Food Research 4 (3) : 679 - 689 (June 2020)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Evaluation of storage temperature, packaging system and storage duration on postharvest quality of straw mushroom (*Volvariella volvacea*)

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Article history:
Received: 21 October 2019
Received in revised form: 3 January 2020
Accepted: 5 January 2020
Available Online: 17 January 2020

Keywords:
Volvariella volvacea,
Straw mushroom,
Postharvest,
Packaging,
Storage temperature

DOI:
https://doi.org/10.26656/fr.2017.4(3).349

Abstract

Volvariella volvacea is an edible mushroom, highly perishable and has a very short shelf life (1-2 days) at room temperature (RT). This research was conducted to determine the postharvest qualities at different storage temperatures (10, 15ºC, and RT) and storage durations (0, 2, 4, 6 and 8 days) in perforated polyethylene (PE) films. *V. volvacea* stored at 15°C showed lower weight loss, no veil opening and retained higher firmness. Thus, the mushrooms were expanded to examine the optimum packaging systems (perforation, PVC film wrap, vacuum and control) applied to *V. volvacea* for 0-8 d at 15ºC. PVC film was shown to maintain higher firmness, lower weight loss, browning degree, and PPO enzyme activity compared to other packaging. Minor damages and ultrastructure tissue shrivelling were seen in PVC film packaging. Overall, *V. volvacea* was best stored at 15°C in PVC film to retain their quality and extend its shelf life.

1. Introduction

Mushroom generally is highly perishable compared to other fresh commodities due to high respiration and transpiration rate (Taghizadeh *et al*., 2010; Jafri *et al*., 2013; Dhalsamant *et al*., 2015: Rux *et al*., 2015; Azevedo *et al*., 2017; Gholami *et al*., 2017; Wang *et al*., 2017). This might be due to its high metabolic activities that occur in the fruiting body of the mushroom. Mushrooms produced 200-500 mg/kg h at 20ºC during respiration (Kim *et al*., 2006). *Volvariella volvacea* has a very short shelf life at room temperature which is 1-2 days. Pine mushroom also has only 1-2 days shelf life at ambient temperature (Wei *et al*., 2017). The storage life of mushrooms can be extended if the appropriate condition is applied. For example, the shelf life of *Agaricus bisporus* at room temperature was up to 3-4 days (Jiang, 2013), however storing *A. bisporus* at 4°C would be greatly advantageous as the quality of attributes and their physical appearance could be maintained up to 22 days of storage (Gholami *et al*., 2017). High demanding of mushrooms has awake the mushroom industry to continuously supply of mushroom towards consumers. Therefore, the mushrooms should be kept in long term storage especially to fulfill the needs for far distance market. In this case, storage temperatures for mushroom give a greater impact for retaining its quality.

Temperature can be categorized as the most powerful major factors in retaining the quality of mushrooms after harvest. It could be a great influence on postharvest losses during storage periods (Jamjumroon *et al*., 2012). In India, improper storage at ambient temperature for more than a day exposure caused a huge post-harvest loss and affects the mushroom industrial economic (Rai and Arumuganathan, 2008). It causes massive deterioration in quality and induced veil opening problem. In addition, temperature plays a vital role in maintaining the quality components on the mushrooms along with the storage time (Azevedo *et al*., 2017; Gholami *et al*., 2017; Joshi *et al*., 2018; Singh *et al*., 2018). According to Azevedo *et al*. (2017), the lower temperature decreased weight loss along the storage period. Furthermore, mushrooms have higher respiration rates compared to other leafy vegetables or commodities (Kim *et al*., 2006). Temperature highly correlated towards the metabolism and bacterial activity engaged with the mushrooms (Dhalsamant *et al*., 2015).

After harvest, mushrooms continue to grow. Postharvest problems such as physical colour changes, tissue damages, decreasing turgidity, microbial attack, and flavourless happened due to the higher metabolic rate in mushroom which eventually leads to senescence (Gholami *et al*., 2017). Apart from the application of storage temperatures after harvest, packaging systems also highly contribute to the postharvest shelf life of straw mushroom. Effective packaging systems would

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eISSN: 2550-2166

reduce the deterioration rate and minimize their exposure to the undesirable environment during storage durations (Dhalsamant *et al*., 2015). High metabolic activities occur inside the packaging such as transpiration and respiration processes contribute to water loss in the mushroom (Rux *et al*., 2015; Azevedo *et al*., 2017). Eventually, it stimulates enzymatic browning followed by rapid deterioration.

Recently, packaging became a main useful tool in extending the postharvest life of mushroom. This includes implementing various types of packaging systems which directly or indirectly introduced modified packaging systems (MAP) in the research area. Modified atmosphere packaging (MAP) is one of the best solutions applied for the mushroom to extend its shelf life (Azevedo *et al*., 2017; Gholami *et al*., 2017). According to Gholami *et al*. (2017), packaging systems and materials used could contribute to the successful impact in maintaining the quality of mushrooms. Packaging systems commonly applied in the mushroom industry included perforated packaging, vacuum packaging, film wrap (Gorris and Peppelenbos, 1992; Kim *et al*., 2006; Dhalsamant *et al*., 2015). However, mushrooms were also sold directly without packaging in the market after harvest (Rai and Arumuganathan, 2008). Polyvinyl chloride (PVC) and polyethylene (PE) also widely used in mushroom packaging, however, PE was found to reduce the browning activities, alleviate water loss, and slower respiration rate in pine mushrooms (*Tricholoma matsutake Sing*.) (Wei *et al*., 2017). Postharvest approaches with suitable temperature and packaging systems excellently maintaining the quality attributes and extending the shelf life of straw mushroom. Therefore, this study was carried out to examine the optimum storage temperature and packaging systems applied to *V. volvacea* along storage period.

2. Materials and methods

2.1 Materials

2.1.1 Cultivation

Spawn used for cultivation of *V. volvacea* was purchased from Department of Agriculture, Padang Terap, Malaysia. The spores were grown in that spawn medium consist of cotton, paddy straw and other agricultural waste that contain cellulose until it is ready to use on cultivation bed. The cultivation of *V. volvacea* was conducted in Herbal Garden, Taman Pertanian Universiti, Universiti Putra Malaysia. Fresh empty fruit bunches (EFB) of palm oil about 1 day from its fruit bunch removing process were composted for 9 days and watered every three days with temperature around 30-38ºC. The EFB was turned over alternately with an adequate amount of water were used until the EFB surface was moistened evenly during compost. The average weight of EFB used was within 3-5 kg with the height of compost EFB about 15 cm from the ground. Size of EFB bed was about 0.8 x 2.1 m. They were incubated with poly sheet plastic for 8 days. The stake of curved pipes was arranged on the EFB bed for mushroom growth space. The mushroom production could be seen growing on the EFB bed medium within one week and ready to harvest. The mushroom took about a month to produce the yield starts from the bed composting.

2.1.2 Postharvest handling

Volvariella volvacea at button stage was harvested. A uniform size of 3-4 cm diameter and free-damage mushroom was selected and the dirt attached to mushroom were removed before storage. The mushrooms were handled gently and immediately to avoid major physical damage and deterioration. One hundred gram of mushrooms for each treatment were packed in zipped lock polyethylene (PE) bag (7x15 cm) with 12 perforations (diameter hole: 5 mm) according to the practices commonly applied by producers. The packed samples were stored at 3 different storage temperatures at 10±1ºC, 15±1ºC (RH ~80-85%) and 27±2ºC ambient (RT) (RH ~60-65%). Weight loss, firmness, veil opening, physical features and ultrastructure, browning degree, and PPO enzyme activity were measured at 0-8 d of storage. The study furthered with packaging. One hundred gram of mushrooms for each treatment were packed in 4 different packaging systems which were perforation using polyethylene (PE) (film thickness: 0.08 mm), PVC film wrap (film thickness: 0.017 mm), vacuum using nylon film (film thickness: 0.16 mm), and control (air-exposed) (Figure 1). All the packaging size were about 7 x 15 cm. Perforated packaging was designed similar to the previous experiment with5 mm perforation size and 6 holes at both sides. Mushrooms packed with PVC film were wrapped fully together with polystyrene foam at the base. Mushrooms packed with vacuum packaging was conducted by completely remove the air inside the packaging using vacuum machine and sealed tightly. All the samples were then stored at 15ºC (RH ~80-85%).

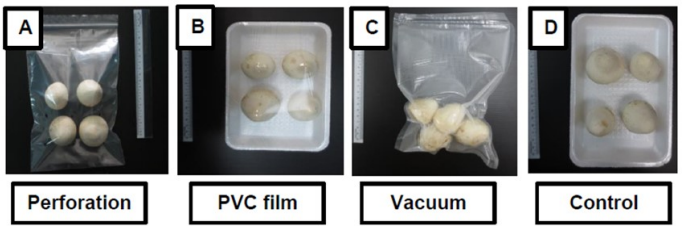


Figure 1. Packaging systems for storage of *V. volvacea*.

2.2 Methods

2.2.1 Weight loss

The weight of the mushroom samples was measured at two days interval until day eight. The initial weight and final weight of the samples were recorded. The percentage of weight loss was calculated with respect to the initial weight based on the formula:

Percentage of weight loss (%) = $[(W_i - W_f / W_i) \times 100\%]$

Where W_i = Initial weight of sample (g), and W_f = Final weight of sample (g) (Ul Haq *et al*., 2011)

2.2.2 Firmness

The firmness of the mushroom was measured by using the method described by Zivanovic *et al*. (2003) with slight modification with an Instron Universal Testing Machine (Model 5543P5995, Instron Corp. Minneapolis, USA). The measurement was conducted by piercing on the upper surface of the button tips and at the center of the pileus part during veil opening stage. The mushrooms were punched with 6 mm diameter probe, 3 mm depth with a crosshead speed of 20 mm min^{-1}. The reading was recorded in Newton (N) using Instron Merlin software version M12-13664-EN.

2.2.3 Veil opening

The number of veil opening of the intact button stage was measured based on the cracked or broken on the mushroom's volva. The percentages of veil opening were calculated using the formula (Dhalsamant *et al*., 2015):

Percentage of veil opening (%) = $[(V_i - V_f / V_i) \times 100\%]$

Where V_i =Total number of mushrooms (g), and V_f = Number of veil opened (g)

2.2.4 Physical appearance and ultrastructure

The ultrastructure of lamella or gills in both button and veil opening stages were observed under the scanning electron microscope (SEM) using Karnovsky's fixative by the method of Zivanovic *et al*. (2000) with slight modification. Samples were cut into slices and fixed in 2% glutaraldehyde for 2 hrs at 4°C followed by washing the samples with 0.1 M sodium cacodylate buffer about 3 times for 30 mins interval each. Thereafter, samples were fixed in 1% osmium tetraoxide for 2 hrs at 4°C. Samples were repeatedly washed with 0.1 M sodium cacodylate buffer for three changes. Dehydration of samples was conducted using dehydration series of 35%, 50%, 75%, 95%, and 100% acetone at room temperature. Lastly, the samples were dried through critical point dried (CPD) followed by mounting on the stub and sputter-coated with gold. The samples were observed under JSM-IT 100 InTouch Scope™ JEOL SEM, Japan.

2.2.5 Browning degree

Mushrooms were extracted and measured using Li *et al*. (2017) method with slight modification. A total of 500 mg of fresh mushroom samples were homogenized and grounded with 5 mL sodium acetate buffer (pH 4.8, 50 mmol L^{-1}). The supernatants were collected with centrifugation with 16128 g at 4°C for subsequent analysis.

Browning degree was measured using UV spectrophotometer (Fisher Thermo Scientific, Multiskan Go, United Kingdom) at 410 nm. Browning degree of the mushroom was expressed as A_{410nm}.

2.2.6 Polyphenol oxidase (PPO) enzyme activity

The PPO enzyme activity of the mushroom extracts was determined by the method described by Li *et al*. (2017) with slight modifications. The mixture of 3.9 mL sodium acetate buffer (pH 4.8, 50 mmol L^{-1}), 1 mL of catechol (0.1 M) was homogenized with 0.8 mL of extracted enzyme solution from the mushroom samples. The solutions were measured at 420 nm using the spectrophotometer (Fisher Thermo Scientific, Multiskan Go, United Kingdom). 1 unit (U) of PPO enzyme activity was defined as the amount of enzyme which increased the absorbance by 0.01 min^{-1} under the assay condition. The actual value of PPO activity was expressed equivalent to the amount of mg of FW min^{-1}.

2.3 Experimental design and statistical analysis

The experiment was conducted using a randomized complete block design in a two factorial arrangement of treatments with three replications. The treatments involved were storage temperatures (10, 15ºC and RT) and storage durations (0-8 days). For packaging, the mushrooms were kept in four different packaging systems (perforations, PVC film, vacuum and control) at 15ºC and storage durations (0-8 days). The results were analyzed using analysis of variance (ANOVA) and mean comparison of least significant difference (LSD) test at $P \leq 0.05$ by using SAS 9.4 version.

3. Results and discussion

3.1 Effect of storage temperatures on physical quality of Volvariella volvacea

3.1.1 Weight loss

Weight loss is mainly due to the water loss caused by transpiration and respiration processes. Figure 2a shows that there was an increment of weight loss along storage periods.

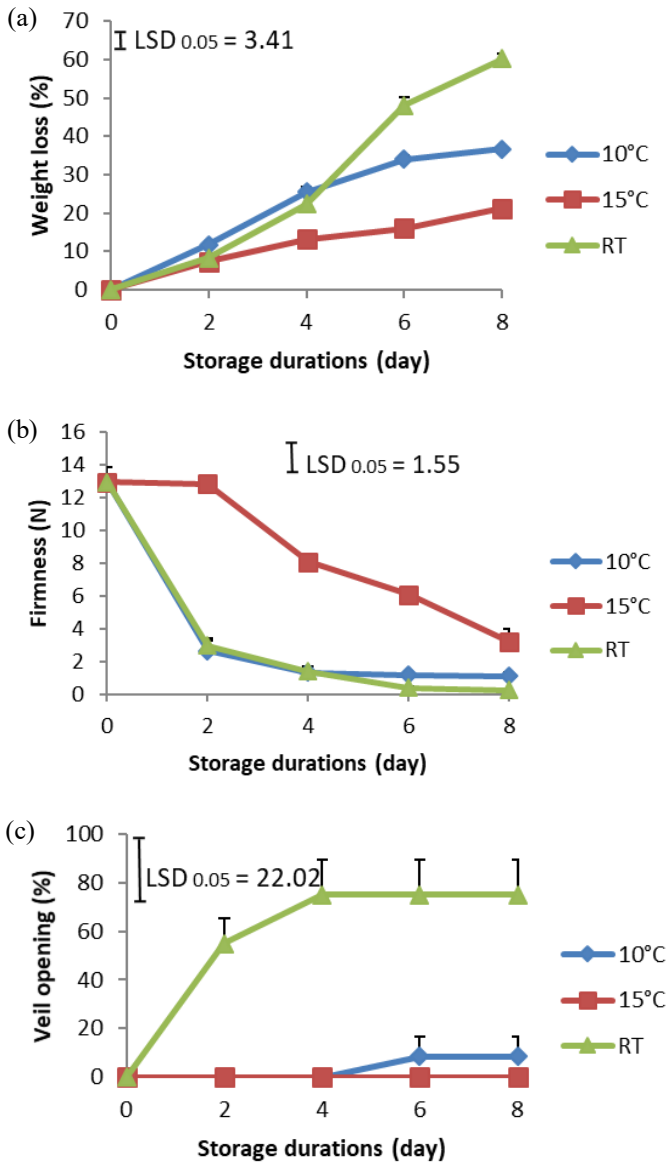


Figure 2. Relationships between storage durations (0, 2, 4, 6, and 8 days) towards the weight loss, firmness and percentage of veil opening at different storage temperatures (10, 15ºC, and RT) at P ≤ 0.05 (mean ± SE).

Percentage of weight loss was significantly increased along storage periods for all mushrooms at different storage temperatures. As mentioned by Aday (2016) there were gradual losses in weight of *A. bisporus* mushroom along the storage period. The weight loss of mushrooms at RT was significantly higher as compared to mushrooms stored at 10 and 15ºC (Figure 2a). Mushroom contains about 85-95% water and they have a very high transpiration rate. There were no barriers to water loss from their surface (Singh *et al.*, 2018). The temperature on the surface of fresh mushrooms could greatly affect the transpiration rate and water loss of the commodities. Higher temperature would produce a higher rate of water loss to the surrounding in relation to its relative humidity (Azedevo *et al.*, 2017). Mushrooms stored at 15ºC showed a significant lowest weight loss with only 21.23% increment from day 0 to day 8 compared to mushrooms stored at 10ºC and RT which were 36.61% and 60.10%, respectively. According to Antmann *et al.* (2008) and Mahajan *et al.* (2008), mushrooms that exceed 15% of weight loss were not acceptable as a good quality mushroom. Therefore, the mushrooms stored at 15ºC for 6 days reached an acceptable limit by showing 15.98% weight loss.

On top of that, weight loss also related to relative humidity (RH). Low RH stimulates water loss from the mushroom to the surrounding and thus affects the quality especially the firmness of mushroom during storage period (Mahajan *et al.*, 2008; Rux *et al.*, 2015; and Azevedo *et al.*, 2017). Indirectly, higher storage temperature increases the RH at the surrounding atmosphere which eventually causes an increase in water loss to the produces. According to Alikhani-Koupaei *et al.* (2014), the gradient of water vapour pressure highly associated with vapour-phase diffusion in different environment contributes to the percentage of weight loss. Therefore, the higher temperature at RT and lower RH causes the atmospheric water demand to transpire out moisture from the mushroom samples towards the environment. However, the higher weight loss was found in 10ºC (36.61%) as compared to 15ºC (21.23%) which might be due to chilling injuries (CI) formation at mushrooms stored at 10ºC. The mushrooms became vulnerable to that chilling temperature and caused shriveling on the tissue and water loss. In addition, thin structure of mushroom's epidermal layer did not provide support to prevent rapid water transpires to the external environment which eventually leads to dehydration along storage period (Singh *et al.*, 2018). The outer surface structure of mushrooms also could cause a great effect of the increment of water loss compared to leafy vegetables that have stomata for equilibrium transpiration and respiration.

3.1.2 Firmness

Firmness represents the indication of textural quality in postharvest studies. According to Alikhani-Koupaei *et al.* (2014), firmness best describes as changes in metabolic activity and water content in the mushroom. There were significant interaction effects (P ≤ 0.05) on the firmness of the mushroom between storage temperatures and storage durations.

The firmness of mushrooms indicates their quality, freshness and shelf life and can be counted as main attributes that might affect consumer preferences and satisfaction. Higher firmness indicates the better textural quality of mushroom. Mushrooms undergo firmness degradation from initial firmness after harvest at day 0 (Figure 2b). This result similar to Alikhani-Koupaei *et al.* (2014), whereby there was decrement of firmness at

A. bisporus after harvest. The *V. volvacea* stored at 15ºC showed significantly highest firmness throughout the storage period where the decrement was about 75.20%. The firmness of the mushroom at 15ºC maintained until day 2 with only slight decrement about 0.62% compared to the firmness of mushroom at other storage temperatures which up to 77.09%-79.48% at the same storage day. This finding indicated that storage of *V. volvacea* at 15ºC could preserve the tissue structure and thus retain the quality. Meanwhile, mushrooms stored at 10ºC and RT showed the same trend of rapid firmness decrement as early as day 2. The firmness declined dramatically until day 4 storage which about 93.45% and 89.35% at 10ºC and RT, respectively followed by gradually decreased thereafter. The dramatic firmness decrement of the mushrooms indicated that it was totally unacceptable for consumption and literally lost its marketability. The deterioration of *V. volvacea* at 10ºC might be due to chilling injuries. Thapa *et al*. (2016) stated that storage of *V. volvacea* in refrigerator more than 15-24 hours would deteriorate its quality. Besides that, the bacterial enzymes exist in the mushroom might lead to the structural changes of tissues which eventually exhibit shrivelling of cell walls. This phenomenon also drive from the amplification activity of endogenous autolysins occurred in the mushroom (Zivanovic *et al*., 2000).

Correlation analysis was conducted to observe the relationship between attributes. There was a significant, negative and strong correlation (r = -0.78) between the percentage of weight loss and firmness. This indicated that the increasing percentage of weight loss leads to decreasing in firmness. This result was similar to the finding by Gholami *et al*. (2017) and Singh *et al*. (2018) which stated that alleviation pattern of firmness can be seen along the storage period due to the effect of water loss and enzymatic activity.

3.1.3 Veil opening

Figure 2c shows *V. volvacea* stored at RT sparked a significantly higher percentage of veil opening as compared to other mushrooms stored at refrigerated temperatures at 10 and 15ºC. There were rapid increments of veil opening from day 0 to 4 about 75% at RT and became plateau throughout the storage period until day 8.

The *V. volvacea* stored at 10ºC only started the veil opening at day 6 accounted for 8.33% and fixed until the end of the storage period. Meanwhile, there was no veil opening occurred at *V. volvacea* stored at 15ºC. Opening of veil happened due to dryness from water loss which results in decrease cohesive forces and hydrophilic molecules such as protein, responsible for the intact condition of the mushroom veil (Alikhani-Koupaei *et al*., 2014). Besides that, lower temperature gives drying effect to commodities. In addition, the mushrooms continue to grow even after harvest. Thus, these factors triggered the opening of veil towards the mature stage. There was a significant, positive and intermediate correlation (r = 0.56) between the percentage of veil opening and weight loss which indicated increasing weight loss causes increasing of veil opening. This result is in agreement with the study conducted by Alikhani-Koupaei *et al*. (2014) whereby the opening of *A. bisporus* veil was highly correlated with the dryness resulted from vaporisation of water along storage periods. In vice versa, there was a significant, negative and intermediate correlation (r = -0.51) between veil opening and firmness. This means that when veil opening increase, the firmness would decrease.

3.2 Effect of packaging system on postharvest quality of Volvariella volvacea

Through the preliminary study, since the *V. volvacea* retained its quality when stored at 15ºC, the postharvest storage using different packaging systems were conducted using this temperature. Thus, implementation of this optimum temperature could eliminate the unnecessary factors that might exacerbate the mushroom quality during storage.

3.2.1 Weight loss

One of the main causes of the deterioration in the quality of mushroom is water loss (Ye *et al*. 2012; Gholami *et al*., 2017). There were significant interaction effects ($P \leq 0.05$) between packaging systems (perforations, PVC film, vacuum and control) and storage durations at (0, 2, 4, 6, and 8 days) on weight loss of *V. volvacea.*

On the whole, there was still increment of weight loss up to 39% along with the storage durations (Figure 3a). According to Tao *et al*. (2006), weight loss of mushroom increased along storage periods. However, both perforation and PVC film showed lower weight loss of *V. volvacea* after 8 days storage. Figure 3a shows a gradual weight loss of *V. volvacea* when stored in perforation (22.98%) and PVC film (13.18%) packaging. PVC film showed the lowest percentage of weight loss due to their good permeability of gaseous exchange between inside and outside environment of packaging. According to Ladaniya (2008), PVC film is a non-fogging film and transparent, which was suitable for marketing and consumer-friendly. In addition, cooling and packaging technology are the two important factors that helped in delaying mushroom's senescence and retain its quality (Singh *et al*., 2010; Kumari and

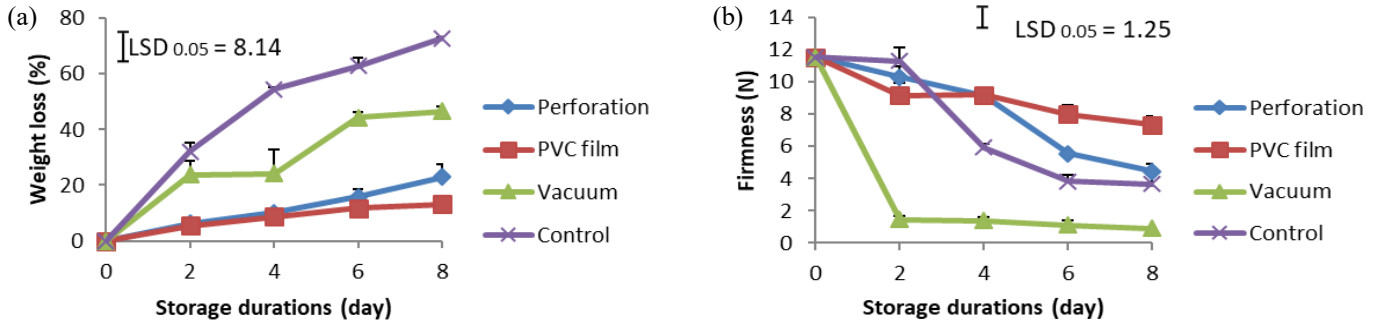


Figure 3. Relationships between storage durations (0, 2, 4, 6, and 8 days) towards weight loss and firmness at different packaging systems (perforated, PVC film, vacuum and control) at P ≤ 0.05 (mean ± SE).

Baskaran 2015).

V. volvacea stored in control packaging leads the highest increment of weight loss up to 72.83% within 8 days storage periods. According to Kumari and Baskaran (2015), mushrooms have no protective layer to prevent water from evaporating to the surrounding environment. Control packaging loss its acceptability in postharvest quality within 2 days because the percentage of weight loss exceeded 15% from the initial storage. This was due to the control packaging has no coated film that covers the mushrooms. The mushroom was exposed to surrounding air and thus causes the permeability barrier to became 100% changes with the environment. The mushrooms became dried and harden at the end of storage days. The factors that contribute to weight loss in mushrooms were the condition of mushrooms, air humidity, gaseous components and atmospheric pressure (Kumari and Baskaran, 2015). In addition, Dhalsamant *et al*. (2015) emphasized that a lower respiration rate in the packaging could lower the water loss to surrounding and hence the percentage of weight loss would be lower. All these factors affect the quality of mushroom after harvest.

V. volvacea stored in vacuum packaging showed lower weight loss (37.15%) compared to control (Figure 3a). The weight loss in vacuum packaging was majorly contributed by the elimination of water out from the fruiting body of mushroom during storage. Vacuum packaging provides zero air components inside the packaging at initial. Due to high metabolic activity, transpiration and respiration rate, the mushrooms produced unbearable watery condition and developed gaseous component inside the packaging. Alikhani-Koupaei *et al*. (2014) reported that the watery environment inside the packaging was due to condensation. This happens when there were high transpiration rate and lack of vapour permeability of packaging creates high humidity and eventually leads to the accumulation of water. Therefore, the vacuum type of packaging is not practical for storage of *V. volvacea*.

3.2.2 Firmness

Firmness degradation happens along storage periods due to rupture of cell walls caused by bacterial enzymes and endogenous autolysins occurred in *V. volvacea* (Zivanovic *et al*., 2000). There are significant interaction effects (P≤0.05) between packaging systems and storage durations on firmness of *V. volvacea*. In general, *V. volvacea* packed in all types of packaging showed a decrement in firmness along storage periods (Figure 3b).

Firmness is one of the important components measured to determine the postharvest physical quality of mushroom. The firmness of *V. volvacea* at vacuum packaging decreased sharply as early as day 2 about 87.22% followed by a gradual decrease by about 39.30% until day 8 (Figure 3b). Compression from vacuum packaging is not the main factor affecting the mushroom's firmness since the plastic film has unaltered or undamaged the mushroom shape after vacuum. The watery conditions inside packaging moisten the whole mushroom tissues and thus making the texture spongy and tender. This is in agreement with Dhalsamant *et al*. (2015) who reported that firmness of *V. volvacea* reduced when subjected to zero perforated packaging. Besides that, *V. volvacea* stored in control packaging also recorded a drastic decrement (47.36%) in firmness from day 2 to 4. This is due to the zero barrier of water vapour for transpiration to occur without packaging film.

V. volvacea stored in perforation packaging showed moderate decreased (61.30%) in firmness along storage periods. Mushrooms packaging in microporous or perforated film aid in uncontrolled weight loss and thus improved postharvest quality and storage life (Kumari and Baskaran, 2015). The perforated on the packaging might affect the firmness when there was a water loss throughout the perforations (Dhalsamant *et al*., 2015). This caused minor tissue shrinkage as depicted in Figure 5b and thus changed the firmness while the probe was pressed on the sample. Whilst, *V. volvacea* stored in PVC film showed significantly lowest firmness degradation (36.32%) along storage periods. This was

also might be closely related due to the permeability of gaseous exchanges inside and outside the packaging. During respiration, carbon dioxide (CO_2) rises followed by drops in oxygen (Kumari and Baskaran, 2015). When their respiration and transpiration reached equilibrium, they could control the movement of water particles from the mushrooms to the outside environment or within the PVC film wrap packaging. In addition, an adequate amount of CO_2 might preserve the firmness of the mushroom compared to control (Gholami *et al*., 2017). Thus, they would produce better textural quality and good eating experience to the consumer.

There was a highly significant, negative and intermediate correlation (r = -0.67) between the weight loss and firmness. This indicates that an increase in weight loss causes decreasing in firmness of *V. volvacea* along with storage durations. After harvest, the mushrooms changes in texture, exposed to weight loss, browning degree, senescence and wilting (Kumari and Baskaran, 2015).

3.2.3 Physical appearance and ultrastructure

The mushrooms undergo deterioration towards the end of the storage period (Figure 4). *Volvariella volvacea* right after harvest are round, firm, whitish colour and aromatic (Figure 5a). However, there was an improvement on the physical features of mushroom when further treated with different packaging systems compared to mushrooms treated only with different storage temperatures. Generally, darkening appearance and veil opening seem to be reduced. These include a reduction in slimy-coated layer, stickiness and pungent at the end of storage durations. Commonly, mushrooms are susceptible to lose their aroma and flavour after harvest (Kumari and Baskaran 2015). The packaging helps the mushrooms to breathe steadily due to their permeability to exchange gaseous within surrounding (Dhalsamant *et al*., 2015). According to Ye *et al*. (2012), high respiration rate could elevate the utilization of nutrients and thus accelerate deterioration and decrease their quality. Passive modified atmosphere packaging (MAP) would response through decrease in oxygen (O_2) content with an increase in CO_2 concentrations which turns the result in decrease the respiration rate of mushroom in the packaging and thus prolong shelf-life. Besides that, it retained the freshness and quality by controlling the microbial activity. The MAP can effectively control the respiration rate of fresh produces. Mangaraj *et al*. (2009) emphasize that MAP helps in controlling moisture content from the changes in CO_2 and O_2 level in a packaging.

The ultrastructure image showed that no tissue shrinkage and bacterial contamination after harvest were

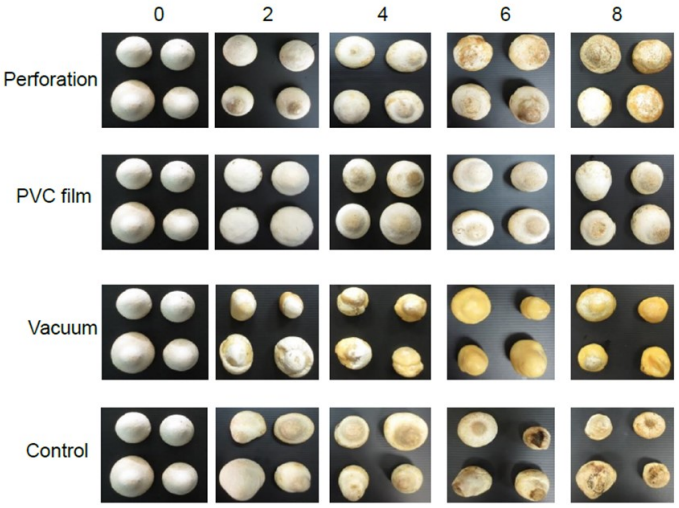


Figure 4. The physical structure of mushroom where down vertical represents effect of different packaging systems (perforation, PVC film, vacuum and control) along storage period until day 8 at horizontal.

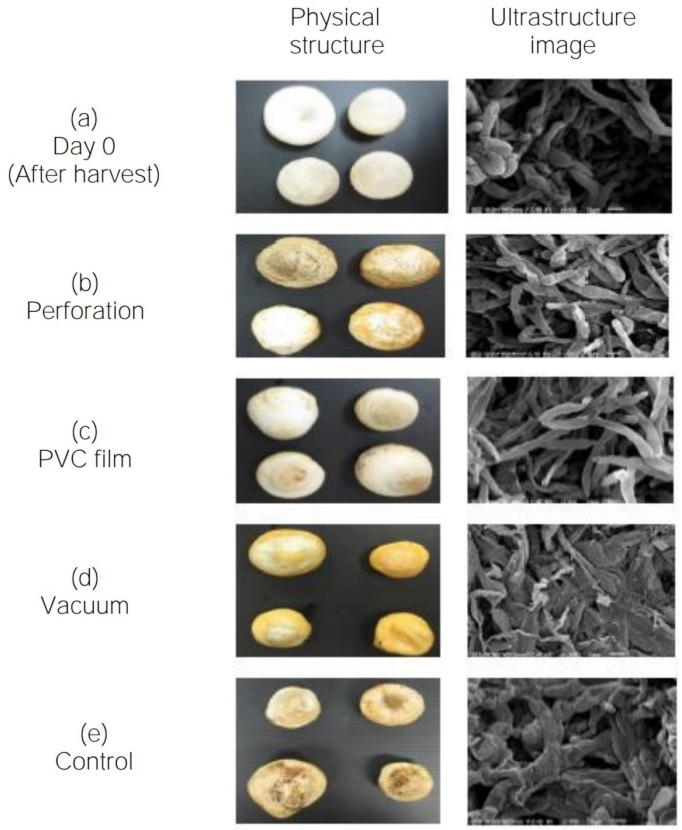


Figure 5. Physical appearance compared to ultrastructure image of mushroom on the surface of the volva under SEM at 1000x magnification at the end of their shelf life at day 8. Day 0 represents the image of control.

observed (Figure 5a). This result is in agreement with the previous study conducted by Zivanovic *et al*. (2000) where there was no contamination seen on the first day of harvest at control. Following storage periods, there were changes in the structure of *V. volvacea*. There were tissue shrinkage and bacteria contamination seen on the cap surface of *V. volvacea* in all packaging on day 8. The wilting and shrivelling of mushroom caused in the decrement of its postharvest quality. The ultrastructure

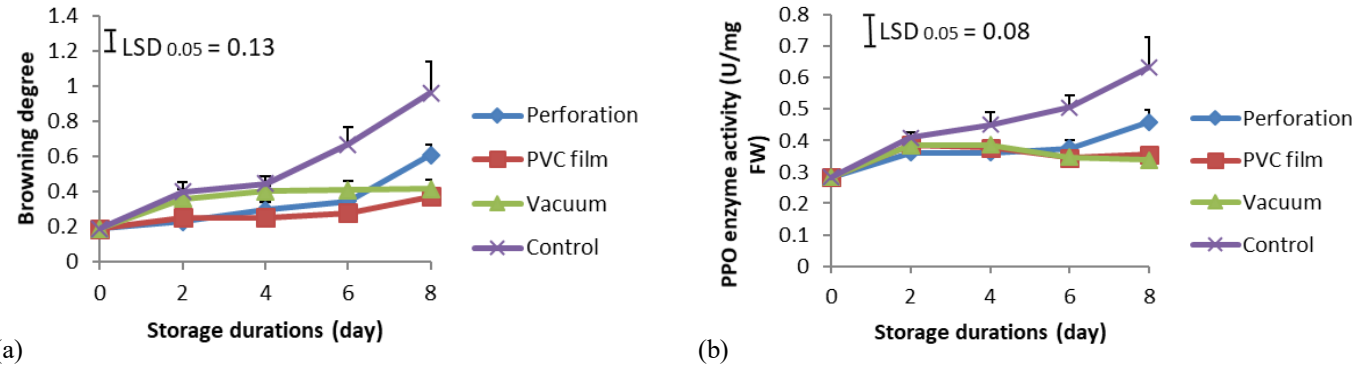
images when stored in perforation (Figure 5b) and PVC film (Figure 5c) have no much difference. Nevertheless, the tissue cells are expanded followed by a teary effect especially at the mushroom in vacuum packaging (Figure 5d). This might due to the mushrooms were immersed in the watery condition in vacuum packaging until day 8. According to Dhalsamant *et al*. (2015), fully sealed packaging with no perforation would sharply reduce the firmness as a consequence of water accumulation. This is because the mushrooms continue to develop and respire even after harvest. Mushroom has high metabolism and respiration rate. Thus, this causes the mushrooms' tissues were soaked in the watery environment which eliminated from its fruiting body during storage period. The tissue was expanded and thus less porous structure formed (Figure 5d). In addition, Kim and Seo (2018) stated that the porous and breathable film packaging decreased the metabolic activity from anaerobic respiration, CO_2 inside packaging. Whilst the ultrastructure of *V. volvacea* stored at control also showed shrivelling and heavy wrinkles at the tissue (Figure 5e). This was due to the hardening structure from the effect of water loss to the surrounding.

3.2.4 Browning degree

Browning became a major factor that reduced the marketability (Mohapatra *et al*., 2008). Browning is an important component affecting the acceptability of consumers and crucial issues in the mushroom industry. There were significant interaction effects ($P \leq 0.05$) between packaging systems and storage durations on browning degree of *V. volvacea*. The browning degree was significantly lowest in PVC film packaging with gradual increment of about 97.78% throughout storage periods (Figure 6a). The browning degree in perforated packaging was much higher about 225.22% which has no significant difference to vacuum packaging about 121.53% along the storage periods (Figure 6a). The trend of browning was similar to the previous study reported by Tsang (1999) where the browning of *V. volvacea* stored in vacuum flask increased at day 2 and became constant thereafter. Browning degree was significantly highest in *V. volvacea* stored in control packaging with 414.19% browning increment from the initial to the end of storage periods (Figure 6a).

After harvest, mushrooms are highly perishable and susceptible to enzymatic browning (Kumar *et al*., 2013; Kumari and Baskaran, 2015). Browning was found significantly highest in control packaging and lowest in PVC film packaging (Figure 6a). Browning pigments appeared when O_2 react with enzyme present in the mushroom (Kumari and Baskaran, 2015). In addition, the phenolic substrate oxidation process triggered by PPO enzyme contributed to the browning effect (Alikhani-Koupaei *et al*., 2014). This means that there was a high accumulation of O_2 and PPO enzyme in the fruiting body when stored at control packaging. Depletion of O_2 in enclosed packaging was controlled by the permeability barrier of packaging films. Many types of packaging have been implemented to increase the shelf life of mushroom after harvest. However, PVC film found to be the most suitable packaging film for mushroom (Taghizadeh *et al*., 2010). Oyster mushroom (*Pleurotus florida*) treated with MAP packaging with condition O_2 10%: CO_2 5% showed significantly lower browning degree compared to untreated packaging without MAP which is 1.5 and 4.0 respectively observed at day 25 (Jafri *et al*., 2013). Thus, this showed that the introduction of MAP whether in passive or active packaging systems gives significant effect to preserve the mushroom quality. Different packaging systems would create different permeability and air component inside the packaging.

The firmness was significant, negative and intermediate correlation with browning degree ($r = -0.60$) and PPO enzyme activity ($r = -0.43$). The decrement of firmness resulted in the increment of browning degree and PPO enzyme accumulation. This will eventually faster the deterioration and thus degrade postharvest qualities in *V. volvacea*.



(a) (b)

Figure 6. Relationships between storage durations (0, 2, 4, 6, and 8 days) towards browning degree PPO enzyme activity at different packaging systems (perforated, PVC film, vacuum and control) at $P \leq 0.05$ (mean ± SE).

3.2.5 Polyphenol oxidase (PPO) enzyme activity

There were significant interaction effects (P≤0.05) between packaging systems and storage durations on PPO enzyme activity of *V. volvacea*. PPO enzyme activity in control packaging was found significantly higher (123.76%) compared to another packaging (Figure 6b). This was due to the mushrooms were exposed directly to the environment, associated with higher transpiration and weight loss and thus exacerbated with the increment of PPO enzyme in that stressful condition. However, *V. volvacea* stored in perforation, PVC film and vacuum packaging showed no significant difference of PPO enzyme activity. Towards the end of storage, there was about 22.45% sharp increment of PPO at perforation packaging (Figure 6b). The PPO fluctuated gradually along storage periods. This showed that packaging film helped in reducing the PPO enzyme activity compared to exposed air at control. Li *et al*. (2017) also reported some fluctuations of PPO enzyme in *V. volvacea* mushroom along 96 hours of storage durations. From previous study, aside of *V. volvacea*, oyster mushrooms also found to be best stored in PVC wrapped film at 8-10ºC storage temperature (Kumari and Baskaran, 2015). Dhalsamant *et al*. (2015) stated that packaging with less perforation or high physical barrier would increase the chances of oxygen depletion in the package. Depend on the types of packaging, the gaseous components will be modified automatically through the respiration process. Thus, it will regulate the metabolic activity such as activation of PPO enzyme in the fruiting body.

There was a highly significant, positive and strong correlation (r = 0.88) between browning degree and PPO enzyme activity. This means that higher PPO enzyme activity resulted in higher browning degree in *V. volvacea*. Besides storage durations, physical damage such as cutting of tissue is one of the causes of elevation in PPO, PAL and POD enzymatic activity which eventually resulted in shorter shelf life (Saltveit, 2000). There was also a significant, positive and strong correlation (r = 0.83) between weight loss and browning degree. Whereas, correlation between weight loss and PPO enzyme activity was significant, positive and intermediate (r = 0.72). This showed that the increment of weight loss would lead to an increment of browning degree and PPO enzyme activity.

4. Conclusion

Storage at 10ºC exhibited CI symptoms while at RT causes rapid and major rotting incidence in *V. volvacea*. Whilst, the rate of postharvest qualities can be portrayed as PVC film > perforated > vacuum > control packaging. Therefore, Postharvest quality of *V. volvacea* could be retained by storage at 15ºC with PVC film packaging for up to 6 days storage. This combination of practices could contribute to the effective cooling chain in the mushroom industry and thus improved the economy by reducing the postharvest losses. However, further research could be conducted to retain their quality for longer storage durations by implementing edible coatings on *V. volvacea* or treatment during pre-harvest to improve fruiting body texture and production during cultivation. Besides that, the cultivation of this mushroom should be conducted during hot climate season rather than rainy season which in turn help larger yield quantity and thus increase its production.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

The authors would like to thank Fundamental Research Grant Scheme (FRGS/1/2016/ WAB01/ UPM/15) for funding this research. Special thanks to Mybrain15 scholarship by Ministry of Education, Malaysia.

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eISSN: 2550-2166