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Synergistic effects of pre-treatments and storage conditions on the shelf-life of white button mushrooms (*Agaricus bisporus*) packaged in biodegradable packaging

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Abstract
This research explores the efficacy of combining hydrogen peroxide washing treatments with innovative, eco-friendly packaging solutions to extend the shelf-life of white button mushrooms. The study evaluates the impact of these treatments under varying storage conditions, emphasizing their potential to maintain product quality and safety while reducing waste and reliance on conventional plastics. Mushrooms were washed with tap water, distilled water, and hydrogen peroxide solutions (1%, 1.5%, and 2% w/v) before packaging. For packaging, a biodegradable container made from sugarcane bagasse and a biodegradable film developed at ICAR-CIAE, Bhopal using polylactic acid (PLA), corn starch and glycerol via. modified solvent casting techniques. The mushrooms sample were packed and stored at ambient (25±2 °C) and recommended low temperatures (5±1 °C) and the results demonstrated that hydrogen peroxide washing significantly improved shelf life, extending it from 1 to 3 days at 25±2 °C and from 5 to 12 days at 5±1 °C. Among all treatments, washing with a 1.5% hydrogen peroxide solution and using biobased biodegradable packaging was the most effective, maintaining mushroom quality for up to 12 days at 5 °C. This approach effectively controlled weight loss, pH, firmness, maturity index, microbial counts, total soluble solids, and colour. The findings highlight the viability of this approach as a commercially promising alternative for the mushroom industry, mushrooms treated with natural preservatives aligns with current sustainability goals and contributing to the development of sustainable packaging systems that support both environmental and economic objectives.

Keywords: White button mushroom, washing, Hydrogen peroxide, biodegradable packaging, Shelf-life, Storage temperature

1. Introduction
Mushrooms have been consumed from centuries back due to their medicinal and nutritional benefits (Bringye *et al.*, 2021) [6]. Mushrooms are a rich source of vitamins, particularly vitamin B and also a high in protein, minerals, and essential amino acids, with low in calories (Singh, 2023) [34]. Among the numerous edible mushroom species available, only a few, including white button mushroom, oyster mushroom, paddy straw mushroom, and milky mushroom are cultivated and consumed commercially in India. Among these species, white button mushroom stands out as the most widely consumed and cultivated mushroom globally, contributing to around 40% of the world's total mushroom production (Moradian *et al.*, 2018) [24]. Due to its physiology, *Agaricus bisporus* is highly susceptible to spoilage and damage after its harvest, as its cap has no cuticle to shield it from physical harm, water loss, and microbial invasion (Subrahmanyam *et al.*, 2023) [36]. Mushrooms have a higher respiration rate than other horticultural crops resulting in a shorter post-harvest life and which faces problems in marketability. Several factors characterize the postharvest quality of mushrooms, including shape, size, colour, maturity, microbial growth, physiological weight loss etc. One of the critical issue, particularly for commercially sold mushrooms, is the loss of their original white colour during storage. Colour is the first thing played a role in customer acceptance and largely controls mushroom business growth. Since the use of compost in mushroom cultivation creates a huge contamination, in the form of dirt and bacterial adherence to the mushroom.

These contaminations can be removed by washing or rinsing to prevent microbial spoilage (Verma *et al*., 2020) [43]. However, washing increases the moisture content of mushrooms, making them more prone to microbial growth and browning. Traditional washing methods for button mushrooms, such as chlorine or water-based treatments, often fail to sufficiently maintain the quality and extend the shelf-life of mushrooms. These methods can result in undesirable changes, such as browning, texture softening, and increased microbial load, reducing marketability and consumer acceptance. Additionally, the use of chlorine poses health risks due to potential carcinogenic byproducts and is environmentally concerning. Therefore, it is a way to add anti-browning and antimicrobial agents in water for washing the mushrooms (Zhang *et al*., 2018) [46]. Using hydrogen peroxide, sodium hypochlorite, hydrogen peroxide, malic acid, acetic acid, and ascorbic acid in mushroom washing processes can effectively reduce microbial activity (Sahoo *et al*., 2021; Sami *et al*., 2021) [28, 29]. However, these treatments still have limitations in enhancing the shelf life of mushrooms. Among the many reported treatments, washing of fresh button mushrooms with aqueous hydrogen peroxide is considered an easy and cost-effective because it is less likely to cause the leaching of essential nutrients and bioactive compounds. Additionally, its use as a natural preservative aligns with the growing consumer demand for clean-label and chemical-free food products.

Apart from the application of washing treatment, packaging technique also contribute to the improvement in the quality and shelf life of mushrooms. In the mushroom industry, commonly used packaging techniques include perforated packaging, vacuum sealing, and film wrapping (Dawadi *et al*., 2022; Sharma *et al*., 2024) [10, 31]. However, after the mushrooms are harvested, they are also sold directly in the market without packaging (Castellanos-Reyes *et al*., 2021) [7]. Polyethylene (PE) and polyvinyl chloride (PVC) are commonly used for mushroom packaging, but PE is effective in reducing browning, minimizing water loss, and slowing the respiration rate of pine mushrooms (Feng *et al*., 2023) [11]. By monitoring the exchanges of gases and vapours with the outside atmosphere and monitoring the needs of the food, plastic packaging helps to maintain the mushroom quality during storage and enhances safety by reducing the risk of contamination and food-borne illnesses.

The use of plastic packaging offers key benefits such as reducing food waste with extending shelf life. Additionally, its lightweight nature helps to save energy and fuel, thereby lowering greenhouse gas emissions during transportation (Boone *et al*., 2023) [4]. However, despite these advantages, the leakage of millions of tons of plastic into the environment has led to significant pollution and incurred substantial economic costs. Developing sustainable packaging alternatives made from bio-based and biodegradable materials may help to mitigate plastic waste (Tweneboah Kodua *et al*., 2022) [40]. Polylactic acid (PLA), derived from renewable resources like starch and sugar, is a compostable polymer increasingly used for food packaging due to its sustainability benefits (Swetha *et al*., 2023) [37]. However, PLA is brittle under stress, undergoes significant physical aging, and is relatively expensive compared to traditional polymers (Ferdináand *et al*., 2023) [12]. To improve its properties and make it more cost-effective, PLA is often blended with other agents or used with plasticizers, such as glycerol or formamide, to enhance flexibility and mechanical strength (Shahbazi & Shavisi, 2018) [30]. PLA-starch blend films are a promising option for biodegradable packaging, but they still require further development to improve their mechanical properties (Da Silva Pens *et al*., 2024) [9]. Despite the interest, there is limited research on using PLA and PLA-starch films for packaging fresh vegetables, particularly for fresh white button mushrooms (Yaradoddi *et al*., 2020) [44].

Effective postharvest management, appropriate temperature control, and advanced packaging technologies are crucial for preserving the quality and extending the shelf life of white button mushrooms. However, the impact of washing these mushrooms with varying concentrations of hydrogen peroxide solutions, followed by packaging in bio-based and biodegradable materials, on their postharvest quality during storage has not been thoroughly studied. The use of hydrogen peroxide as a washing treatment is compatible with biodegradable packaging, which is increasingly preferred for sustainable food packaging solutions. The combination of hydrogen peroxide treatment and biobased packaging materials can further enhance the preservation of mushrooms by creating a synergistic effect. This study is important as it explores the effectiveness of hydrogen peroxide washing in varying concentrations to improve the shelf life and quality of white button mushrooms during storage at both ambient (25±2 °C) and low (5±1 °C) temperatures. By reducing microbial load and inhibiting enzymatic browning, hydrogen peroxide treatment can enhance product safety and visual appeal. Packaging the treated mushrooms in biobased-biodegradable films aligns with sustainability goals, reducing environmental impact. The findings could lead to commercially viable solutions that combine natural preservatives with eco-friendly packaging, offering an improved, sustainable alternative for the mushroom industry.

2. Materials and Methods

2.1 Study location

The experiments were conducted at the Agricultural Produce Processing Division of the ICAR-Central Institute of Agricultural Engineering, Bhopal, India (coordinates: 23°15'35.75" N, 77°24'45.40" E) in January 2024.

2.2 Collection, washing, film preparation, packaging, and storage of white button mushroom

Freshly harvested, unwashed, whole white button mushrooms were collected from the identified farm situated near to Bhopal city of Madhya Pradesh, India. The individual mushrooms, at its closed cap stage with a pileus diameter of 40-50 mm, were cut from parent media and safely transported to reach in to the laboratory and stored at 5 °C with 90% relative humidity until further use. Before using it for experiments, the stem of each mushroom was trimmed to 10 mm length, and selected only those with uniform size, no defects, undamaged caps, and a cap diameter of 40 to 45 mm. The selected mushrooms were then washed in aqueous solutions of 1%, 1.5%, and 2% (w/v) hydrogen peroxide for 2 minutes with gentle shaking to remove soil adherents and minimize bruising. All types of washing solutions were prepared at ambient temperature. After rinsing, the mushrooms were placed on absorbent paper for 10 minutes to remove the excess surface moisture. The selected treatment for washing of mushrooms includes:

unwashed/control, washed in tap water, washed in distilled water, and washed in 1%, 1.5%, and 2% (w/v) hydrogen peroxide solutions. Further, the mushrooms were packaged in biobased-biodegradable (made from sugarcane bagasse) trays (approx. 100g/tray) of 0.72 mm thick with box dimensions of 140×100×40 mm which was procured from local market. The trays were overwrapped with laboratory prepared biodegradable films (Figure 1). Biodegradable film (combination of polylactic acid, corn starch and glycerol) developed (Mangaraj *et al*., 2022) [22] with some modifications using modified solvent casting (Yousefnia Pasha *et al*., 2021) [45]. The developed biodegradable film showed the thickness: 0.043±0.006 mm, tensile strength (TS): 56.38±2.84 (Mpa), haze percentage: 26.37%, oxygen transmission rate (OTR): 318.25±6.23 ($cc.m^{-2}.day^{-1}$) and water vapour transmission rate (WVTR): 146.85±4.67 ($g.m^{-2}.day^{-1}$).

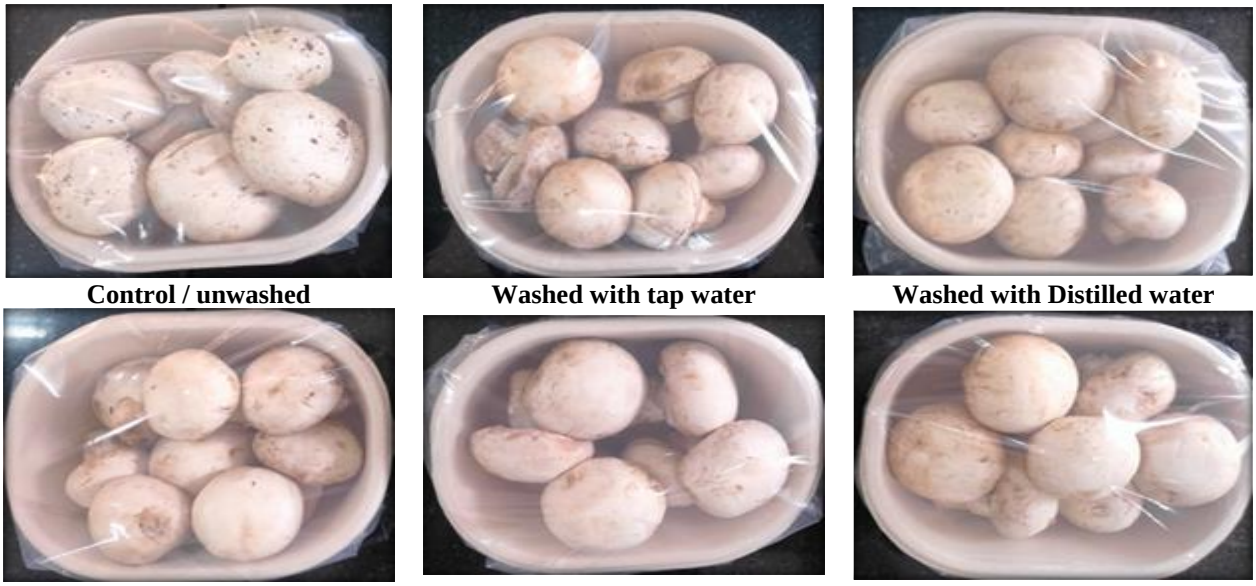


Fig 1: White button mushroom in biobased-biodegradable packaging with different washing treatments.

The mushroom trays were split into two equal groups and stored at two distinct temperatures: ambient (25±2 °C) in BOD incubator and low (5±1 °C) in refrigerator. The treatments applied to the mushrooms during the experiments are mentioned in Table 1. The experimental samples washed, packaged and stored as per the experimental conditions were analyzed at every 2 days of interval for its selected quality attributes like change in physiological loss in weight (PLW,%), pH, firmness, maturity index, microbial count, total soluble solids, colour and browning index till the spoilage in stored samples.

Table 1: Different codes (T_1 - T_{12}) showing the washing treatment and storage conditions of mushroom samples

Treatment codes	Stored at ambient temperature (25±2°C)	Treatment codes	Stored at low temperature (5±1°C)
T_1	Control / unwashed	T_7	Control / unwashed
T_2	Washing with tap water	T_8	Washing with tap water
T_3	Washing with Distilled water	T_9	Washing with Distilled water
T_4	Washing with 1% hydrogen peroxide (w/v)	T_{10}	Washing with 1% hydrogen peroxide (w/v)
T_5	Washing with 1.5% hydrogen peroxide (w/v)	T_{11}	Washing with 1.5% hydrogen peroxide (w/v)
T_6	Washing with 2% hydrogen peroxide (w/v)	T_{12}	Washing with 2% hydrogen peroxide (w/v)

2.3 Quality attributes

2.3.1 Physiological Loss in Weight (PLW,%):

The weight loss of all experimental samples of mushroom was measured through a digital weighing balance (Mettler Toledo, Switzerland), with an accuracy of ± 0.001 g. The percentage (%) of physiological loss in weight was calculated as follows:

$$PLW\ (\%) = \left(\frac{W_i - W_f}{W_i}\right) \times 100$$

Where,
PLW- Physiological loss in weight, (%)
W_i - Initial weight of mushrooms, (g)
W_f - Final weight of mushrooms. (g)

2.3.2 pH value

The stored mushroom samples with an equal quantity of distilled water (w/v) were crushed in pestle mortar, and the supernatant was filtered. The pH of the filtered mushroom juice was estimated with the help of a digital pH meter (Model: LT 5001, Labtronics, India) with a resolution of 0.01, after calibration with standard pH 4, 7, and 9 buffer solutions (Thakur *et al*., 2020) [38].

2.3.3 Firmness

Firmness of the stored samples were assessed using a texture analyzer (TA-133 XT, Stable Micro Systems, UK) equipped with a 250 N load cell. A cylindrical probe (P-12.5) of a 12.5 mm diameter was used for testing of whole mushroom. During testing, the probe was operated at a constant speed of 2 mm/s, with defined penetration depth of

5 mm. The mean firmness was determined by averaging the firmness values obtained from different positions of the mushroom relative to the probe (Moradian *et al*., 2018) [24].

2.3.4 Maturity index
The maturity index of mushrooms, which indicates the level of maturity and generally increases after harvest, was assessed based on cap opening using a 7-point scale (Ban *et al*., 2014) [3]. To evaluate the ageing of white button mushrooms, five mushrooms were randomly selected from each package and manually inspected through visual observation and the veil opening rate was used as a key parameter. This rate is defined as the ratio of mushrooms with open caps to the total number of mushrooms.

2.3.5 Microbial load
The total microbial load on the mushroom samples was monitored at two-days intervals during storage to evaluate the incidents of microbial spoilage. The colony count method is used for microbial load calculations in terms of CFU. During the estimation, nutrient agar based culture medium was used for bacterial growth, employing the pour plate method. Serial dilutions were prepared for bacterial colony counting. For each experiment, 1 gram of the sample was thoroughly mixed in a sterile test tube filled with 9 millilitres of distilled water. The medium was then poured to cover approximately one-third of the plate's depth and spread evenly. From each diluted sample, pour 1 millilitre of diluted solution into a sterile petri plate. After allowing the plates to cool, they were sealed with parafilm and incubated for 24-48 hours at 37 °C. Colony counts were performed by dividing the plate into four sections, counting the colonies in one section using a digital colony counter, and then multiplying by four to estimate the total colony count (Ranganna, 1986) [27]. The colony forming units (CFU) per gram of sample were then calculated.

2.3.6 Total soluble solids (TSS)
The total soluble solids of the mushrooms were determined from the extracted mushroom juice using a portable digital refractometer (HM SCM-1000, HM Digital, India) with a measurement range of 0 to 45% (López-Gómez *et al*., 2021) [18].

2.3.7 Color measurements
Mushroom sample images with high resolution were captured using a 24.2-megapixel DSLR camera (Model D-3500, Nikon) with a pixel resolution of 23.5×15.6 mm. The images were then uploaded in Adobe Photoshop software (version 21.1) to determine the colour coordinates (L, a, and b values). Since the L, a, and b values are not standardized, they were converted to L*, a*, and b* values for evaluation (Thakur *et al*., 2020) [38].

2.3.8 Browning Index
The browning index of the mushroom during the storage period as per the given standard procedure (Mohapatra *et al*., 2010) [23].

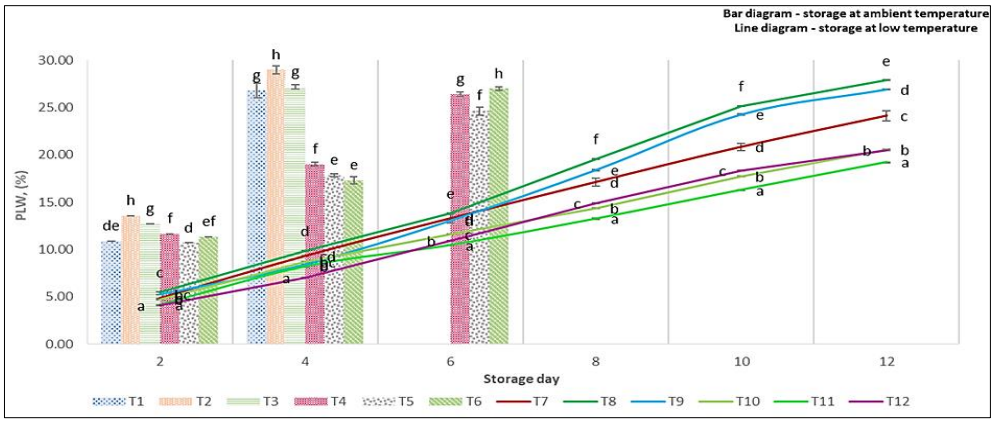
$$\text{Browning Index (BI)} = 100 \times \left[\frac{X - 0.31}{0.17}\right]$$

Where:

$$X = \frac{a + 1.75L}{5.645L + a - 3.012b}$$

2.4 Statistics
Statistical analysis, including Duncan's Multiple Range Test (DMRT) and ANOVA, was evaluated to find out the effects of washing treatments and storage temperatures on statistical differences of these quality parameters. These analyses were performed using SPSS 19.0 statistical software.

3. Results and Discussion
3.1 Effect of washing treatments and storage conditions on quality attributes of white button mushrooms
3.1.1 Physiological loss in weight (PLW,%)
Physiological loss in weight, resulting from water loss from the epidermal layer during postharvest storage, significantly affects mushroom quality (Cavusoglu *et al*., 2021) [8]. In the present investigation, a notable difference in physiological weight loss ($p<0.05$) was observed between washed and unwashed mushrooms stored at both ambient (25±2 °C) and low (5±1 °C) temperatures. Weight loss was notably influenced by storage temperature, being twice as much at ambient temperature compared to selected low temperature. For all mushroom samples, PLW,% increased over elapse in storage time (Figure 2), evident from their dehydrated appearance on surface. However, washing treatments played a crucial role in reducing weight loss, likely by decreasing respiration and transpiration rates (Figure 2) (Lufu *et al*., 2020) [19].



D*: Samples discarded due to spoilage

Fig 2: Effect on physiological loss in weight (PLW,%) of white button mushroom during storage period (days) at ambient (25±2 °C) and low (5±1 °C) temperatures. (*Values are given as means (n = 3). Different lowercase letters (a-h) indicate the significance ($p \leq 0.05$) analyzed by DMRT*).

At ambient temperature, the highest weight loss was observed in sample T_2, with approximately 29% loss after 4 days of storage. In contrast, sample T_5 exhibited the lowest weight loss, with a reduction of 24.63% after 6 days of storage. The increase in weight loss during storage duration was generally higher in higher temperatures (Castellanos-Reyes *et al*., 2021) [7]. This corresponds to a reduction in the vapour transmission of films, along with a decrease in the transpiration and respiration rates of the mushrooms, regardless of the treatment and packaging materials used (Gholami *et al*., 2020) [14]. Washing the mushroom with hydrogen peroxide at all concentration samples (T_4, T_5, T_6) showed less weight loss compared to control (T_1), washing with tap water (T_2) and distilled water. At low temperatures, mushrooms washed with 2% hydrogen peroxide (T_{11}) showed the lowest weight loss of 19.21% followed by samples T_{12} and T_{10} after the 12th day of storage. At ambient temperature (25±2 °C), hydrogen peroxide-washed mushroom samples were rejected after 6th day of storage, whereas their counterparts stored at low temperatures (5±1 °C) lasted longer till 12th day of storage. Storage of mushrooms at low temperatures was also effective in reducing the weight loss for control and washing with tap and distilled water. Mushrooms stored under refrigerated conditions retained more weight compared to those stored at ambient conditions (Dawadi *et al*., 2022) [10]. Washing with 2% (w/v) hydrogen peroxide and storing at low temperatures (T_{11}) was more effective in minimizing weight loss compared to the control and other washing treatments. Additionally, pretreatments with H_2O_2 have been found to significantly reduce weight loss in pepper fruits during storage at both ambient and low temperatures (Tiamiyu *et al*., 2023) [39].

3.1.2 pH

pH plays a critical role in physiological activities of the fresh agro commodities during storage and further influences on its shelf-life. In the present investigation, the initial pH of stored fresh button mushroom (T_1) was reported as 6.52. Same range (6.50-6.92) of pH was also reported in literature for fresh mushroom (Aday, 2016) [1]. At day 0, the pH of hydrogen peroxide-washed mushrooms in the treatment of T_4, T_5 and T_6 was 5.13, 4.65 and 4.53 which was lower than the without washed control (T_1), and washed with tap (T_2) and distilled water (T_3). The lower pH values observed in the hydrogen peroxide-washed mushrooms were attributed to the acidic nature of hydrogen peroxide, which likely to be adsorbed onto the external surface of the mushrooms during washing. During grinding and centrifugation, hydrogen peroxide may transferred to the supernatant, contributing to the lowering in pH values. Further an increase in pH was observed for samples of all the treatments over elapsed in storage period under both ambient and low-temperature conditions (Table 2).

Table 2: Effect on pH of white button mushroom during storage (days) at ambient (25±2 °C) and low (5±1 °C) temperatures. (*Values are given as means (n = 3). Different lowercase letters (a-l) indicate the significance (p ≤ 0.05) within the same row for each group analyzed by DMRT*).

Days	25±2°C						5±1°C					
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_8**	**T_9**	**T_{10}**	**T_{11}**	**T_{12}**
0	6.52^{f}	6.40^{e}	6.01^{d}	5.13^{c}	4.65^{b}	4.53^{a}	6.52^{f}	6.40^{e}	6.01^{d}	5.13^{c}	4.65^{b}	4.53^{a}
2	6.68^{l}	6.57^{j}	6.35^{h}	5.36^{f}	4.96^{d}	4.84^{c}	6.61^{k}	6.45^{i}	6.07^{g}	5.20^{e}	4.72^{b}	4.59^{a}
4	6.97^{k}	6.81^{j}	6.50^{h}	5.47^{f}	5.13^{d}	5.03^{c}	6.71^{i}	6.52^{h}	6.15^{g}	5.34^{e}	4.81^{b}	4.74^{a}
6	D*	D*	D*	5.94^{f}	5.36^{d}	5.31^{c}	6.76^{i}	6.77^{h}	6.26^{g}	5.49^{e}	4.95^{b}	4.87^{a}
8				D*	D*	D*	6.84^{f}	6.68^{e}	6.33^{d}	5.64^{c}	5.06^{b}	4.99^{a}
10							6.91^{f}	6.76^{e}	6.41^{d}	5.79^{c}	5.23^{b}	5.13^{a}
12							7.02^{f}	6.86^{e}	6.51^{d}	5.96^{c}	5.40^{b}	5.36^{a}

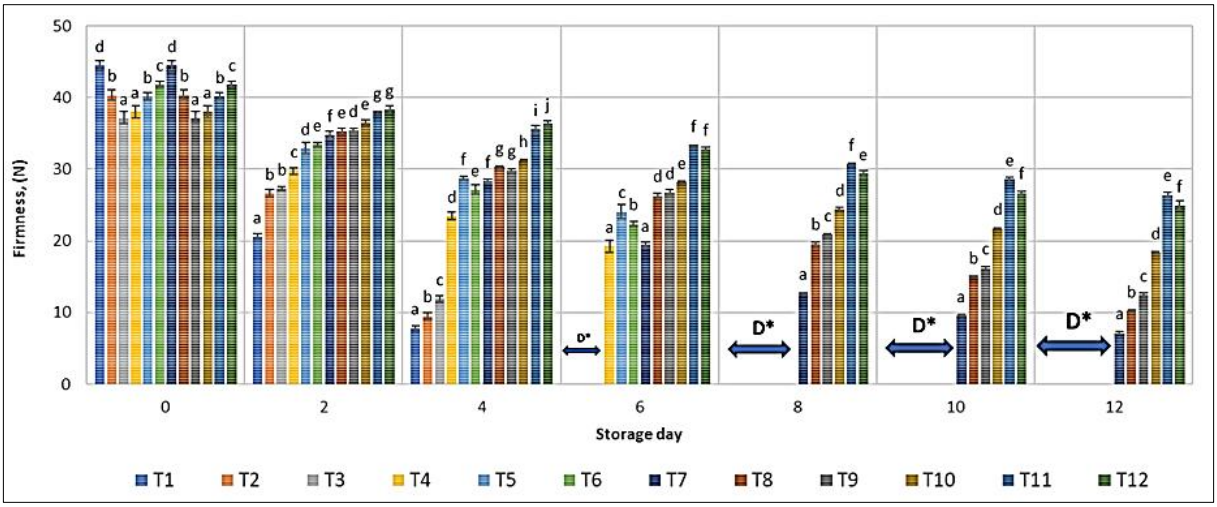
D*: Samples discarded due to spoilage

The pH values are responsible for microbial growth, spoilage and the subsequent production of organic acids (Snyder *et al*., 2018) [35]. Washing treatments and storage temperatures significantly influenced on pH ($P < 0.05$) of the stored mushroom samples (Table 2). At ambient temperature, T_5 treatment showed the lowest pH value of 5.31 after 6 days of storage, which is less than the pH value of fresh mushrooms at 0th days. At low temperatures, mushrooms washed with 3% hydrogen peroxide (T_{12}) showed the lowest pH value of 5.36 followed by samples T_{11} (5.40) and T_{10} (5.96) after the 12th day of storage. Washing button mushrooms with 3% (w/v) hydrogen peroxide and stored at low temperature (T_{12}) was found effective in reducing pH level compared to control and other washing treatments.

3.1.3 Firmness

The firmness of white button mushrooms is one of the critical quality parameter for consumers acceptance., The change in textural attributes of the mushroom is also one of the key indicator for deterioration (Borges *et al*., 2023) [5]. The initial firmness of fresh mushrooms (T_1) was found to be 44.49 ±3.64 N. The firmness values of all the treated and stored samples was gradually decreased with increase in storage time for both ambient and low temperatures storage conditions (Figure 3).

The treatment parameters like treatment combinations, storage temperature, and duration significantly ($P< 0.05$) effected on the firmness values of the mushroom. Mushroom softening or loss of firmness is also linked to the degradation of proteins and polysaccharides, shrinkage of hyphae, disruption of central vacuoles, and expansion of intercellular spaces on the surface of the piles (Qiu *et al*., 2022) [26].



D*: Samples discarded due to spoilage

Fig 3: Effect on firmness (N) of white button mushroom during storage period (days) at ambient (T_1 to T_6) and low temperatures (T_7 to T_{12}). (*Values are given as means (n = 3). Different lowercase letters (a-h) indicate the significance ($p \leq 0.05$) analyzed by DMRT*).

Under ambient conditions, the mushrooms washed with hydrogen peroxide shown better retention of firmness even after 6th day of storage as compared to control without washing sample and distilled water washed sample shown at 4th day of storage (Figure 3). These observation might be due to the hydrogen peroxide washing appeared to mitigate softening caused by bacterial activity and water loss. Similarly, at low temperature storage, mushrooms washed with hydrogen peroxide showed significantly higher firmness ($p \leq 0.05$) compared to the control and those washed with tap and distilled water after the 12th day of storage. This results raveled that, hydrogen peroxide washing, when combined with suitable packaging, helps to delay the mushroom softening during storage. Additionally, the use of PVC film for packaging was observed to slow down changes in firmness by increasing CO_2 levels within the package and hence reduction in respiration rates (Mangaraj *et al*., 2014) [20]. Under low-temperature conditions, after 12th days of storage, the firmness of the control (T_7) decreased to 7.15 N, whereas the firmness values for T_{10}, T_{11}, and T_{12} were 18.36 N, 26.42 N, and 24.89 N, respectively. Minimal changes in textural parameters were also noted for T_{11} followed by T_{12}, highlighting the effectiveness of hydrogen peroxide washing and biodegradable packaging to maintain the texture of mushroom.

3.1.4 Maturity index

The opening of button mushroom cap during storage is an indicator of maturity, represents the internal moisture loss. During the maturity, tissues of upper surface of mushroom dry out and further leads to increased water loss. This water loss further causes the caps and veil to lose their original structure (Cavusoglu *et al*., 2021) [8]. The washing treatments with hydrogen peroxide found to be effective in preserving the integrity of the veil until the 6th days of storage at ambient temperature and up to the 12th days under low-temperature conditions (Table 3). At the start of the experiment (Day 0), all mushroom samples exhibited a maturity index of 0, as each selected mushroom was at the closed-cap stage. The experimental results presented in table 3, shows that, hydrogen peroxide solution of 3% (w/v) concentration used to wash the mushrooms observed to be the most effective approach to delayed cap opening.

Table 3: Effect on maturity index of white button mushroom during storage (days) at ambient (25±2 °C) and low (5±1 °C) temperatures. (*Values are given as means (n = 3). Different lowercase letters (a-k) indicate the significance ($p \leq 0.05$) within the same row for each group analyzed by DMRT*).

Days	25±2 °C						5±1 °C					
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
2	2.95^{k}	1.96^{j}	1.88^{i}	1.52^{h}	1.46^{g}	1.41^{e}	1.43^{f}	1.40^{e}	1.37^{d}	1.13^{c}	1.09^{b}	1.01^{a}
4	4.11^{k}	4.08^{j}	3.97^{i}	2.86^{h}	2.76^{g}	2.70^{f}	2.59^{e}	2.36^{d}	2.23^{c}	1.92^{b}	1.79^{a}	1.77^{a}
6	D*	D*	D*	3.98^{h}	3.86^{g}	3.85^{g}	3.26^{f}	3.10^{e}	2.98^{d}	2.51^{c}	2.38^{a}	2.41^{b}
8				D*	D*	D*	4.13^{d}	3.88^{c}	3.88^{c}	2.92^{b}	2.87^{a}	2.91^{b}
10							D*	D*	D*	3.58^{c}	3.42^{a}	3.51^{b}
12										3.93^{c}	3.81^{b}	3.79^{a}

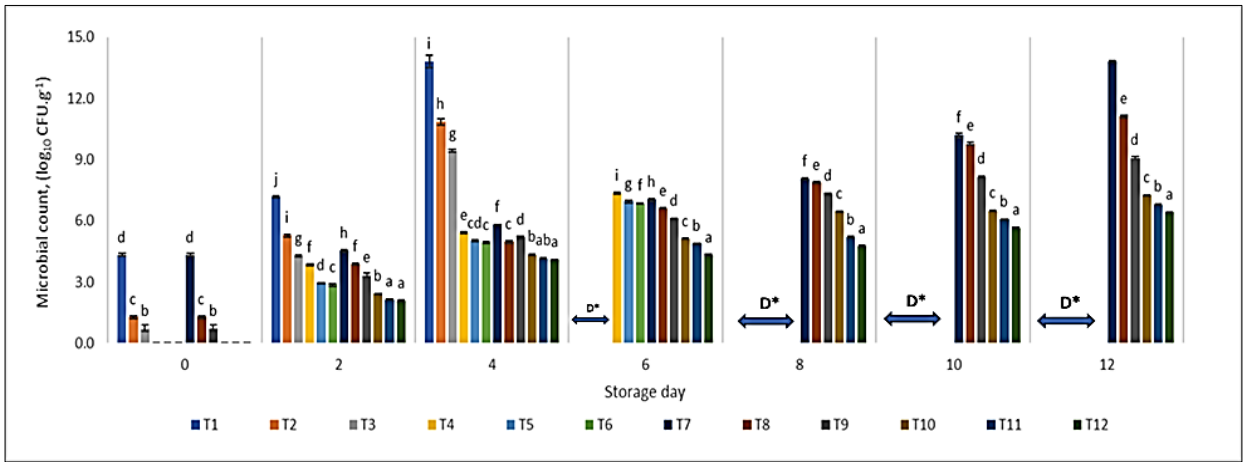
D*: Samples discarded due to spoilage

The maturity index found to be increased with the values of 3.93 in T_{10}, 3.81 in T_{11}, and 3.79 in T_{12} at 12th day of storage. Despite the hydrogen peroxide washing treatment, the incidences of veil opening was visible in mushrooms stored at ambient temperature storage. Among all treatments, washing with a 3% (w/v) hydrogen peroxide solution and storing the mushrooms at a low temperature (i.e T_{12}) was found the most effective with lower values of maturity index and far better than both the control and other selected washing treatments.

3.1.5 Microbial load

The microbial load, particularly *Pseudomonas spp*., plays a vital role in determining the postharvest quality of mushrooms (Venturini *et al*., 2011) [42]. In this study, the initial *Pseudomonas spp*. count on fresh white button

mushrooms was reported in the tune of 4.33 log_{10} CFU.g^{-1}, which is also in range with previous studies which reported it in the range of 3.9 to 6.1 log_{10} CFU.g^{-1} at harvest time (Simón *et al*., 2005) [33]. With elapsed in storage period, the *Pseudomonas spp*. count found to be increased in the stored freshly harvested mushroom samples. However, washing the mushrooms with hydrogen peroxide and packaging them in biodegradable packaging significantly reduced the microbial counts, due to antimicrobial effect of hydrogen peroxide's (Figure 4). The inhibitory impact of sodium citrate (SC) on microbial growth reported by Allende *et al*. (2009) [2] in which the reduction of total aerobic mesophilic bacteria by 3 log_{10} CFU.g^{-1} on fresh-cut cilantro after washing with a 1 g.L^{-1} SC solution (Allende *et al*., 2009) [2]. The washing methods shown the significant ($p \leq 0.05$) effect on the *Pseudomonas spp*. counts in treated and stored mushrooms as per the experimental conditions.



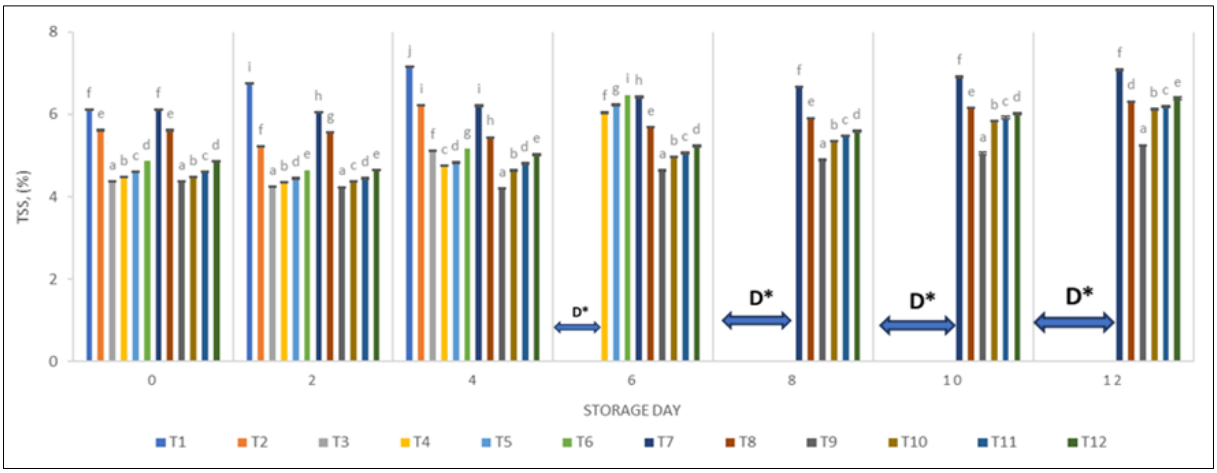
D*: Samples discarded due to spoilage

Fig 4: Effect on microbial load (*Pseudomonas spp*.) of white button mushroom during storage period (days) at ambient (T_1 to T_6) and low temperatures (T_7 to T_{12}). (*Values are given as means (n = 3). Different lowercase letters (a-j) indicate the significance ($p \leq 0.05$) analyzed by DMRT*).

The highest microbial count was observed in the control samples, in the range of 13.80 to 13.81 log_{10} CFU.g^{-1} after 4 days of ambient storage and 12 days of low-temperature storage, respectively. Among the selected treatments, washing of mushrooms with 3% (w/v) hydrogen peroxide solution proved the most effective in curbing microbial growth. At the 6th day of storage, the microbial count in the T_6 treatment was 6.84 log_{10} CFU.g^{-1}, and it get change up to 6.42 log_{10} CFU.g^{-1} at 12th days of storage. Although the total microbial count in various treatments was found to be increased with increase in storage time, still the values are in the acceptable limits given by IS: 7463-2004. The findings indicate that washing of button mushrooms with a 3% (w/v) hydrogen peroxide solution, followed by storage at a low temperature (T_{12}), are more effective in controlling the microbial growth as compared to the control (without washing) and other washing treatments.

3.1.6 Total soluble solids (TSS)

The TSS of mushroom found to be increased with increase in storage as presented in figure 5. This increasing trend in TSS is the indicator of elevated respiration rate and progression of ripening process (Moreno *et al*., 2021) [25]. The changes in TSS were more significant in the control samples than in the treatment samples. On day 0, also washing of mushrooms with tap water, distilled water, and 1%, 1.5%, and 2% (w/v) hydrogen peroxide solutions shows the lower TSS value compared to the control without washed sample (Figure 5).



D*: Samples discarded due to spoilage

Fig 5: Effect on total soluble solids (%) of white button mushroom during storage period (days) at ambient (T_1 to T_6) and low temperatures (T_7 to T_{12}). (*Values are given as means (n = 3). Different lowercase letters (a-j) indicate the significance ($p \leq 0.05$) analyzed by DMRT*).

At ambient temperature, the maximum TSS value was reported in sample of treatment T_1 i.e., 7.15%. While the sample of T_4 treatment showed less TSS value i.e., 6.04% even after 4th days of storage. At low-temperature storage, mushrooms washed with distilled water (T_{10}) showed the lowest TSS value (5.25%) followed by samples T_{10} and T_{11} after the 12th day of storage. Similar trend of change in TSS was also reported by Shirvani *et al*. (2023) [32] in modified atmosphere (MA) packaged conditions fresh-cut mangosteen, purple and yellow plums, and pears (Shirvani *et al*., 2023; Mangaraj *et al*., 2011) [32, 21].

3.1.7 Colour

Change in L*

Colour change in mushrooms is one of the major and primary indicator for consumer preference and acceptance. In the experimental data, the L* value, representing the lightness or darkness of the mushroom samples, decreased with respect to increase in storage time in both ambient and low-temperature storage (Table 4). Similar trend was also reported by Vazirianjafari *et al.* (2023) [41] for button mushroom (Vazirianjafari *et al*., 2023) [41]. Washing treatments mainly enhance the L* value and hence help to maintain the whiteness of the button mushroom. On day 0, the L* value of the fresh white button mushrooms (control) was 87.53, and this value fond to be gradually increased in all treated samples after washing. At 4th days of storage at ambient temperature, the L* value of the control sample (T_1) was significantly ($p \leq 0.05$) lower than the washed sample with hydrogen peroxide. Among the treatments, washing with a 2% (w/v) hydrogen peroxide solution was most effective for maintaining the whiteness of the mushrooms after 6th days of storage, likely due to its bleaching properties (Table 4). However, higher concentrations of hydrogen peroxide caused a slight yellowing of the mushrooms. The hydrogen peroxide wash resulted in the smallest reduction in L* value at the beginning of storage, and it gets decline over time (Gupta & Bhat, 2016) [15].

Table 4: Effect on L* values of white button mushroom during storage (days) at ambient (25±2 ℃) and low (5±1 ℃) temperatures. (*Values are given as means (n = 5). Different lowercase letters (a-h) indicate the significance ($p \leq 0.05$) within the same row for each group analyzed by DMRT*).

Days	25±2℃						5±1℃					
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
0	87.53^{a}	90.15^{b}	90.30^{bc}	91.66^{cd}	92.03^{d}	92.49^{d}	87.53^{a}	90.15^{b}	90.30^{bc}	91.66^{cd}	92.03^{d}	92.49^{d}
2	80.94^{a}	83.66^{b}	84.53^{b}	86.71^{d}	88.75^{ef}	89.56^{fg}	84.51^{b}	85.74^{c}	88.39^{e}	90.09^{g}	91.41^{h}	91.59^{h}
4	75.11^{a}	76.59^{a}	79.61^{b}	82.80^{cd}	83.82^{de}	84.71^{def}	80.60^{bc}	81.12^{bc}	85.67^{ef}	86.29^{f}	89.72^{g}	90.13^{g}
6	D*	D*	D*	78.69^{b}	81.50^{d}	80.06^{c}	77.60^{a}	78.90^{b}	81.68^{d}	86.80^{e}	97.98^{f}	88.47^{f}
8				D*	D*	D*	74.61^{a}	75.35^{b}	79.08^{c}	85.05^{d}	86.48^{e}	86.40^{e}
10							70.87^{a}	72.15^{b}	76.96^{c}	83.86^{d}	84.77^{e}	83.85^{d}
12							67.21^{a}	70.01^{b}	74.02^{c}	80.08^{d}	82.48^{f}	81.95^{e}

D*: Samples discarded due to spoilage

After the 12th day of storage at low temperatures, the highest L* value was recorded at 82.48 for treatment T_{11}, followed by 81.95 for T_{12} treatment, and 80.08 for T_{10}. Mushrooms with L* value below 80 are generally considered unacceptable at the wholesale level, as reported by Lagnika *et al*. (2014) [17]; Gao *et al*. (2014) [13]. This criterion is commonly used to assess the shelf life of mushrooms in both industry and research. Therefore, washing mushrooms with a 2% (w/v) hydrogen peroxide solution and storing them at a low temperature is the most effective method for minimizing the colour changes in white button mushrooms.

3.1.8 Browning index

The browning index of mushrooms is influenced by changes in L*, a*, and b* values of it. As storage time progressed, the browning index of the mushrooms increased consistently across all the selected treatments (Table 5). This change in browning index might be due to the natural ageing and respiration in the mushrooms. The washing treatments had a significant effect ($p \leq 0.05$) on the extent of mushroom browning with respect to increase in storage duration. At ambient temperature, the browning index in control (T_1) without washed sample was 15.37 which was significantly higher than the sample washed with 2% (w/v) hydrogen peroxide concentration and measured values at 4th days of storage i.e., 7.60.

Table 5: Effect on Browning index of white button mushroom during storage (days) at ambient (25±2 ℃) and low (5±1 ℃) temperatures. (*Values are given as means (n = 5). Different lowercase letters (a-i) indicate the significance ($p \leq 0.05$) within the same row for each group analyzed by DMRT*).

Days	25±2℃						5±1℃					
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
0	1.85^{e}	1.55^{d}	1.25^{bc}	1.07^{a}	1.17^{ab}	1.32^{c}	1.85^{e}	1.55^{d}	1.25^{bc}	1.07^{a}	1.17^{ab}	1.32^{c}
2	6.78^{g}	5.66^{f}	5.17^{f}	1.88^{bcd}	1.97^{cd}	2.45^{de}	2.65^{e}	2.34^{de}	2.04^{cd}	1.19^{a}	1.32^{ab}	1.47^{abc}
4	15.37^{i}	13.20^{h}	10.69^{g}	4.00^{de}	4.31^{e}	4.89^{f}	4.23^{de}	3.88^{d}	3.13^{c}	1.87^{a}	2.06^{ab}	2.35^{b}
6	D*	D*	D*	8.53^{f}	7.60^{e}	10.24^{g}	6.80^{d}	6.31^{c}	5.39^{b}	2.61^{a}	2.73^{a}	3.05^{a}
8				D*	D*	D*	9.96^{f}	8.56^{e}	7.59^{d}	3.73^{a}	4.14^{b}	4.52^{c}
10							12.39^{e}	10.88^{d}	9.82^{c}	4.83^{a}	5.61^{b}	5.76^{b}
12							15.85^{f}	13.45^{e}	11.69^{d}	7.97^{b}	6.70^{a}	9.27^{c}

D*: Samples discarded due to spoilage

Similar observations were observed for button mushrooms stored at low temperatures. During low temperature storage, T_7, T_8, and T_9 samples had browning index scores of 15.85, 13.45, and 11.69, respectively, at the same time there is development of off-colour on the 12th days of storage especially in the low-temperature storage compared to their

counterparts stored at ambient temperature condition at day 4. Mushrooms stored at low temperatures showed better performance compared to those stored at room temperature. This improvement is likely due to the lower temperature reducing the rates of respiration, ageing, biochemical reactions, and enzymatic activity (Castellanos-Reyes *et al*., 2021) [7]. At low temperatures, all the hydrogen peroxide-treated mushroom samples were effective in controlling the browning up to the 12th day of storage. However, mushrooms washed with 2% (w/v) hydrogen peroxide concentrations scored better than all other selected treatments. The browning of mushrooms is primarily caused by the oxidation of phenolic compounds by polyphenol oxidase enzymes (Kim *et al*., 2020) [16].

4. Conclusion

Mushroom is one of the best sources of protein for vegetarian diet. However, it has shelf life of 1 day under ambient conditions, which might be due to high rate of respiration and microbial contamination at field level. Hence, the study aimed at finding a solution for washing treatment to reduce the microbial contamination and biodegradable packaging solutions for enhancing shelf life with sustainable environmental safety. During the investigation, selected mushrooms were treated with different washing techniques and stored at ambient and low temperatures. The study demonstrates that washing white button mushrooms with hydrogen peroxide and storing them at low temperatures effectively maintains their quality. Washing with 2% hydrogen peroxide (T_{11}) minimized weight loss, while 3% hydrogen peroxide (T_{12}) reduced pH levels more effectively than the control and other treatments. Texture preservation was best in T_{11} and T_{12}, emphasizing the combined benefits of hydrogen peroxide washing and biodegradable packaging. Furthermore, T_{12} resulted in the lowest maturity index and superior microbial control. Hydrogen peroxide-treated mushrooms, particularly those washed with 2% and stored at low temperatures, also showed minimal browning and better color retention, making these methods optimal for extending shelf-life. Among the treatments, washing the mushrooms with 2% (w/v) hydrogen peroxide followed by biodegradable packaging and storing at low temperatures (5±1°C) effectively preserved freshness and prevented spoilage up to 12 days. These findings suggest that hydrogen peroxide washing and biodegradable packaging systems could serve as viable alternatives for enhancing the shelf life of button mushrooms while reducing the environmental impact of synthetic plastics.

Author Contributions

Aman Kumar: Conceptualization, methodology, data collection, analysis, and manuscript drafting. He was primarily responsible for designing the study and conducting the primary data analysis, as well as writing the initial draft of the manuscript. Deepak Kumar: Data curation, formal analysis, and review & editing. He assisted with the statistical analysis, contributed to the interpretation of the results, and participated in revising the manuscript critically for important intellectual content.

Conflict of Interest

The authors affirm that they have no financial or personal conflicts of interest that might have influenced the research presented in this article.

Data availability statement

The data (developed film data, and mushroom quality data after different washing treatments) will be available by the corresponding author on receiving a reasonable request.

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