





Effect of Ethylene Scavenging-Active Packaging on Quality and Shelf Life of Button Mushrooms (*Agaricus bisporus*)

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ABSTRACT

The aim of this study was to evaluate the effect of an ethylene absorber (zeolites) in a packaging material on the shelf life of button mushrooms (*Agaricus bisporus*). Mushrooms were packaged under a passive modified atmosphere in low-density polyethylene (LDPE) bags with or without zeolites. Alongside the unpackaged control, three treatment groups were stored at 4°C and 50% RH for 16 days. Headspace gas composition, physical (weight loss, color and texture), chemical (pH, total soluble solids) and sensory analyses were carried out every 4 days of cold storage. Mushrooms packaged in LDPE bags without zeolites and LDPE with zeolites showed almost no significant change in weight during the storage time whereas the unpackaged mushrooms reached almost 20% weight loss by the end of the storage. Sensory attributes for mushrooms packaged in LDPE bags without zeolites and LDPE with zeolites were also still acceptable after the 8th day while unpackaged mushrooms lost their acceptability by day 4. Although no mentionable difference in shelf life was observed between samples packaged in LDPE bags without zeolites and LDPE with zeolites when physical, chemical and sensory properties were considered, the active material was better than the control material in terms of color, with less browning index and higher whiteness index.

Keywords: Active packaging, Zeolites, Shelf life, Button mushrooms, *Agaricus bisporus*

Etilen Tutucu İçeren Aktif Ambalajlamanın Mantarın (*Agaricus bisporus*) Kalitesi ve Raf Ömrüne Etkisi

ÖZ

Bu çalışmanın amacı etilen tutucu olarak zeolite içeren ambalaj materyalinin mantarın (*Agaricus bisporus*) raf ömrüne etkisini belirlemektir. Mantar pasif modifiye atmosfer altında zeolit içeren ve içermeyen düşük yoğunluklu polietilen (LDPE) torba ambalajlarda ambalajlanmıştır. Ambalajsız grupla birlikte toplam üç uygulamaya ait ürünler 4°C'de %50 bağıl nemde 16 gün depolanmıştır. Depolamanın her dört gününde bir tepe boşluğu gaz analizi, fiziksel (ağırlık kaybı, renk ve tekstür), kimyasal (pH, toplam çözünebilir kuru madde) ve duyu analizler gerçekleştirilmiştir. Zeolit içeren ve içermeyen LDPE ambalajlarda ağırlık kaybı açısından depolamanın sonunda önemli bir değişim ortaya çıkmamıştır, ancak ambalajsız mantarlarda %20'ye yakın bir kayıp ortaya çıkmıştır. Ambalajsız mantarların duyu analizi olarak kabul edilebilirliği 4 günle sınırlıyken, bu süre zeolit içeren ve içermeyen ambalajlarda 8 gün olarak tespit edilmiştir. Fiziksel, kimyasal ve duyu özellikler açısından değerlendirildiğinde zeolit içeren ve içermeyen ambalajlar arasında raf ömrü açısından çok büyük bir fark ortaya çıkmamasına rağmen, aktif ambalajlanmış mantarda beyazlık indeksi daha yüksek, esmerleşme indeksi daha düşük bulunmuştur.

Anahtar Kelimeler: Aktif ambalajlama, Zeolit, Raf ömrü, Mantar, *Agaricus bisporus*

INTRODUCTION

Agaricus bisporus (also known as button mushroom) is the most widely cultivated mushroom globally, contributing to as much as 30% of the total mushroom production worldwide [1]. This unrivaled popularity is due to its high nutritional value, sensory properties, medicinal attributes, ease of harvesting, and low price compared to other mushrooms [2]. Unfortunately, mushrooms have a great disadvantage: it is highly perishable. Mushrooms have a short shelf-life compared to other fresh food commodities, which was reported to be 1 to 3 days when stored at room temperature (20-25°C), 5 to 7 days when stored at 0 to 2°C, or about 8 days when stored under refrigerated conditions [3-5].

Many internal factors (i.e., water activity, respiration rate and microbial activity) and external factors (related to storage conditions) play a role in the perishable nature of button mushrooms [5]. One significant factor directly contributing to the browning of mushroom caps is ethylene. Although mushrooms produce a little amount of ethylene, they are highly sensitive to ethylene, which makes storing or shipping mushrooms challenging, since mushrooms can be affected by the ethylene produced by other fresh fruits and vegetables if shipped together [1]. As stated by Zhang, many researchers have developed methods that succeeded in reducing postharvest deterioration and achieved a shelf-life extension of mushrooms [5]. However, the studies that concentrated on eliminating ethylene as the deteriorating factor are limited.

The removal of ethylene from the product environment using an ethylene scavenger slows the ripening process, thus prolongs the shelf-life of the produce. Ethylene scavenging is done either by adding a sachet containing the active material to the package or incorporation of it in the package structure itself. The most widely used ethylene scavenger in sachets is potassium permanganate (KMnO₄). However, it cannot come in direct contact with food due to its high toxicity [6-8]. Another system for scavenging ethylene depends on metals and metal oxides, which is activated by UV light or visible light or both. However, the fact that UV exposure can affect food quality and should be taken into consideration [8]. Considering the previously mentioned reasons, zeolite-based materials are a good choice as ethylene scavengers since they have no adverse effects on the safety or quality of food products. However, the literature is unsatisfactory for using zeolite-based materials as ethylene scavengers for fresh produce.

Zeolite based films were successfully used for the packaging of broccoli [9], mango [10], and kiwifruit [11]. However, literature lacks the application of such packaging on mushrooms.

The aim of this study was to investigate the effects of zeolite-added LDPE packaging on the overall quality parameters (physical, chemical and sensory) as well as the extension of the shelf-life of button mushrooms.

MATERIALS AND METHODS

Materials

Freshly harvested mushrooms (*A. bisporus*) were purchased from the producer 1 day before the study and were kept under refrigerated conditions (4°C). After damaged, dirty and mushrooms with broken stems were excluded, mushrooms with similar size and color were chosen to get uniform samples.

The packaging materials used are low density polyethylene (LDPE) bags without zeolite and LDPE bags with zeolites. The dimensions of both types are 35 cm × 27 cm. The oxygen transmission rates (OTR) and the water vapor transmission rates (WVTR) are 3303 cc/m²/24hr and 5.24 g/m²/24hr for LDPE without zeolites and 5664 cc/m²/24hr and 17.5 g/m²/24hr for LDPE with zeolites, respectively. The amount of zeolite is about 10%.

Packaging of Mushrooms

Mushrooms were divided randomly into 3 treatment groups: (M1) LDPE bags without zeolite, (M2) LDPE bags with zeolites and (C) unpackaged mushrooms. 500 grams of mushrooms were weighed in LDPE bags and thermally sealed under a passive modified atmosphere with a product to headspace ratio of 1:1. Two parallels were prepared for each treatment. The samples were stored at 4°C for 16 days, and physiological (headspace gas composition), physical (weight loss, texture and color), chemical (pH, total soluble solids) and sensory analyses were carried out every 4 days.

Headspace Gas Composition

The concentrations of O₂ and CO₂ were detected using a PBI Dansensor (CheckPoint O₂/CO₂, Ringsted, Denmark). Two samples from each group were analyzed by inserting a thin needle into the packages and a pump draws a small sample of headspace gas into the analyzer equipment. The headspace gas comes into contact with a sensor that can measure the concentration of residual oxygen or carbon dioxide in the headspace gas sample. Two readings were taken from each parallel package for each treatment [1].

Weight Loss

The mushrooms were weighed using an electronic weighing scale (GE 2101, Sartorius, Germany) with ± 0.1 g accuracy every 4 days. The results were then expressed as a percentage of weight loss with the respect to the initial weight [1].

Color

The caps of 10 mushrooms from two parallel bags of each treatment group were analyzed using a colorimeter device (Hunter Laboratories, Reston, VA, USA) and L*a*b* values were obtained (L*: light/dark, a*: red/green, and b*: yellow/blue).

Using the formulas below, the whiteness index (WI) and the browning index (BI) were calculated [2].

$$WI = L - 3b + 3a$$

$$BI = \frac{100(x - 0.31)}{0.17}$$

$$\text{Where: } x = \frac{a+1.75L}{5.645L+a-3.012b}$$

Texture

Ten mushrooms were taken from two parallel bags of each treatment group. After removing the stems, the firmness of mushroom caps was measured using a TA-XT Plus texture analyzer (Stable Micro Systems, Surrey, England) equipped with a 2mm diameter cylindrical probe. Samples were penetrated with a speed of 5 mm s⁻¹, and results were expressed as Newton (N) [12].

pH and Total Soluble Solids (TSS)

The pH values were determined by taking 3 mushrooms from each treatment. After being homogenized and squeezed with the aid of a hand press, 10 g of the resulting juice were taken and diluted by 10 folds. Using a pH-meter (WTW-315i, Weilheim, Germany), four pH readings for each treatment were taken. Using a digital refractometer (Atago N-50E, Tokyo, Japan), the Brix value of the resulting juice was determined [2].

Cap Opening

Cap opening (%) is a quality criterion based on the development of the umbrella-like shape of the cap, followed by a detachment of the veil [12]. Cap opening was determined based on a 5 points scale (1=Caps completely closed, 2=caps slightly open, 3=caps half-open, 4=caps mostly open, 5=caps completely open). The result for each value was expressed as percentage. The percentage open caps were determined from the known number of mushrooms as:

$$\% \text{Open caps} = \frac{\text{Noc}}{\text{Nt}} \times 100$$

Where: Nt=total number of mushrooms; Noc=number of open capped mushrooms.

Sensory Analysis

On each day of analysis, 6 experienced panelists evaluated the mushrooms using 5 point scale in terms of color (1:the cap and the stem are brown, 3: acceptable, 5:the surface is white), overall appearance (1:creased surface, the formation of a sticky layer, 3: acceptable, 5:smooth surface) and overall acceptance (1:bad, 3: acceptable, 5:excellent), the acceptability level is 3 out of 5 points at room temperature. The samples were coded with three-digit random numbers and served in different orders to each panelist to eliminate the order effect.

Statistical Analysis

Data were expressed as the mean ± SD (standard deviation). Two-way analysis of variance (ANOVA) was performed by SPSS (IBM SPSS version 20) and the difference was considered to be statistically significant if p<0.05.

RESULTS and DISCUSSION

Headspace Gas Composition

Mushrooms' respiration rate is high in comparison with other fresh commodities, which contributes directly to its postharvest quality degradation [13, 14]. Both respiration and the gas permeability of the packaging material play a role in the change of the headspace gas composition [14]. Changes in O₂ and CO₂ concentrations are shown in Fig. 1 (A and B, respectively). O₂ concentration started with the ambient concentration (around 21% in both packaging materials) and decreased rapidly during the first 4 days of storage in M1 (0.2-0.1%) and continued decreasing in M2 until it was undetectable by the 16th day of storage. It is worth mentioning though that it is possible for O₂ to be trapped in the caps of the mushrooms. In contrast, the CO₂ concentration increased sharply during the first four days of storage in both packages, corresponding to the sharp decrease in O₂ concentration. The increase of CO₂ was followed by a sharp depletion between the 4th and 8th days of storage until it reached equilibrium in both packages, with a noticeably higher concentration in M1 compared to M2, which can be attributed to the lower barrier properties of M1. This pattern of change in headspace composition is in an agreement with other reports in the literature [2, 13, 14].

Weight Loss (%)

Weight loss is an important indicator when it comes to the quality of mushrooms, leading to the reduction of consumer's acceptability [15]. This weight loss is mainly due to the continuous loss of moisture in mushrooms [5], which is caused by both the thin epidermal structure of mushrooms and their high transpiration rate [14,16]. Fig. 2 shows the weight loss in mushrooms packaged in LDPE bags without zeolites (M1), LDPE bags with zeolites (M2) as well as the unpackaged mushrooms (C). As mentioned by Gantner, weight loss greater than 3% reduces the consumer's acceptability [15]. The weight loss for unpackaged mushrooms reaches almost 20% at the end of the storage, but it already reached unacceptable quality after the 4th day of storage. On the other hand, M1 and M2 showed a gradual but barely mentionable weight loss (less than 1%), thus M1 and M2 can be considered economically efficient. These results may be due to the low WVTR of PE films controlling the moisture loss [2, 14].

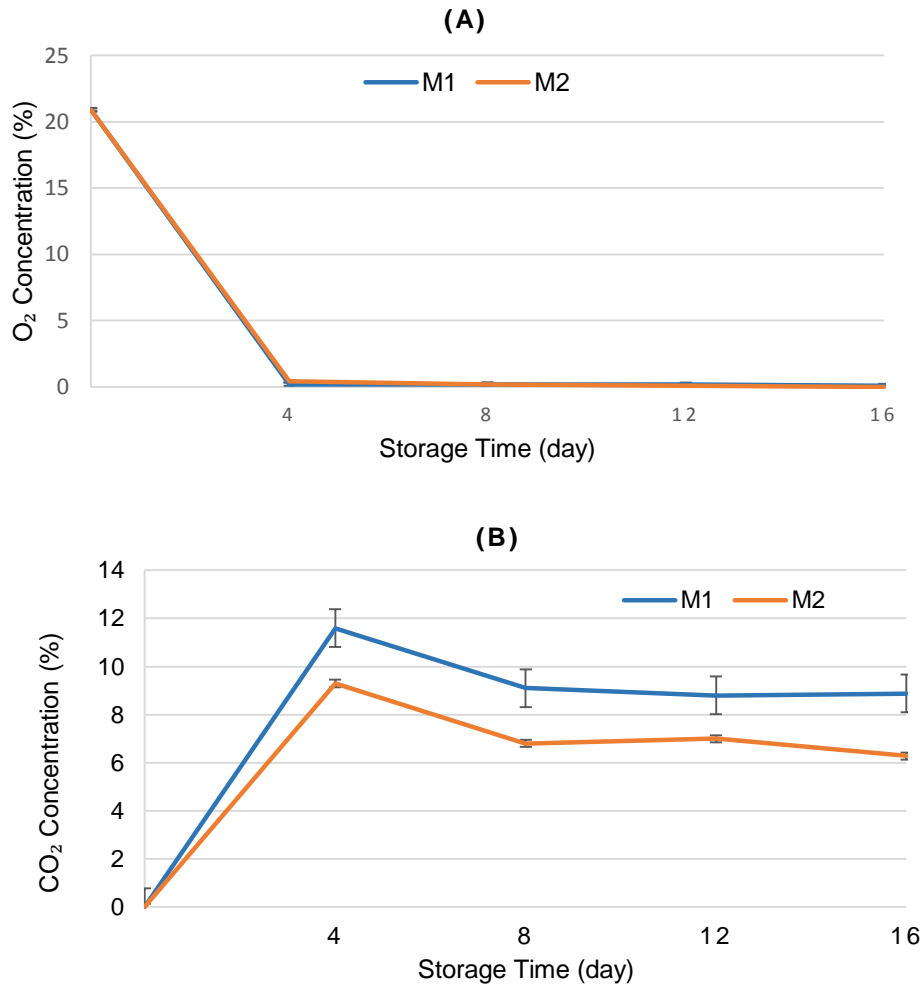


Figure 1. (A) Change in concentration of O₂ (%) and (B) Change in CO₂ (%) concentration during cold storage (4°C, 50% RH). (M1: LDPE bags without zeolites, M2: LDPE bags with zeolites).

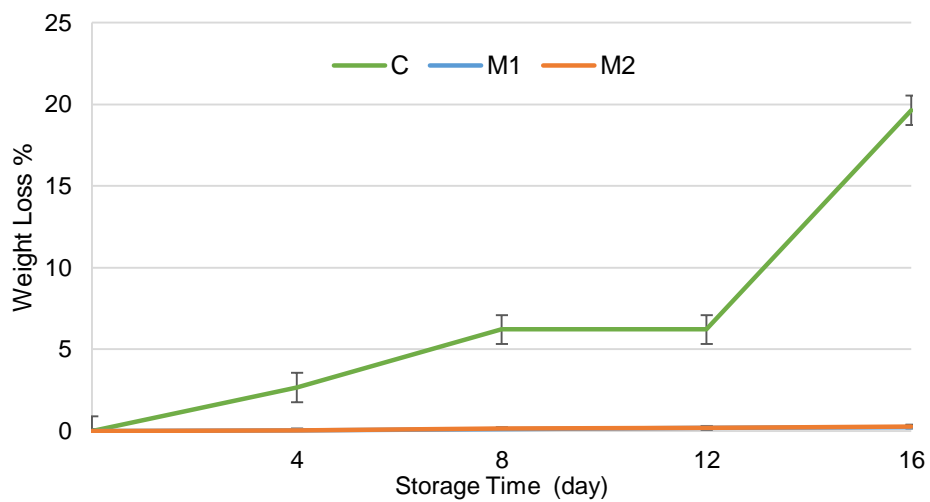


Figure 2. Effect of packaging on weight loss (%) during cold storage (4°C, 50% RH). (C: Unpackaged mushrooms M1: LDPE bags without zeolites, M2: LDPE bags with zeolites)

Color

Color is considered the most important quality attribute in mushrooms since it directly contributes to the consumer's acceptance. Browning occurs in mushrooms due to either enzymatic activities or/and microbial contamination [2, 5, 12, 14, 17]. Hence, the whiteness index (WI) and browning index (BI) are important to evaluate color changes in mushrooms. Table 1 shows the change in the whiteness index while (B) shows the change in the browning index. As the whiteness index decreases with storage time the browning index increases. These trends occur regardless of the packaging material used and come in agreement with other researchers' previous works [13, 14, 16]. M2

showed the best results with the lowest decrease in WI and the lowest increase in BI. M1 did not show a significant difference in terms of WI in comparison with the control ($p > 0.05$). This could be attributed to the higher CO₂ concentration in this package (M1), leading to the damaging of mushroom cap surface tissue, and such results were reported in the literature [2, 18].

As for the browning index, M2 maintained the lowest browning index during storage the compared to other two groups (M1 and control group) (Table 2). There is no significant difference observed between LDPE with no zeolite and unpackaged groups during storage in terms of the browning index.

Table 1. The effect of different packaging materials on the whiteness index (WI) of mushrooms during cold storage (4°C, 50% RH)

Treatment	Whiteness index (WI)				
	Storage day				
	0	4	8	12	16
C	60.11±4.44 ^{Aa}	42.89±7.78 ^{ABb}	36.02±6.10 ^{Ac}	25.07±8.72 ^{Bd}	28.17±4.56 ^{Bd}
M1	60.11±4.44 ^{Aa}	40.06±7.18 ^{Bb}	35.69±5.41 ^{Ac}	25.71±7.04 ^{Bd}	27.68±6.25 ^{Bd}
M2	60.11±4.44 ^{Aa}	47.98±9.78 ^{Ab}	38.78±5.57 ^{Ac}	33.78±6.07 ^{Ad}	37.09±4.54 ^{Accd}

*Uppercase letters indicate significant differences ($p < 0.05$) between different treatment groups, lowercase letters indicate significant differences ($p < 0.05$) within the same treatment group. C: Unpackaged mushrooms M1: LDPE bags without zeolites, M2: LDPE bags with zeolites.

Table 2. The effect of different packaging materials on the browning index (BI) of mushrooms during cold storage (4°C, 50% RH)

Treatment	Browning index (BI)				
	Storage day				
	0	4	8	12	16
C	16.79±2.89 ^{Ae}	27.90±5.97 ^{ABd}	36.14±7.46 ^{Ac}	47.66±8.32 ^{Aa}	41.01±4.98 ^{Ab}
M1	16.79±2.89 ^{Ac}	29.15±5.29 ^{Ab}	32.52±4.82 ^{Abb}	44.81±7.09 ^{Aa}	42.66±6.65 ^{Aa}
M2	16.79±2.89 ^{Ac}	24.03±8.08 ^{Bb}	32.17±5.22 ^{Ba}	34.53±4.46 ^{Ba}	32.33±3.64 ^{Ba}

*Uppercase letters indicate significant differences ($p < 0.05$) between different treatment groups, lowercase letters indicate significant differences ($p < 0.05$) within the same treatment group. C: Unpackaged mushrooms M1: LDPE bags without zeolites, M2: LDPE bags with zeolites.

Texture (Firmness)

Enzymatic activity and water loss cause texture changes in mushrooms, thus leading to quality deterioration and less consumer acceptability [2, 17]. Table 3 shows the change in the penetration force (N) during storage time. All treatments experienced a loss in firmness during the storage time. The unpackaged mushrooms changed texture quickly, requiring higher penetration force on the 4th and 8th days, probably due to the massive loss of

moisture and drying out, causing the caps to become more elastic and thus requiring more force. In comparison, the other packaged products in both packages (M1 and M2) lost firmness slightly and showed no significant difference ($p > 0.05$) since the 4th day to the end of the storage time, which may be a sign that the change in the texture in these treatments is purely due to internal enzymatic reactions that cause the mushrooms to soften, not due to the loss of moisture.

Table 3. The effect of different packaging materials on firmness (penetration force, N) of mushroom during cold storage (4°C, 50%RH)

Treatment	Penetration force (N)				
	Storage day				
	0	4	8	12	16
C	3.94±1.28 ^{Abc}	4.49±0.80 ^{Aab}	4.79±1.17 ^{Aa}	3.57±1.12 ^{Ac}	2.62±0.82 ^{Ad}
M1	3.94±1.28 ^{Aa}	2.58±0.43 ^{Bb}	2.64±0.43 ^{Bb}	2.68±1.06 ^{Bb}	2.71±0.82 ^{Ab}
M2	3.94±1.28 ^{Aa}	2.50±0.31 ^{Bc}	2.22±0.30 ^{Bc}	3.10±1.13 ^{ABb}	2.54±0.56 ^{Ac}

*Uppercase letters indicate significant differences ($p < 0.05$) between different treatment groups, lowercase letters indicate significant differences ($p < 0.05$) within the same treatment group. C: Unpackaged mushrooms M1: LDPE bags without zeolites, M2: LDPE bags with zeolites.

pH and TSS

Table 4 shows the change in pH values in the different packages during the storage time. pH values dropped slightly but not significantly in all treatment groups. Table 5 shows the change in total soluble solids content over storage. As expected, the Brix value in the unpackaged

mushrooms increased significantly at first corresponding to the massive loss of moisture. In comparison, no significant difference ($p < 0.05$) was found between M1 and M2 in terms of Brix values during the storage time, which further proves the efficiency of LDPE bags in retaining the moisture of the mushrooms.

Table 4. The effect of different packaging materials on the pH of mushroom during cold storage (4°C, 50%RH)

Treatment	pH				
	Storage day				
	0	4	8	12	16
C	6.86±0.03 ^{Aa}	6.72±0.05 ^{Cb}	6.80±0.18 ^{Aab}	6.40±0.01 ^{Bc}	6.39±0.03 ^{Cc}
M1	6.86±0.03 ^{Ab}	6.88±0.03 ^{Bb}	6.95±0.04 ^{Aa}	6.54±0.01 ^{Ad}	6.64±0.02 ^{Ac}
M2	6.86±0.03 ^{Ab}	7.03±0.06 ^{Aa}	6.82±0.02 ^{Ab}	6.54±0.05 ^{Ac}	6.56±0.04 ^{Bc}

*Uppercase letters indicate significant differences ($p < 0.05$) between different treatment groups, lowercase letters indicate significant differences ($p < 0.05$) within the same treatment group. C: Unpackaged mushrooms M1: LDPE bags without zeolites, M2: LDPE bags with zeolites.

Table 5. The effect of packaging materials on the Brix value of mushroom during cold storage (4°C, 50%RH)

Treatment	Brix (%)				
	Storage day				
	0	4	8	12	16
C	4.55±0.06 ^{Ab}	5.50±0.58 ^{Aab}	7.63±3.03 ^{Aa}	6.50±0.80 ^{Aab}	6.13±1.37 ^{Aab}
M1	4.55±0.06 ^{Aa}	4.33±0.74 ^{Ba}	4.43±0.32 ^{Ba}	4.33±0.10 ^{Ba}	4.03±0.10 ^{Ba}
M2	4.55±0.06 ^{Aa}	4.43±0.21 ^{Bab}	4.08±0.03 ^{Bb}	4.23±0.21 ^{Bab}	4.55±0.37 ^{Bab}

*Uppercase letters indicate significant differences ($p < 0.05$) between different treatment groups, lowercase letters indicate significant differences ($p < 0.05$) within the same treatment group. C: Unpackaged mushrooms M1: LDPE bags without zeolites, M2: LDPE bags with zeolites.

Cap Opening

Mushrooms lose their internal moisture during storage, leading to the drying of the caps and veil. Thus, the mushrooms cap opening percentage is considered as one of the important quality parameters indicating the freshness of mushrooms [2]. As can be seen in Table 6, the percentage of completely closed caps decreased with the storage time regardless of the packaging material. Such results were also obtained by other researchers [2, 12]. As expected, the control witnessed the highest drop in closed caps percentage with storage time, which comes in correlation with weight loss results explained earlier. M2 showed a superior performance with 79% completely closed caps compared to 69% in M1 at the end of the storage.

Sensory Evaluation

Sensory evaluation is crucial in assessing the postharvest quality of mushrooms [18]. As Table 7 shows, sensory scores declined in all packages with extended storage time. These results come in agreement with other researchers [1, 12, 18]. In terms of color, both M1 and M2 are still acceptable on the 8th day while the unpackaged mushrooms lose their acceptability on the 4th day. For overall appearance, packaged mushrooms were acceptable on the storage

day of 8, but it is limited to 4 days for unpackaged control product. The same trend was observed for the overall product acceptability. Although there was a significant color difference between active packages and control packages in terms of whitening and browning index in the favor of the active package, it was not reflected by the color evaluation by the sensory panelists.

CONCLUSIONS

In conclusion, weight loss was the most affected by the packaging. The weight loss reached around 20% in the unpackaged samples by the end of the study while there was not any significant weight loss in M1 and M2. Although no significant difference in performance was achieved in weight loss and extending the shelf-life between M1 and M2, M2 showed superiority regarding color with higher whiteness and lower browning, retained closed caps for longer storage time (16 days). Regarding shelf-life, the estimated shelf-life of the unpackaged mushroom is 4 days, since it lost acceptance in all sensory attributes after the 4th day. Although no mentionable difference in sensory attributes was observed between samples packaged in LDPE bags without zeolites and LDPE with zeolites, the active material was better than the control material in terms of color with less browning and higher whiteness index.

Table 6. The effect of different packaging materials on the cap opening of mushrooms during cold storage (4°C, 50% RH)

Treatment	Score	Cap opening (%)				
		Day 0	Day 4	Day 8	Day 12	Day 16
C	1	100.00	76.47	72.50	66.15	61.63
	2	0.00	19.61	22.50	22.94	22.97
	3	0.00	3.92	3.34	1.78	9.71
	4	0.00	0.00	1.67	5.35	3.70
	5	0.00	0.00	0.00	3.79	2.00
M1	1	100.00	85.25	82.51	87.96	69.05
	2	0.00	11.48	10.47	3.94	18.12
	3	0.00	3.28	5.30	3.94	5.49
	4	0.00	0.00	1.73	2.09	7.34
	5	0.00	0.00	0.00	2.09	0.00
M2	1	100.00	76.27	76.70	75.86	79.00
	2	0.00	20.34	15.31	12.07	19.28
	3	0.00	3.39	3.03	5.17	0.00
	4	0.00	0.00	3.24	6.90	1.73
	5	0.00	0.00	1.73	0.00	0.00

1: Completely open, 2: Partially open, 3: Half open, 4: Mostly open, 5: Completely open
C: Unpackaged mushrooms M1: LDPE bags without zeolites, M2: LDPE bags with zeolites.

Table 7. The effect of different packaging on sensory attributes of mushrooms during cold storage (4°C, 50% RH).

Attribute	Time (d)	Sensory evaluation		
		Treatment		
		C	M1	M2
Color	0	5.00±0.00	5.00±0.00	5.00±0.00
	4	3.33±0.52	3.00±0.63	3.67±0.52
	8	1.83±0.41	3.83±0.41	3.00±0.00
	12	3.00±0.00	1.83±0.41	2.1 ±0.41
	16	1.00±0.00	1.00±0.00	2.33±0.52
Overall appearance	0	5.00±0.00	5.00±0.00	5.00±0.00
	4	4.17±0.41	3.50±0.84	3.83±0.75
	8	1.83±0.41	3.50±0.55	3.17±0.41
	12	2.17±0.41	2.00±0.00	2.17±0.41
	16	1.00±0.00	1.00±0.00	2.33±0.52
Overall acceptance	0	5.00±0.00	5.00±0.00	5.00±0.00
	4	4.00±0.00	3.50±0.89	4.00±0.63
	8	1.83±0.41	3.50±0.55	3.00±0.00
	12	2.67±0.52	2.00±0.00	2.00±0.00
	16	1.00±0.00	1.00±0.00	2.00±0.00

C: Unpackaged mushrooms M1: LDPE bags without zeolites, M2: LDPE bags with zeolites

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