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Hydrogen Peroxide Washing Induced Changes in Postharvest Quality of Button Mushrooms (*Agaricus bisporus)* during Storage

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Abstract: Present study hypothesized that washing of button mushrooms (*Agaricus bisporus*) with aqueous solution of H_2O_2 retains their postharvest quality in terms of weight loss, maturity, color, flavor, taste, disease incidence and overall acceptability during storage. To test this hypothesis, fresh button mushrooms were washed with aqueous solution of H_2O_2 at different concentrations (3, 4 and 5%) for 2 minutes and stored under two temperature conditions namely; low (5°C) and room temperature (25-35°C). Results evidenced that washing treatments with different concentrations of H_2O_2 were found effective in retaining the quality of stored button mushrooms for extended period (4-14 days) when compared with unwashed button mushrooms (3 days). With the advancement in storage period, significant increase in weight loss, maturity index and microbial growth of button mushrooms was observed. Among all the treatments, washing with 5% H_2O_2 followed by storage at 5°C temperature was found to be the most effective in controlling the weight loss, maturity index, microbial growth and overall quality of button mushrooms for up to a period of 14 days.

Keywords: Button mushroom, hydrogen peroxide, washing treatments, storage, shelf-life

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1. Introduction

Edible mushrooms form an important part of human diet since antiquity because of their striking color, distinct flavor and peculiar aroma. Mushrooms are a form of edible fungi that contains high quality proteins, rich in essential amino acids, minerals and vitamins with low calories (Kale *et al.* 2018). Although twenty genera of mushrooms are being cultivated throughout the world, only

four types, *viz*., white button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus* spp.), milky mushroom (*Calocybe indica)* and paddy straw mushroom (*Volvariella volvacea*) are grown commercially in India. The button mushroom (*Agaricus bisporus*) is the most widely cultivated and consumed mushroom throughout the world and includes about 40% of total world mushroom production and 85 to 90% of the country's production.

Post-harvest management of button mushrooms is very critical due to their short shelf-life. Considering its physiology, it is one of the most sensitive agricultural crops after harvesting, because there is no cuticle on the cap surface to protect it from physical damage, water evaporation and microbial attack (Brennan *et al.* 2000; Sapers *et al.* 2001). Owing to perishable nature of mushrooms they get easily spoiled due to wilting, maturing, weight loss, diseases, browning, liquefaction, loss of texture, aroma, flavor, etc. making them unsalable. Delicate texture and high moisture of most of the mushrooms is the reason behind their short shelf life. Respiration rate of mushrooms is high in comparison to the other horticultural crops which results in their short postharvest existence and decreases their marketability.

There are several indicators that determine the postharvest quality of mushrooms namely; physical appearance, size, color, veil opening, microbial growth and physiological weight loss. Loss of original color or whiteness during storage is particularly deleterious for the mushroom market. Color as first perceived by the consumers and largely regulate the mushroom industry. In order to improve the color and enhance the shelf-life, washing of mushrooms with various anti browning agents is suggested. There are many methods to extend the shelf-life of mushrooms. They include modified atmosphere packaging (MAP) (Roy *et al.* 1995), controlled atmosphere storage (CAS) (Lopez-Briones *et al.* 1992), edible coating (Nussinovitch and Kampf, 1993), refrigeration (Gomley, 1975), cultivating with calcium chloride solution (Miklus and Beelman, 1996) and using sorbitol (Roy *et al.* 1995). In some researches, the effects of different anti-microbial and anti-browning solutions on the sensory attributes and microbial quality of the whole or sliced mushrooms have been studied. These solutions included ascorbic acid and its derivatives as anti-browning agents (Hsu *et al.* 1988); ascorbic, citric and erythorbic acids as browning inhibitors (Sapers *et al.* 1994); citric acid and hydrogen peroxide (H_2O_2) as anti-bacterial agents (Brennan *et al.* 2000); H_2O_2 as an anti-bacterial agent along with sodium erythorbate as an anti-browning agent (Sapers *et al.* 2001); citric acid as an anti-microbial agent (Simon and Gonzalez-Fandos, 2010); H_2O_2 and ClO_2 as anti-bacterial agents and sodium

D-isoascorbate monohydrate as an enzymatic browning inhibitor; and citric acid along with sodium D, L-isoascorbate as anti-browning agents (Simon and Gonzalez-Fandos, 2010).

Among several reported treatments, washing the button mushrooms with aqueous solution of H_2O_2 is considered to be an easy and cost effective alternative. Hence, present research was conducted to study the effect of washing the button mushrooms with different concentrations of H_2O_2 on color, odor, disease incidence, physiological weight loss, maturity index and microbial count.

2. Materials and Methods

Freshly harvested button mushrooms (*Agaricus bisporus*) were procured from a farmer's field in Abohar, Punjab during the month of March 2019. Mushrooms were harvested from the first and second break (1st and 2nd crop from the same bed), loose packed in polyethylene bags and brought to the laboratory of ICAR-Central Institute of Postharvest Engineering and Technology, Abohar, Punjab for further experiments**.** Mushrooms (initial moisture content: 87-88%) of uniform size, intact veil and healthy fruits in the stage 1 or 2 according to Guthrie's scale were carefully selected (Roy *et al.* 1995). The pilei diameter of the selected mushrooms was 4 to 5 cm. Mushrooms were kept in cold store operating at 4±0.5°C temperature and 85% relative humidity (RH) for 15-16 h to narrow down the respiration rate.

Before applying the washing treatments, mushrooms were thoroughly cleaned with distilled water to remove surface contamination. After washing with water, mushrooms were surface dried with the help of tissue paper. Hydrogen peroxide (H_2O_2) (SD Fine-Chem Ltd, Mumbai, India) was used as an anti-browning/anti-bacterial agent at three different concentrations *viz.* 3, 4 and 5% (v/v). Mushrooms were treated by dipping them in H_2O_2 aqueous solutions for 2 minutes followed by surface drying under the fan. Washed mushrooms were packed in polypropylene bags, PP (75 µ thickness and 100 g pack size) with 10 to 12 holes in each packet for air exchange. Approximately, 100 g of mushrooms were packed in single packet. Mushroom packets were equally divided into two lots and stored at two different storage temperatures namely; low temperature (5±0.5°C) and room temperature (temperature range of 25-35°C). Low temperature (5±0.5°C) was achieved by keeping the packed mushrooms in cold store (M/s Winchester Enterprises, Chandigarh, India) operating at 5±0.5°C and 85±5% relative humidity. Details of the treatments applied during study are given in Table 1.

The observations on change in color (off-color), odor (off-odor), moisture content (%), disease incidence (%), maturity index, physiological weight loss (PLW, %) and microbiological analysis were recorded each day of storage till its spoilage. The moisture content was determined by hot air oven method, using hot air oven (M/s Popular Traders, Ambala, India). The off-color and off-flavor were studied on a scale ranging from 1 to 4 with 1 equals to none (no change) to 4 means high (Gupta *et al.* 2017). Disease incidence was calculated by the formula (Eq.1),

$$Distance\ incidence(\%) = \frac{Number\ of\ infected\ fruiting\ body}{Total\ number\ of\ fruiting\ body} \times 100 \quad (1)$$

The maturity index of mushrooms is a sign of maturity which increases after harvest and can be observed on the basis of cap opening. It was measured according to the method suggested by Guthire (1984) on the basis of scale ranging from 1 (veil intact) to 7 (cap open, gills flat). Aging in white mushrooms can be characterized by the veil opening rate. Five mushrooms were selected randomly from each packet and were evaluated manually by visual observation. PLW was determined by weighing the contents of the package before and after storage and was expressed as the percent loss of weight with respect to the initial weight (Eq.2).

$$PLW(\%) = \frac{w_i - w_f}{w_i} \times 100 \quad (2)$$

Where, W_i was the initial weight of the mushrooms before treatment and W_f was the final weight of mushrooms after predetermined storage interval.

Microbiological spoilage of fresh and stored mushroom samples was assessed in terms of total plate count. For estimating the microbial population in mushroom samples, dilution plate method was followed and was carried out up to six dilutions. In order to isolate the microorganisms, various bacteriological media chemicals and reagents used in the present study were obtained from Hi-media. All media used in the present study were prepared according to the instructions provided by the manufacturing firms and checked for sterility. Microbial count was recorded as cfu/g. All the quality parameters were measured in triplicate. Duncan's multiple range test (DMRT) and ANOVA were performed to evaluate the statistical differences in these parameters as affected by washing treatment and storage temperature. SPSS 16.0 software was used to conduct the DMRT and ANOVA tests. The significance was accepted at 5% levels of significance ($p<0.05$).

3. Results and Discussions

The data pertaining to effect of H_2O_2 treatment and storage temperature on moisture content (%) of button mushrooms is presented in Table 2. Moisture content in button mushrooms stored at ambient conditions and low temperature ranged from 87.23 to 92.98% and 87.23 to 90.90%, respectively. Moisture plays a great role in the shelf-life of any food commodity. Food material with higher moisture content spoils easily due to microbial attack. Mushrooms washed with 3, 4 and 5% H_2O_2 at ambient temperature showed lesser moisture content than mushroom that was not washed at all and other washed with tap water. It is evident from the results that treated button mushrooms stored at room temperature spoiled in six days in comparison to those stored at low temperature.

H_2O_2 concentration had a significant ($p<0.05$) impact on the development of off flavor at both ambient and low temperature (Table 3). Scores for off-flavor in mushrooms stored at ambient temperature ranged 1 to 3.5. Maximum score for 3, 4 and 5% H_2O_2 treated mushrooms was observed as 3.5, 3.1 and 3.0, respectively on 5th day of storage. Among all the concentration of H_2O_2 used, its 5% dose was found to be the most effective in controlling off-flavor development with a score of 3.0 on 5th day of storage at ambient temperature. Results vary with respect to score for off-flavor obtained for H_2O_2 pretreated mushrooms at low temperature. At low temperature, H_2O_2 at 5% proved to be highly effective in controlling off flavor with a score of 2.4 on 14th day of storage. On comparison, it was observed that mushrooms stored at low temperature scored better in terms of off flavor development than that stored at ambient temperature. Also, H_2O_2 treated mushrooms scored better at both ambient and low temperature.

Gradual change in color of mushrooms from white to brown and tones of black occurs with the duration of storage. Washing showed significant variation in the retention of color during storage. The effect of washing treatments on button mushroom color is compared with control treatment and data is presented in Table 4. Washing treatments significantly ($p<0.05$) affected color changes as the storage period progressed. The deterioration of color was observed in all treatments at varying rates. At ambient temperature, the off-color development was much faster in control (T1) and mushrooms washed with tap water (T2) in comparison to H_2O_2 treated mushrooms. As the duration of storage proceeded, there was consistent increase in color values in all the treatments which might be due to natural inherent senescence and respiration in mushrooms. However the off-color development was more in control as

Table 1: Treatment codes and their description

Treatment code	*Ambient temperature storage*	*Treatment code*	*Low temperature storage*
T_1	No washing/control	T_6	No washing/control
T_2	Tap water	T_7	Tap water
T_3	3% H_2O_2	T_8	3% H_2O_2
T_4	4% H_2O_2	T_9	4% H_2O_2
T_5	5% H_2O_2	T_{10}	5% H_2O_2

Table 2: Moisture content (%) of button mushrooms subjected to different treatments

Days	*T1*	*T2*	*T3*	*T4*	*T5*	*T6*	*T7*	*T8*	*T9*	*T10*
0	87.23a	87.23a	87.23a	87.23a	87.23a	87.23a	87.23a	87.23a	87.23a	87.23a
1	87.28a	90.06c	90.07c	88.02b	86.92a	87.12a	87.14a	88.64b	87.71a	87.23a
2	88.08c	89.69d	86.29a	88.46c	88.90c	89.02d	88.02c	87.40b	88.54c	86.53a
3	R*	R*	88.72a	88.96a	89.35b	88.22a	89.24b	90.69c	89.24b	90.97c
4			91.45c	91.22c	91.76c	91.28c	87.82a	90.67c	89.83b	89.06b
5			R*	89.36b	89.94b	87.92a	89.43b	89.83b	90.66c	89.91b
6				R*	91.52	88.44	89.14	89.58	88.83	88.52
7					R*	86.86a	88.61b	90.77c	89.02b	89.24b
8						88.01	90.2	88.42	89.13	90.05
10						R*	R*	92.46b	88.76a	88.99a
12								87.97a	89.69b	87.65a
14								88.20a	90.01b	88.32a

*R: rejected
Values in a row followed by same letter do not differ significantly (α=0.05)

Table 3: Off-flavor value of button mushrooms subjected to different treatments

Days	*T1*	*T2*	*T3*	*T4*	***T5***	***T6***	***T7***	***T8***	***T9***	***T10***
0	1.0a	1.0a	1.0a	1.0a	1.0a	1.0a	1.0a	1.0a	1.0a	1.0a
1	2.0c	1.6b	1.5b	1.5b	1.5b	1.0a	1.0a	1.0a	1.0a	1.0a
2	3.0c	2.9b	2.4b	2.6b	2.3b	2.2b	2.2b	1.8a	1.6a	1.6a
3	R*	R*	2.8b	2.8b	2.7b	2.3b	2.3b	1.8a	1.8a	1.8a
4			3.4	3.5c	3.4c	2.4b	2.3b	2.0a	1.9a	1.9a
5			3.5c	3.1c	3.0c	2.4b	2.3b	2.0a	1.9a	1.9a
6			R*	R*	3.3c	2.4b	2.3b	2.0a	1.9a	1.9a
7					R*	2.5b	2.4b	2.2a	2.0a	2.0a
8						2.6b	2.5b	2.3b	2.0a	2.0a
10						R*	R*	2.4a	2.2a	2.2a
12								2.5a	2.4a	2.3a
14								2.7a	2.5a	2.4a

*R: rejected
Values in a row followed by same letter do not differ significantly (α=0.05)

Table 4: Off-color values of button mushrooms subjected to different treatments

Days	*T1*	*T2*	*T3*	*T4*	*T5*	*T6*	***T7***	***T8***	***T9***	***T10***
0	1.0a	1.0a	1.0a	1.0a	1.0a	1.0a	1.0a	1.0a	1.0a	1.0a
1	1.4a	1.2a	1.3a	1.3a	1.3a	1.0a	1.0a	1.0a	1.0a	1.0a
2	3.0b	2.7b	2.7b	1.8a	1.7a	2.0a	1.8a	2.0a	1.6a	1.5a
3	R*	R*	2.9b	3.7b	2.3a	2.3a	2.0a	2.1a	1.9a	1.8a
4			3.7c	3.8c	2.8b	2.4a	2.1a	2.2a	2.0a	1.9a
5			R*	4.0c	3.1b	2.4a	2.5a	2.2a	2.0a	2.0a
6				R*	3.5c	2.6a	2.8b	2.2a	2.0a	2.0a
7					R*	3.2b	3.2b	2.2a	2.1a	2.1a
8						3.6b	3.3b	2.3a	2.1a	2.1a
10						R*	R*	2.4a	2.2a	2.2a
12								2.7a	2.4a	2.3a
14								3.0b	2.5a	2.4a

*R: rejected
Values in a row followed by same letter do not differ significantly (α=0.05)

compared to mushrooms washed with H_2O_2 (3, 4 and 5%). Rajarathnam *et al.* (1983) observed an increase in activities of o-diphenol oxidase and proteases, and a fall in total phenols and an increase in free amino acids, which resulted in the increased discoloration of mushroom with the progress in storage duration. Similar observations were recorded in case of low temperature stored button mushrooms. T6 and T7 treatments scored 3.6 and 3.3 for off color development on 8th day of storage at low temperature in comparison to their counterparts stored at ambient temperature. At low temperature, all the H_2O_2 treatments were effective in maintaining the color up to 14th day of storage. However, mushrooms washed with 5% H_2O_2 scored better of all other treatments. Mushrooms stored at low temperature scored better than those at room temperature this might be due to the fact that at low temperature, rate of respiration, senescence, biochemical reactions and other enzymatic changes slows down.

The disease incidence progressed with the duration of storage of button mushrooms (Table 5). Significant ($p<0.05$) variations in disease incidence was observed between unwashed and washed mushrooms. However, the washed mushrooms had lesser disease as compared to unwashed mushrooms or washed mushrooms with tap water (Table 5). Mushrooms stored at ambient temperature observed more disease incidence in comparison to those stored at low temperature. Unwashed mushrooms (T1) and tap water washed mushrooms (T2) showed 85 and 90% of disease incidence on 2nd day of storage and were rejected thereafter. Washing mushrooms with H_2O_2 delayed disease incidence up to 2nd day of

storage at ambient temperature. T3, T4 and T5 treatments showed 44.44%, 75% and 45.81% of disease levels on 4th, 5th and 6th day, respectively. On the contrary, there was no incidence of disease occurrence in mushrooms washed with H_2O_2 (3, 4, and 5%) and stored at low temperature. However, unwashed (T6) and tap water washed (T7) mushrooms showed disease incidence on 10th day of storage with the score of 85 and 90% followed by rejection.

Significant ($p<0.05$) variations were observed between washed and unwashed mushrooms in respect of PLW at both ambient and low temperature storage. The storage temperature exerted a strong influence on weight loss, the effect being twice at ambient temperature to that at low temperature. PLW values showed inconsistency during storage period. Initially PLW increased in all the treatments but gradually decreased inconsistently. It might be due to the absorption of water vapors by mushroom samples. Water vapors were generated inside the packet through respiration. Some moisture might have been absorbed by mushrooms from humid air. PLW values of 2.16-3.05% and 1.67-1.94% were observed after 2 days of storage in T1 and T2 mushrooms, respectively at ambient temperature. These weight losses were revealed by the dehydrated appearance of the mushrooms. Weight loss in mushrooms is a common phenomenon which occurs mainly due to moisture loss and loss of carbon reserves due to respiration (Du *et al.* 2007). Washing of mushrooms with H_2O_2 had a significant ($p<0.05$) effect in controlling weight loss and it might be because these treatments helped in reducing the rate of respiration and transpiration (Table 6). PLW ranged from 1.41 to 0.52% for unwashed

Table 5: Disease incidence (%) in button mushrooms

Days	*T1*	*T2*	*T3*	*T4*	*T5*	*T6*	***T7***	***T8***	***T9***	***T10***
0	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
1	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
2	85	90	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
3	R*	R*	30.0b	50.0c	10.0b	0.0a	0.0a	0.0a	0.0a	0.0a
4			44.4c	66.7d	22.2b	0.0a	0.0a	0.0a	0.0a	0.0a
5			R*	75.0c	37.5b	0.0a	0.0a	0.0a	0.0a	0.0a
6				R*	45.8b	0.0a	0.0a	0.0a	0.0a	0.0a
7					R*	0.0a	0.0a	0.0a	0.0a	0.0a
8						85.0b	90.0c	0.0a	0.0a	0.0a
10						R*	R*	0.0a	0.0a	0.0a
12								0.0a	0.0a	0.0a
14								0.0a	0.0a	0.0a

*R: rejected

Values in a row followed by same letter do not differ significantly (α=0.05)

Table 6: Physiological weight loss (PLW, %) in button mushrooms

Days	*T1*	*T2*	*T3*	*T4*	*T5*	*T6*	*T7*	*T8*	*T9*	*T10*
0	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
1	3.05d	1.67b	4.25e	2.32c	4.68e	1.41b	0.69a	2.74c	2.76c	4.57e
2	2.16c	1.94b	2.45c	1.89b	2.02c	0.63a	2.39c	0.77a	0.57a	1.21b
3	R*	R*	6.44b	7.88b	7.97b	0.28a	0.25a	0.0a	0.08a	0.21a
4			1.04a	1.37a	3.27b	0.11a	0.0a	0.28a	0.04a	0.07a
5			R*	2.69b	2.93b	0.31a	0.45a	0.46a	0.41a	0.50a
6				R*	3.22b	0.24a	0.32a	0.17a	0.29a	0.07a
7					R*	0.40a	0.71a	0.32a	0.29a	0.50a
8						0.68a	0.52a	0.43a	0.29a	0.50a
10						R*	R*	0.50a	0.21a	0.65a
12								0.21a	0.63a	0.07a
14								0.0a	0.12a	0.44a

*R: rejected
Values in a row followed by same letter do not differ significantly (α=0.05)

Table 7: Maturity index of button mushrooms

Days	*T1*	*T2*	*T3*	*T4*	*T5*	*T6*	*T7*	*T8*	*T9*	*T10*
0	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
1	1.05b	1.67b	1.25b	1.03b	1.01b	0.98a	1.06b	0.88a	0.71a	0.56a
2	2.76	2.94	2.45	1.89	1.55	1.40	1.44	1.03	0.96	0.83
3	R*	R*	3.44c	2.88b	2.11b	1.98a	2.11b	1.42a	1.31a	1.11a
4			4.04	3.37c	2.80b	2.36b	2.59b	1.55a	1.35a	1.24a
5			R*	4.69	3.50c	2.66b	2.88b	1.75a	1.68a	1.52a
6				R*	3.62	3.10b	3.26b	1.90a	1.81a	1.78a
7					R *	3.42b	3.65b	2.44a	2.30a	2.15a
8						3.88b	4.11c	2.84a	2.66a	2.46a
10						R*	R*	3.33b	3.11b	2.89a
12								3.51a	3.30a	3.12a
14								3.66a	3.50a	3.33a

*R: rejected
Values in a row followed by same letter do not differ significantly (α=0.05)

mushrooms and mushrooms washed with tap water during low temperature storage. Mushroom washed with H_2O_2 showed the lowest PLW of 0.12% on 14th day of low temperature storage. At ambient temperature H_2O_2 washed mushroom samples were rejected after 5, 6 and 7th day of storage in comparison to their counterparts stored at low temperature. Our results are in line with the observations made by Das *et al.* (2010). Use of citric acid and H_2O_2 was found to be effective in reducing PLW as compared to EDTA (Das *et al.* 2010). Bayoumi

(2008) also reported that pretreatments with H_2O_2 significantly decreased weight loss in pepper fruits during storage at ambient and low temperature conditions.

Washing treatments with H_2O_2 helped in delaying the cap opening and keeping the veil intact (Table 7). All the treatments were effective in keeping the veil intact up to 3rd day at ambient temperature and up to 6th day at low temperature in washing treatments with H_2O_2. However, H_2O_2 with 5% (T10) was the most effective in delaying cap opening. Maturity index increased with the increase in storage period with a value of 3.66 in T8 to 3.50 in T9 and 3.33 in T10 on 14th day of storage. Veil opening was highest in mushrooms stored at ambient temperature in spite of treatment with H_2O_2.

The total plate count (TPC) in mushrooms was as high as 3.55 (data not shown). The antimicrobial effect of H_2O_2 at different concentrations helped in controlling the microbial growth. Among the various treatments, H_2O_2 (5%) was found to be the most effective in inhibiting microbial growth. On 6th day of storage TPC was 3.55 cfu/g in T5 treatment whereas it was 5.17 cfu/g in T10 on 14th day of storage. The count was highest in control samples (without washing) ranging from 7.21 to 7.30 cfu/g at room and at low temperature storage condition on day 3 and day 8 of storage. The significant effect of H_2O_2 in limiting the spoilage by pathogenic microorganisms has been well documented by Gupta and Bhat (2016); Bayoumi (2008); Brennan *et al.* 2000). The total microbial count though increased with storage, but was lesser than the count of ISI specification (IS: 7463-2004) (Singh *et al.* 2011). The count for yeast and molds and total coliforms was observed as nil during the storage duration in all the samples. All washing treatments helped in maintaining whiteness of mushrooms. Although whiteness decreased as storage progressed, L* value (lightness/darkness) decreased and a* (redness/greenness) and b*value (yellowness/blueness) increased (Data not shown).

4. Conclusions

Overall results confirmed that mushrooms stored at ambient temperature (25-35°C) without washing spoils after 3-4 days whereas mushrooms washed with 5% H_2O_2 water and stored at low temperature remained fresh like till 14 days or more. Therefore it may be concluded that button mushrooms should be washed with 5% H_2O_2 water and stored at low temperature (5°C, 90% RH) in order to retain freshness and to prevent spoilage for longer storage period.

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