India
[2] Amity International Centre for Post-Harvest Technology and Cold Chain Management, Amity University, Noida, Uttar Pradesh, India
[3] Department of Food Science and Technology, Dr. YS Parmar UHF, Nauni, Solan, Himachal Pradesh, India

Abstract
Shelf-life of freshly harvested mushrooms (*Agaricus bisporus*) is limited to 1-3 days at ambient temperature because they transpire at the same rate as the fruiting sporophore. Condensed water on the underside of plastic pouches causes a thin layer of moisture to persist on caps and thus supports growth of fungus which leads to the requirement of moisture absorbers to increase the shelf-life of mushrooms. The present study was conducted to determine the combined effect of packaging material and absorber on quality and shelf-life of fresh packaged mushrooms. Freshly harvested mushrooms were washed with 0.5 % $CaCl_2$ + 0.5 % KMS + 0.5 % NaCl solution, packed in polypropylene with different concentrations of absorbers (desiccant mixture of bentonite 0.55 g + sorbitol 0.25 g + $CaCl_2$ 0.20 g g^{-1}), and were subjected to the storage period of 3 days at ambient condition. During storage, different physical and biochemical parameters were analyzed. Mushrooms packed in polypropylene pouches with 12 g absorber showed best results with respect to weight loss and visual observation. Quality of mushrooms was found to be maintained with moisture absorber. A notable decrease in surface moisture of mushrooms was observed when packed with absorber containing 16 g of desiccant mixture.

Keywords: button mushrooms, degree of whiteness, absorber, packaging

1. Introduction
White Button mushrooms (*Agaricus bisporus*) are very perishable in nature with a usual shelf-life of 1-3 days at ambient condition. They have very high transpiration rates, resulting in a large amount of water vapour accumulation inside the package, thereby, increasing the in-package humidity level (Mahajan *et al*., 2008) [13]. The vapour can built-up in the package, allowing spoilage bacteria to grow and cause the mushrooms to become brown and spotted. Temperature control and modification of atmosphere are two important factors in prolonging shelf-life. Packaging is a fundamental tool in order to retain general quality. But polymeric films used in packaging of fresh produce have lower water vapour transmission rates. This low water transmission rate of packaging films in combination with the high transpiration rate of mushroom results in rapid saturation of the package atmosphere (Roy *et al*., 1996) [19]. Therefore, high humidity conditions prevail in the packages, causing moisture condensation, microbial growth and decay of the product. These conditions may also cause the growth of Pseudomonas tolasii, responsible for browning or yellowing of the sporophore surface, known as bacterial blotch, also making the package unattractive (Jin *et al*., 1994). Condensation of moisture inside the packages may also cause off-odour and off-colour developments.

The use of active packaging with moisture absorbers could be a useful tool in order to cause the absorption of the excess moisture within the package and reduction in speed of spoilage. However, existing moisture absorbers either have low absorption capacity and/or absorb moisture quickly, making them unsuitable for mushrooms. Therefore, the present study was undertaken with an objective to select the storage condition and to standardize the concentration of moisture absorber with the correct moisture holding capacity for prolonging shelf-life of white button mushroom.

2. Materials and Methods
2.1 Preparation of raw material
Fresh button mushrooms used in this study were procured from Mushroom Center, Department of Plant Pathology, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan. After harvesting, sorting and cutting the base, mushrooms were washed with 0.5 % potassium metabisulphite (KMS) + 0.5% sodium chloride (NaCl) + 0.5% calcium chloride ($CaCl_2$) solution for 2 minutes and were air dried at room condition (Singh, Vaidya and Mishra, 2014).

2.2 Packaging with moisture absorber and storage
In order to avoid condensation, sorbitol, bentonite and $CaCl_2$ were used as moisture absorbers to modify the in-package relative humidity in combination with the polypropylene bag (PP, 1500 gauze) and sealed. Desiccant mixture was prepared by mixing bentonite, sorbitol and $CaCl_2$ in the proportions of 0.55, 0.25, 0.20 g g^{-1} (Mahajan *et al.*, 2008) [13]. Different quantities of desiccant mixture i.e. 4, 8, 12 and 16 were prepared and packed in the form of sachet made from synthetic fiber. White button mushrooms were packed in polypropylene packet in quantity of 200g, containing a sachet of moisture absorber and having the sealed dimension of 15 x 20 cm^2. These packets were stored at refrigerated (4±2 °C) conditions. The mushrooms were then analyzed for various physico-chemical changes and sensory attributes at an interval

of 3 days. Each sample was prepared and analyzed in triplicate.

2.3 Quality Parameters

2.3.1 Weight gain by absorber

Weight gain by moisture absorber packed with mushroom in different packaging materials was calculated by deducting the weight of moisture absorber after storage from the initial weight of mushroom absorber. The results were expressed as per cent weight gain by absorber.

$$\text{Weight gain by absorber (\%)} = \frac{(\text{Initial weight of absorber} - \text{Final weight of absorber})}{\text{Initial weight of absorber}} \times 100$$

2.3.2 Weight loss by mushroom

Weight loss in mushroom packed in different packaging materials, with and without moisture absorber, was calculated by deducting the weight of mushroom after storage from the initial weight of mushroom i.e. before storage. The results were expressed as per cent weight loss in mushroom.

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

2.3.3 Moisture content

The moisture content was estimated by drying the weighed sample (5 g) to a constant weight in hot air oven at 70±2 °C. The dried samples were then cooled to room temperature in a desiccator prior to weighing (Ranganna, 2010) [18]. Loss in weight of sample after drying representing the moisture content was expressed as per cent (w/w).

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

2.3.4 Total phenols

The amount of total phenols in the mushroom sample was determined with the Folin-Ciocalteu reagent using catechol as a standard (Bray and Thorpe, 1954) [5]. One gram of sample was taken and grinded with 10 mL of 80 % ethanol in pestle and mortar and centrifuged for 20 min at 1000 rpm and filtered. Filtrate was evaporated in oven up to dryness and dried extract was dissolved in 5 mL distilled water. 0.2-2.0 mL aliquot was taken in separate test tubes and volume was made up to 3 mL. Then 0.5 mL Folin-Ciocalteu reagent was added. After 3 min 2 mL of 20 % Na_2CO_3 was added and mixed. Test tubes were placed in boiling water bath for 1 min and then cooled. Optical density of the sample was recorded at 650 nm with the help of spectrophotometer (Spectronic 20D). The concentration was determined from the standard curve prepared using different concentrations of catechol (8-32 µg mL^{-1}) using the above procedure. The results were expressed as mg per 100 g on fresh weight basis and calculated as given below:

$$\text{Total Phenols (\%)} = \frac{\text{O D of unknown Sample x Phenol value from standard curve (µg)} \times \text{Total volume of extract}}{\text{Aliquot of sample used} \times \text{Weight of sample taken x 1000 x 1000}} \times 100$$

2.3.5 Degree of whiteness

The known weight of sample was macerated with distilled water and then filtered. The increase in absorbance of sample extract at 420 nm as per method was taken. Optical density of filtrate was measured by spectrophotometer (Spectronic 20D), using distilled water as a blank (Ranganna, 2010) [18].

2.3.6 Visual observation

The colour and texture of the washed as well as stored mushroom were analyzed through sensory observation i.e. color through visual observation whereas texture by the hand feel or sense of touch.

2.3.7 Statistical analysis

The data of the experimental observations during the above studies were computed for analysis of variance (ANOVA) using STATISTICA version 7 software of StatSoft Inc., Tulsa, Oklahoma, USA. The ANOVA was performed as per the completely randomized design (CRD). Experiment conducted in this study was replicated thrice.

3. Results and Discussion

3.1 Weight loss by mushroom

An increase in weight loss was recorded with increasing amount of moisture absorber used. Maximum weight loss was recorded with 16 g absorber (A_4) while minimum weight loss was recorded with 4 g (A_1) absorber as shown in Fig. 1. The results were in accordance to Mahajan *et al.* (2008) [13]. Mass loss from packed mushroom that include dry matter and moisture is related to post-harvest respiration and transpiration phenomenon in fresh commodity (Roy *et al.*, 1995) [20]. The transpiration rate of fresh produce increases with water deficit (Roy *et al.*, 1995) [20]. The high rate of transpiration accelerated weight loss. Increase in temperature decreases the RH inside the packages and increases the water vapour deficit in fresh produce (Tano *et al*. 2007) [23]. In control packages, water condensation on the film was observed, forming drops and making poor visibility through the film, whereas very little condensation was observed on the packages containing the moisture absorber.

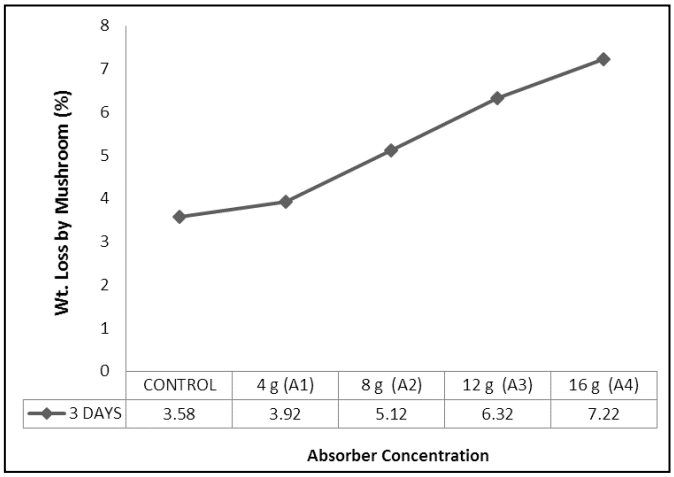

Fig 1: Effect of different concentration of moisture absorber on weight loss (%) by white button mushroom stored at ambient condition (22±2 °C); A= Absorber or desiccant mixture of bentonite 0.55 g + sorbitol 0.25 g + $CaCl_2$ 0.20 g g^{-1}

3.2 Weight gain by moisture absorber

Amongst concentration of the absorbent used maximum weight gain was recorded in mushrooms packed with 4 g desiccant mixture (A_1) as shown in Fig. 2, which may be due to the lower concentration of desiccant mixture. The moisture absorber with concentration of 4 g (A_1) and 8 g (A_2) had maximum gain in weight as it continued to absorb moisture even after turning to liquid form because of their high affinity to water, while it was minimum in 16 g absorber (A_4) at the end of storage period. Mahajan *et al.* (2008) [13] reported that bentonite and CaO stayed in the powder form even after 150 h of storage but absorbed only 0.25 and 0.18 g moisture g^{-1} desiccant. The rate of absorption of bentonite was higher initially, then slowly declined and reached a saturation level after 75 h. The desiccants such as sorbitol and xylitol, exhibit type III sorption isotherm behavior (Brunauer, 1945) [6] which absorbs very little moisture at low RH but at higher RH they absorb very high amount of moisture until they become a saturated solution.

Temperature plays a major role on moisture absorption with a positive effect on kinetic parameter as transpiration rate is lower at high RH and low temperature, yielding less water from the mushroom that is to be absorbed by desiccant. Low RH surrounding the product will increase the transpiration rate thereby yield more moisture, which is required to be absorbed by desiccant at higher sorption rate. Increment in the humidity increases surface active forces necessary to attract the vapour. These forces tend to decrease when the water is absorbed resulting in conditions fall in the sorption rate until equilibrium is attained. These changes were in favour of mushroom growers/sellers as the requirement is to slow down the moisture absorption rate avoiding excess water loss from mushroom at higher RH while requiring a higher moisture holding capacity of desiccant getting most of the excess water absorbed.

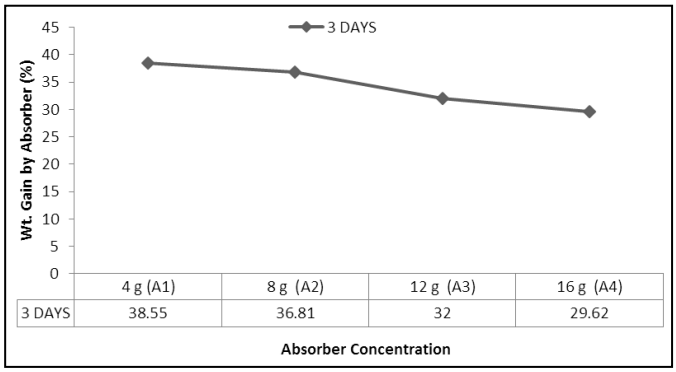

Fig 2: Effect on weight gain (%) by the different concentration of moisture absorber packed with white button mushroom stored at ambient condition (22±2 °C); A= Absorber or desiccant mixture of bentonite 0.55 g + sorbitol 0.25 g + $CaCl_2$ 0.20 g g^{-1}

3.3 Moisture Content

Data given in Table 1 reveals that moisture content decreases with the advancement of storage period. During storage, minimum moisture loss was recorded in mushroom packed with 16 g absorber (A_4) i.e. from 899.7 g kg^{-1} at 0 day storage to 894.6 g kg^{-1} at 3rd day of storage, while maximum moisture content was recorded in control i.e. polypropylene (PP) pouches without absorber (control) i.e. 898.2 kg^{-1} at 3rd day of storage. This can be correlated to the extent of weight loss in mushrooms in presence of absorber. The results were supported by Roy *et al.* (1995) [20] who reported that mushrooms stored with 5 g sorbitol had higher surface moisture content than those stored with 25 g sorbitol after 6 days storage and those stored with 15 g and 20 g sorbitol after 9 days of storage. Rapid decrease in moisture content with 16 g moisture absorber during storage might be an indication of over drying of those mushrooms. Moisture gain in case of control packages may be due to the permeability of packaging material as well as absence of absorber. Diffusion through the packaging film is the only means of moisture loss from the package. Thus, the mushrooms without moisture absorbers had higher moisture content than other treatments throughout the storage period.

Table 1: Effect of washing treatment, packaging and moisture absorber on moisture content (g kg^{-1}) of white button mushroom stored at ambient condition (22±2 °C)

Moisture absorber (A)	Storage Interval (D)		Grand Mean (A)
	0 day	3 days	
Control (without absorber)	899.7	898.2	899.0
A_1: 4 g	899.7	897.4	898.6
A_2: 8 g	899.7	896.2	898.0
A_3: 12 g	899.7	895.1	897.4
A_4: 16 g	899.7	894.6	897.2
Grand Mean (D)	899.7	896.3	
CD (P=0.01)	Moisture Absorber = NS, Storage Interval = 2.54, Interactions = NS		

A= Absorber or desiccant mixture of bentonite 0.55 g + sorbitol 0.25 g + $CaCl_2$ 0.20 g g^{-1} NS= Non-significant

3.4 Total phenols

The total phenols decreases with the increase in moisture absorber concentration and the storage interval. This could be due to the loss in weight during the storage and the concentration of moisture absorber (Table 2). Another factor responsible for the decrease in phenol content could be the degradation of phenol compounds due to different metabolic processes viz., respiration, ethylene production, and enzyme activity. The maximum reduction in total phenols was recorded in control i.e. polypropylene (PP) pouches without absorber when compared with samples packed with different concentrations of moisture absorber. The mushroom packed with 4 g absorber (A_1) showed maximum decrease in phenols with a mean value of 3.6 g kg^{-1} and was minimum in A_4 (16 g absorber) i.e. 4.0 g kg^{-1} when compared with control having mean value of 3.4 g kg^{-1}.

Table 2: Effect of washing treatment, packaging and moisture absorber on total phenols (g kg^{-1}) of white button mushroom stored at ambient condition (22±2 °C)

Moisture absorber (A)	Storage Interval (D)		Grand Mean (A)
	0 day	3 days	
Control (without absorber)	4.2	2.6	3.4
A_1: 4 g	4.2	2.9	3.6
A_2: 8 g	4.2	3.2	3.7
A_3: 12 g	4.2	3.5	3.9
A_4: 16 g	4.2	3.8	4.0
Grand Mean (D)	4.2	3.2	
CD (P=0.01)	Moisture Absorber = NS, Storage Interval = 0.49, Interactions = NS		

A= Absorber or desiccant mixture of bentonite 0.55 g + sorbitol 0.25 g + $CaCl_2$ 0.20 g g^{-1} NS= Non-significant

3.5 Degree of Whiteness (Colour)

There was significant decrease in degree of whiteness of white button mushroom packed with different concentration of moisture absorber with the increase in storage period and moisture absorber concentration (Fig. 3), which could be due to the presence of calcium that is responsible for slowing senescence and helps in maintaining selective permeability of membranes of higher plants (Ferguson, 1984) [9]. The copper containing enzymes tyrosinase, of the PPO group is largely responsible for the enzymatic discoloration of mushrooms (Nerya *et al*., 2006) [16]. Minimum colour loss was recorded in case of 16 g absorber (A_4) as 0.445 followed by A_3 (12 g absorber, 0.447) when compared with control (without absorber) i.e. 0.456. Since mushrooms packed with 12 g moisture absorber had the best overall colour, conditions attained in these packages were considered to be optimal for the best colour of mushrooms during storage.

Duckworth and Coleman (1970) [8] observed that the activity of tyrosinase, responsible for mushroom browning was dependent on O_2 concentration. Thus, a reduction in O_2 below ambient (21.00 %) was expected to reduce activity of the enzyme. Use of sorbitol in conventional packages, resulted in mushrooms with better color than those packaged without sorbitol during 9 days of storage at 12 °C when moisture loss was < 18 per cent (Roy *et al.*, 1995) [20]. He also reported that mushrooms packed with 20 and 25 g sorbitol had lower L-values than those with 10 and 15 g sorbitol after 9 days storage.

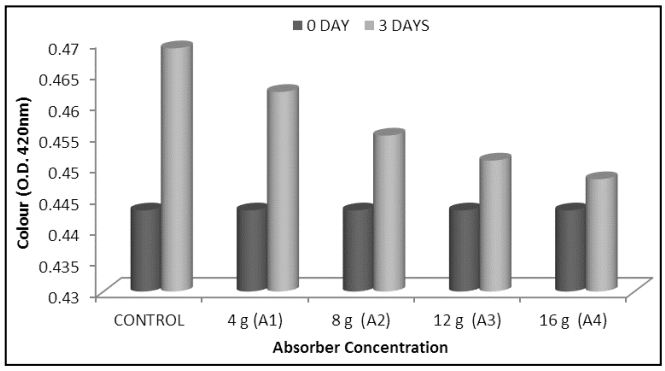

Fig 3: Effect of different concentration of moisture absorber on degree of whiteness (colour) of white button mushroom stored at ambient condition (22±2 °C); A= Absorber or desiccant mixture of bentonite 0.55 g + sorbitol 0.25 g + $CaCl_2$ 0.20 g g^{-1}

3.6 Visual Observation

Visual sensory analysis of the mushrooms showed that mushroom packed with 16 g (A_4) and 12 g moisture absorber (A_3) retained the better quality after 3 days of storage but those packed with 4 g (A_1) and 8 g (A_2) of absorber got spoiled on 3rd day of storage as shown in Table 3. The results were in agreement with Lopez-Briones *et al*. (1992) [12] who reported that mushroom aging can be characterized by a soft and spongy texture, which could be attributed to cell growth and water migration. A significant reduction in firmness was detected after storage when compared with the initial values, in agreement with a report by Murr and Morris (1975) [15] on *Agaricus*. Since the loss of hardness and browning of mushrooms are governed by enzymatic activities, low temperature storage would inactivate the enzyme thus slowing down the metabolic activities and other biochemical process (Mohapatra *et al.*, 2010) [14]. Yellowing of mushroom was observed in case of 4 g absorber (A_1) while shrinkage was noticed in the mushrooms packed with 16 g absorber (A_4).Therefore a concentration of 12 g absorber was found as the best on the basis of colour and texture.

Table 3: Effect of washing treatment, packaging and moisture absorber on visual observation of white button mushroom stored at ambient condition (22±2 °C)

Moisture Absorber (A)	Storage Interval (D)	
	0 day	3 days
Control (Without absorber)	White, firm, unblemished	Whitish brown, firm
A_1: 4 g	White, firm, unblemished	Yellowish white, slight browning, firm
A_2: 8 g	White, firm, unblemished	White, slight browning, firm
A_3: 12 g	White, firm, unblemished	White, slight brown patches, firm
A_4: 16 g	White, firm, unblemished	White, brown patches, shrinkage

A= Absorber or desiccant mixture of bentonite 0.55 g + sorbitol 0.25 g + $CaCl_2$ 0.20 g g^{-1}

4. Conclusions

From the present investigation, it can be concluded that the fresh white button mushrooms treated with 0.5 % KMS + 0.5 % NaCl + 0.5 % $CaCl_2$ and packed in polypropylene (PP, 1500 gauze) with 12 g of moisture absorber was found to be the best for retention of good colour and texture and also in increasing the shelf-life of mushrooms up to 3 days at ambient condition (22±2 °C).

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