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The Effects of Preharvest Silicon Treatment and Passive MAP on Quality and Shelf Life of White Button Mushrooms in Thermoformed Recycled PET Packaging System

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Abstract: A crop pretreatment with silicon was combined with passive modified atmosphere packaging (PMAP) in a thermoformed recycled PET packaging format as a novel approach to minimize the quality degradation in mushrooms. This study was aimed to evaluate the effects of (a) two preharvest treatments, namely preharvest control (PHTC) and preharvest silicon treatment (PHTS) and (b) four packaging lid formats, namely PMAP1: a single hole of 1.1 mm size, PMAP2: two holes of 0.53 mm size, PMAP3: three holes of 0.53 mm size, and PMAPC: OMNI-PW micro perforated cling film as a control on the quality and shelf life of mushrooms during five days of storage at 4 °C and 99.9% RH. The results of the analysis of variance showed that packaging type, storage days, and the double interaction effects of storage days × packaging type had significant effects ($p < 0.0001$) on the changes in O_2, CO_2, colour L^* and a^* values, ΔE, total soluble solids (TSS), and the density of mushrooms. Density, electrolyte leakage (EL), and TSS were significantly affected by the double interaction effects of preharvest treatment × packaging type. Overall, PMAP1, PMAP2, and PMAP3 resulted in lower O_2 + higher CO_2 within packages compared with the conventional control. A preharvest silicon treatment had little overall effect. PMAP 1, 2 and 3 had a significantly lower ΔE (=better quality) after 5 days storage compared to PMAPC which had the highest ΔE (lowest quality) overall. PMAP1 and PMAP2 had the lowest EL values compared to PMAP3 and PMAPC. PMAP1, PMAP2, and PMAP3 all gave better TSS levels and density compared to PMAPC. Notably, this study proved that a perforation-mediated MAP design for mushrooms packaged in a thermoformed recycled PET packaging format maintained improved CO_2, lowered O_2, and reduced EL while maintaining TSS and the density of the mushrooms during the storage period.

Keywords: passive MAP; mushroom; silicon treatment; rPET; electrolyte leakage; thermoformer; quality; colour; density; food waste reduction

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1. Introduction

The white button mushroom, *Agaricus bisporus*, is one of several widely grown edible mushroom species. It is cultivated in over 70 countries worldwide, and accounted for 11% of the world's total mushroom supply in 2018–2019 [1]. In Ireland, it is the most important horticultural crop with a production of around 68,000 tonnes per annum [2]. Teagasc [2] reports that around 80% of the mushrooms are exported to the UK and 20% are used to supply the domestic market, contributing approximately EUR 130 million per annum to the Irish economy. However, exporting to continental Europe is more challenging

due to the short shelf life of mushrooms, high transport costs, and long transport duration. Mushrooms have high respiratory and metabolic rates leading to a very short shelf life—typically 3 days under ambient conditions and 5 to 8 days in a cold storage system [3].

The main causes of mushrooms' short shelf life are their high moisture content (81.8%–94.8%), lack of a cuticle, and high enzyme activity, which facilitates high respiratory and metabolic activity in the harvested fruit body [4]. The susceptibility of mushrooms to mechanical damage and microbial contamination is increased by these factors, leading to browning and a decrease in quality [5–7]. This leads to a decline in both their physiological and morphological quality, including water loss, cap opening, stipe elongation, cell deterioration, texture damage, and the growth of microbes during storage and transportation [8,9]. As a result, there is a considerable reduction in both the nutritional and market value of the mushrooms.

Various packaging types are widely used to extend the shelf life of mushrooms, including nano-packaging and polysaccharide nanoparticle preservation, modified atmosphere packaging, active packaging, edible film packaging, and nanocomposite packaging [3,10–14]. Of these, modified atmosphere packaging (MAP) is a safe, environmentally friendly, simple and cost-effective packaging technique for maintaining the postharvest quality of fresh edible mushrooms.

The modification of the atmosphere inside the package (MAP) for fresh produce occurs through the interaction of the product's respiration and gas transfer through the packaging. This results in an atmosphere with higher CO_2 and lower O_2 levels, which has the potential to decrease the respiration rate, sensitivity to ethylene, decay, and oxidation [15]. Research indicates that using modified atmosphere packaging in combination with low-temperature (4 °C) storage conditions can have positive effects, such as reducing the respiration and transpiration, delaying senescence, and extending the shelf life by preventing wilting and shrivelling [15–17].

MAP is regarded as an efficient, straightforward, and relatively inexpensive packaging technique [9]. The polymeric films of packaging bags/lidding films/trays can take three different types of the MAP system: (a) micro perforated, (b) macro-perforated, and (c) perforation-mediated packaging systems [16,18–20]. Achieving MAP for fresh produce involves using micro perforated polymeric films to maintain low oxygen O_2 and CO_2 levels. These films are suitable for preserving less CO_2-tolerant fruits like mangoes, bananas, grapes, and apples. On the other hand, macro-perforated films and packaging systems with perforations have a higher permeability rate but a lower CO_2 to O_2 permeability ratio, approaching unity. Thus, perforated films with low oxygen and high carbon dioxide levels are highly sought after for preserving fresh produce such as mushrooms [16,18,20].

In Europe, fresh edible mushrooms are frequently retailed in polypropylene trays wrapped in a stretchable Polyvinyl Chloride (PVC) cling film with a label on the top. This kind of film not only has high permeability to O_2 and CO_2 but is also subject to environmental concerns due to its non-recyclability, contributing to massive packaging plastic waste that goes to either landfill or is incinerated. The packaging material suppliers in Ireland emphasized that the mushroom industries are currently introducing recyclable packaging films such as biaxially oriented polypropylene (BOPP) with polypropylene trays (communication with the Leaf No Waste Packaging Material Supplier advisory board). The packaging film should be environmentally friendly and adapted to the O_2 and CO_2 requirements of the commodity, which largely depend on the storage temperature [15].

Sustainable packaging options like recycled polyethylene terephthalate (rPET) and monoPET film support the circular economy by using post-consumer recycled materials and minimizing waste. Mono PET film contains 30% post-consumer recyclable materials, while rPET food trays are made with 80% post-consumer recyclable materials, significantly reducing the carbon footprint and producing the lowest greenhouse gas emissions, water usage, and total energy usage of all plastics in manufacturing [21]. These packaging materials are lightweight, rigid, and have excellent barrier performance, making them suitable for MAP to extend the shelf life and providing a clear presentation of products

with glass-like clarity, especially for fresh produce packaging. Thermoformed packaging materials will help to reduce the warehousing costs of the trays currently utilized by the industry, but implementing a thermoforming packaging system at the packing line within the mushroom industry would be necessary. The successful use of PET mono materials could eliminate the need for PVC and PP in mushroom packaging. This research supports Ireland's recycling targets of achieving 65% of all packaging by 2025 and 70% by 2030, as mandated by EU Packaging Regulations (SI 322/2020) [21].

Numerous studies have demonstrated that the inclusion of silver, titanium, and silicon nanoparticles in nanocomposite packaging materials can effectively delay the degradation of membrane lipids, as well as reduce polyphenol oxidase activity, tyrosinase activity, and reactive oxygen in mushrooms [22–24]. For instance, a nanocomposite film comprising chitosan, nanosilica, and 1% nisin was found to enhance the antimicrobial effectiveness, minimize polyphenol oxidase activity and weight loss, and preserve the colour, pH, and total soluble solids of *Agaricus bisporus* mushrooms [25].

The silicon fertilization in crops such as strawberries and tomatoes has been linked to the improved crop yield and quality, which can be linked to the increased shelf life [26–28]. Silicon applications have also proven effective against biotic stresses, such as disease, associated with silicon polymerization between plant cells [29]. Silicon as a biostimulant increases the peel firmness, total soluble content, total acidity, ascorbic acid, total phenols and antioxidant capacity, and reduced fruit decay in clementine mandarin [30]; reduced weight loss and colour change, increased firmness, total soluble solids, and titratable acidity in Hass Avocado and Mango Fruit [31]; and reduced sweet cherry cracking [32]. In mushrooms, the preharvest supplementation of calcium silicate in the oyster mushroom (*Pleurotus ostreatus*) during two harvest flushes resulted in an increase in vitamin D2 and tocopherol content [33]. Silicon as a preharvest treatment combined with passive MAP (PMAP) is a novel approach that could help to minimize the physiological and morphological quality degradation in mushrooms. For mushrooms, the use of monomaterials designed with post-consumer recyclable materials of 80% for rPET trays and 30% for monoPET films in a thermoforming packaging system is a novel packaging format. Therefore, this study aimed to investigate the effects of the preharvest silicon treatment and passive modified atmosphere packaging (PMAP) in a thermoformed recycled PET on the quality and shelf life of white button mushrooms.

2. Materials and Methods

2.1. Mushroom Cultivation

A crop trial was carried out in an environmentally controlled mushroom tunnel located in the Mushroom Research Unit at the Teagasc Food Research Centre (Dublin, Ireland). Crates (400 mm × 600 mm × 300 mm) with an internal surface area of 0.2 m^2 were filled with 16 kg of substrate, colonized with *Agaricus bisporus* strain Sylvan A15 (Carbury Compost, Kildare, Ireland), and then covered with a 5 cm layer of peat-based mushroom casing (Harte Peat Ltd., Clones, Ireland). The growing conditions in the mushroom tunnel were maintained using the Fancom environmental control system (www.fancommushroom.com) according to the standard commercial practices. The crop was ready to harvest after 17 days, at which stage the temperature and relative humidity in the room were maintained at 18 °C and 88%–90%, respectively, for the remainder of the crop cycle. Mushrooms were harvested over three weeks in three distinct 'flushes', each lasting three days. For this study, third flush mushrooms in the size range of 40–50 mm were used. Mushrooms were harvested into 2 kg punnets and transported to the National Prepared Food Consumer Center packaging suite of the Food Industry Development Department at Teagasc for packaging.

2.2. Silicon Treatment Applications

A silicon treatment was applied using the commercial product, Actisil (Yara, Pocklington, UK) which contains 5.0 g/L silicon, in the form of orthosilicic acid in a liquid formulation. The product was applied at a rate of 2 mL/m^2 in a volume of 2 L H_2O/m^2.

Silicon was applied on three occasions during the crop cycle: the last watering on Day 5, the last watering between flush 1 and 2, and the last watering between flush 2 and 3. Six replicate crates were prepared for each treatment and positioned in the growing room according to a randomized block design.

2.3. Optimization of the Passive Modified Atmosphere Packaging (MAP) Conditions

The optimization of the passive MAP design involves determining the best headspace gas composition to minimize the changes in the product quality parameters. This includes screening polymeric films, identifying potential limitations, and reducing the number of experimental trials. To design a successful passive MAP, it is essential to determine the intrinsic properties of the product such as respiration rate (R), optimal O_2 and CO_2 gas concentrations (y), and film permeability characteristics [16,18,20]. Simulating a passive MAP system is the most suitable method to ensure a correct design and achieve a commercially successful product. Figure 1 depicts a thermoformer packaging system and illustrates fresh mushrooms respiring in a perforation-mediated MAP in an rPET tray with a monoPET lid film.

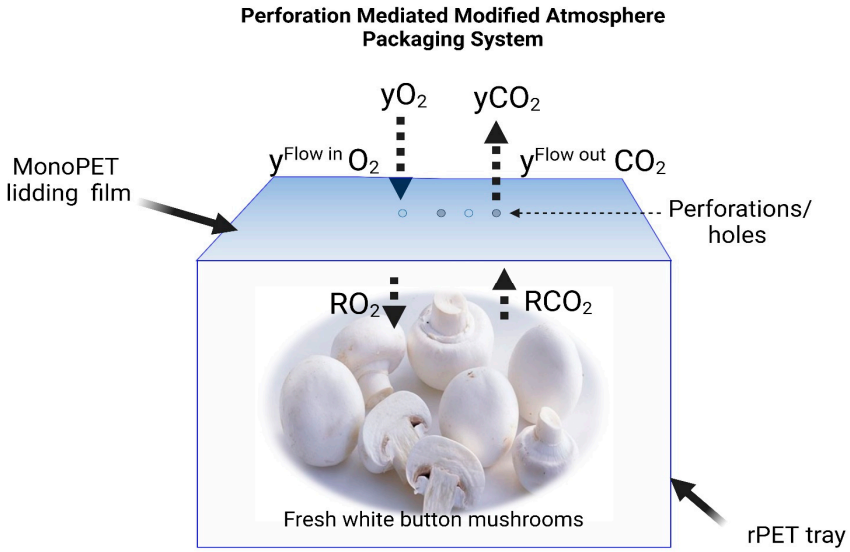


Figure 1. Schematic diagram representing gas exchange in a perforation-mediated MAP for fresh mushrooms. 'y' represents gas exchange through the perforated monoPET lidding film and 'R' represents gas exchange as a result of respiration of the mushrooms.

The plastic film can be used to control the flow of O_2 into the package and the release of CO_2. We can use differential mass balance equations to describe the changes in O_2 Equation (1) and CO_2 Equation (2) concentrations in a package with a respiring product, assuming a constant total pressure and no gas stratification inside the package [18,20]:

$$V_f \frac{d(y_{O_2})}{dt} = \frac{P_{O_2}}{e} A \left({y_{O_2}}^{out} - y_{O_2}\right) - R_{O_2} M \quad (1)$$

$$V_f \frac{d(y_{CO_2})}{dt} = \frac{P_{CO_2}}{e} A \left({y_{CO_2}}^{out} - y_{CO_2}\right) - R_{CO_2} M \quad (2)$$

where,

V_f is the headspace (free volume) in the package
y is the gas concentration (in molar fraction)
e is the thickness of the polymeric film
The package's permeability, denoted as P, is the amount of gas exchanged per unit time and area.

The respiration rate, denoted as R, is the volume of gas produced or consumed per unit time.
M represents the weight of the product.
The subscripts O_2 and CO_2 indicate oxygen and carbon dioxide, respectively.

The simulation of the MAP system in this experiment was carried out using macroscopic perforations in a monoPET lidding film, providing a parallel pathway for gas transport. The apparent permeability applied in Equations (3) and (4) is dependent on the film permeability and the quantity and dimensions of the holes. It has been documented that for holes of the same size [18]:

$$P\prime_{O_2} = P_{O_2} + \frac{\pi R^2{}_H 16.4 \times 10^{-6}}{(e + R_H)} N_H \quad (3)$$

$$P\prime_{CO_2} = P_{CO_2} + \frac{\pi R^2{}_H 16.4 \times 10^{-6}}{(e + R_H)} N_H \quad (4)$$

In Equations (3) and (4), the symbol P' represents the apparent values, where R_H stands for the radius of the holes, and N_H represents the number of holes.

To achieve the best conditions for preserving fresh mushrooms, a polynomial equation was used to analyze the O_2 and CO_2 concentrations in packages with specific macroperforation dimensions. The study revealed that the ideal conditions for packaging fresh mushrooms involved using perforations with a total diameter of 1 mm and a varying number of holes ranging from one to four. Figure 2 illustrates the changes in O_2 and CO_2 levels in the headspace based on the number of perforations and the quantity of mushrooms. The first hole has a diameter of 0.973 mm. There are 2 holes with a diameter of 0.515 mm and 3 holes with a diameter of 0.36 mm. In addition, there are 4 holes with a diameter of 0.282 mm. This arrangement resulted in a gas composition of around 7% O_2 and 10% CO_2 during a storage period of 15 days at 4 °C.

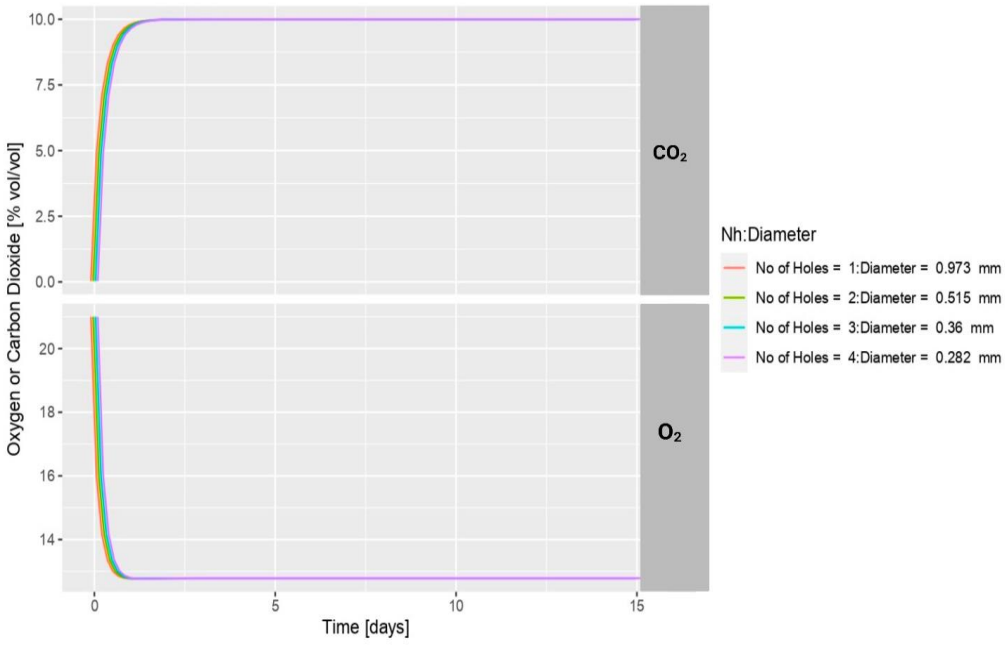


Figure 2. Perforation-mediated MAP optimization for fresh mushrooms with Bottom web rPET of 350 µm thickness and 320 mm width, PET lidding film of 20 µm thickness and 300 mm width, and 149 mm × 149 mm × 60 mm thermoformed packages.

Based on the results of the optimization of the PMAP design as described in Section 2.3 and Figure 3, it was decided to investigate 1 hole × 1.1 mm, 2 holes × 0.53 mm, 3 holes × 0.53 mm, and control packaging systems.

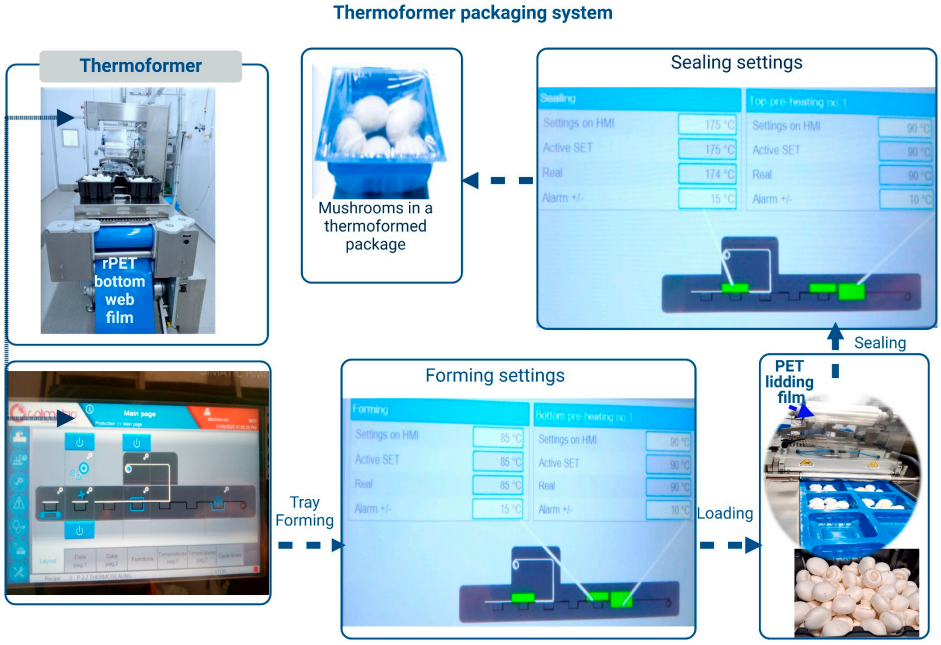


Figure 3. Automatic thermoformer packaging system for fresh white button mushrooms using bottom web rPET of 350 µm thickness and 320 mm width, and PET lidding film of 20 µm thickness and 300 mm width.

2.4. Packaging Treatments

Third flush mushrooms with a size range of 40 to 50 mm were harvested on 27 March 2023 and stored at 4 °C for up to 2–3 h until packaged. The mushrooms were then packaged in 200 g quantities in punnets made using automatic thermoformer settings of forming, namely 85 °C, with bottom pre-heating at 90 °C, and sealing at 175 °C with top pre-heating at 90 °C in bottom web rPET of 350 µm thickness and 320 mm width, and PET lidding film of 20 µm thickness and 300 mm width were used to form the thermoformed packages (149 mm × 149 mm × 60 mm) (Figure 3). The top lidding film was given one, two, or three perforations in the top as described in Figure 1 to give three packaging treatments—PMAP1, PMAP2, and PMAP3. A fourth control packaging type was also included, PMAPC, which consisted of a conventional polypropylene tray (162 mm × 123 mm × 55 mm) wrapped with OMNI-PW micro perforated cling film (Figure 4). The packaged mushrooms were stored for up to 5 days at 4 °C and 99.9% relative humidity. This procedure required that the mushrooms were double handled, first when harvested and again when placed into the punnet for packaging. Generally, mushrooms are only handled once (directly into the final punnets) to minimize any damage to the fragile cap surface.

2.5. Experimental Design

The experiment was set up as a 2 × 4 × 5 factorial design to evaluate the effects of a preharvest silicon treatment and four packaging types on the various quality parameters of mushrooms cold stored over a five-day storage period. In summary, there were (a) two preharvest treatments, namely preharvest control (PHTC) and preharvest silicon treatment (PHTS), (b) four postharvest packaging treatments (PMAP1, PMAP2, and PMAP3) and a conventional control (PMAPC) (Figure 4), and (c) five sampling times (Day 1, Day 2, Day 3, Day 4, and Day 5) corresponding to days 1 to 5 of the storage period. Three replicate punnets with 200 g per punnet were prepared for each treatment combination. The punnets of mushrooms were removed each day for analysis. A range of measurements

were made daily which included the following: concentration of CO_2 and O_2 gases within the punnets, colour parameters L^*, a^*, b^*, and ΔE values, density, electrolyte leakage, and total soluble solids, as outlined below.

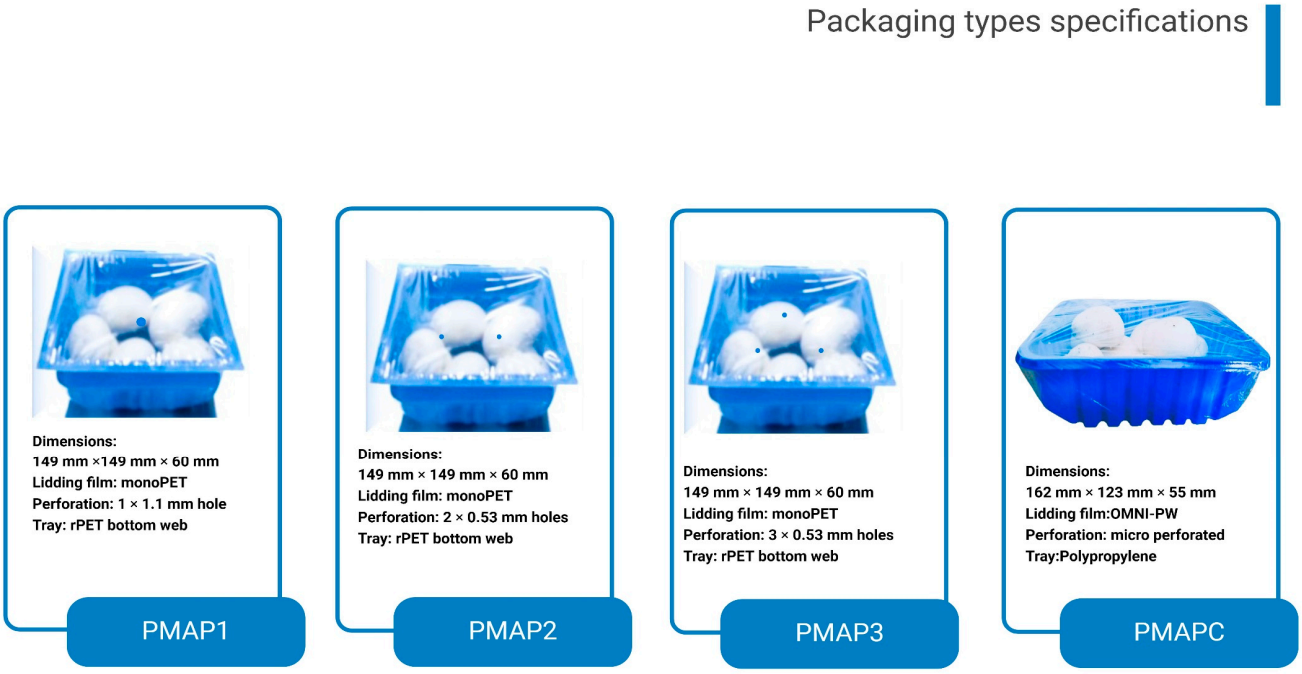


Figure 4. Packaging specifications: PMAP1: one hole of perforation size of 1.1 mm on PET lidding film; PMAP2: two holes of perforation size of 0.53 mm on PET lidding film; PMAP3: three holes of perforation size of 0.53 mm on PET lidding film; PMAPC: polypropylene tray wrapped with OMNI-PW micro perforated cling film as a control conventional packaging system.

2.6. Analytical Methods

Three replicates of mushroom punnets from each of the PMAP packaging treatment and conventional packaging (control) in a randomized complete block design were assessed for changes in internal atmosphere, colour, density, electrolyte leakage, and total soluble solids at one-day intervals in a strict order during a five-day storage period at 4 °C and 99.9% relative humidity. Samples were stored on a shelf labelled with their specific test days and divided between control samples and different treatment samples for easier future testing organization.

2.6.1. Sampling of Gas from the Packaging Atmosphere

The gas samples from each package were obtained using a Dansensor Check-Point 3 by piercing the septum located at the top lidding film of the punnets. This device is designed with an injection needle to penetrate the punnets and monitor the changes in the atmospheric composition inside the package caused by the product's respiration. Just before opening, the device measured the O_2 and CO_2 percentages in the punnets, and the rate of O_2 decline and CO_2 increase indicated the speed of the crop's respiration.

2.6.2. Colour

The colour parameters L^*, a^*, and b^* values were measured from the cap surface on three mushrooms per punnet of mushrooms using an UltraScan Pro (HunterLab, Reston, VA, USA, Stotto Group, Leicester, UK). The visual colour degradation of the mushrooms was expressed in terms of L^*, a^*, and b^* individually, employing ΔE (Equation (5)) as described in I.S. EN ISO/CIE 11664-4:2019 [34].

$$\Delta E = \sqrt{(L_0 - L_1)^2 + (a_0 - a_1)^2 + (b_0 - b_1)^2} \quad (5)$$

where $L_0 = 97$, $a_0 = -2$, $b_0 = 0$ were used as reference values for mushrooms as described by Ajlouni [35].

2.6.3. Density

The density (kg/m^3) of individual mushrooms was measured using Voluscan (Portable micro systems), and three mushrooms per punnet from each treatment were analyzed.

2.6.4. Electrolyte Leakage

Electrolyte Leakage Analysis was adapted from the methods of leaf and fruit-based techniques described by [36]. A circular disc of 12 mm diameter and 10 mm depth was extracted. The obtained cylindrical-shaped mushroom pieces were towel-drained on lint-free sheets, and then placed in 50 mL labelled beakers and filled to 30 mL with ultra-pure water, and the conductivity was recorded using a conductivity meter. Three measurements were conducted at the beginning (e_0), and then the sample was placed on an oscillator and gently mixed for 3 h to allow the electrolyte to permeate (e_1). Subsequently, the contents of the beaker were subjected to heating in a water bath at 75 °C for 20 min to decompose the mushrooms, and a final measurement of electrical conductivity was taken to indicate the overall electrolyte concentration of the mushrooms (e_t). The electrolyte leakage (EL) could then be determined using Equation (6):

$$EL = \frac{(100 \times (e_1 - e_0))}{e_t} \quad (6)$$

2.6.5. Total Soluble Solids (TSS)

Three mushrooms were randomly selected from a punnet and homogenized using a blender, and the obtained extract was centrifuged (4000 rpm for 10 min). TSS in °Brix was measured at 25 °C using an Anton Paar Refractometer (Abbemat 3100, Graz, Austria) with a resolution of 0.1. The test was repeated for each of three replicates per treatment.

2.7. Statistical Analysis

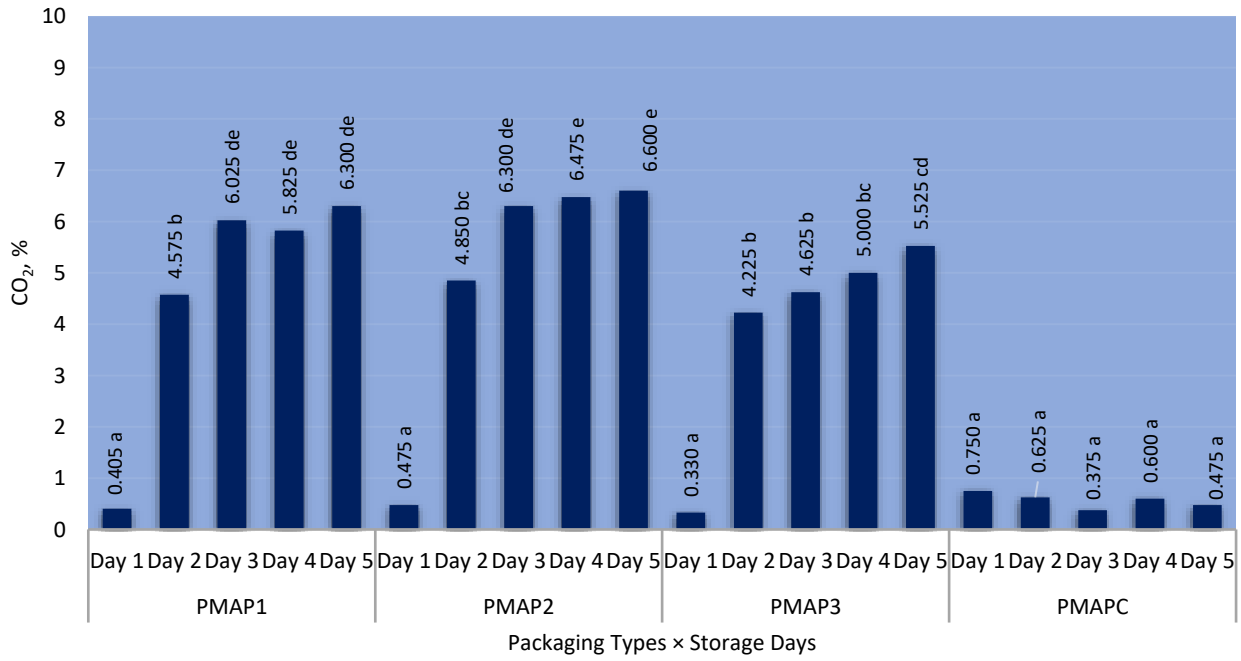
The effects of two preharvest treatments, four PMAP packaging types, and five storage days on the quality of mushrooms were determined using the analysis of variance (ANOVA) for a 2 × 4 × 5 factorial design via the modelling data option (XLSTAT 2023 by AddinSoft™ SARL, Paris, France). To determine significant differences between means, the Fisher Least Significant Difference test (LSD) was performed at a 5% probability level ($p < 0.05$). Additionally, principal component analysis (PCA) was conducted on the observations, or the variables table was analyzed to visually represent the differences among preharvest treatments, packaging types, and storage days using a vector distance plot.

3. Results and Discussion

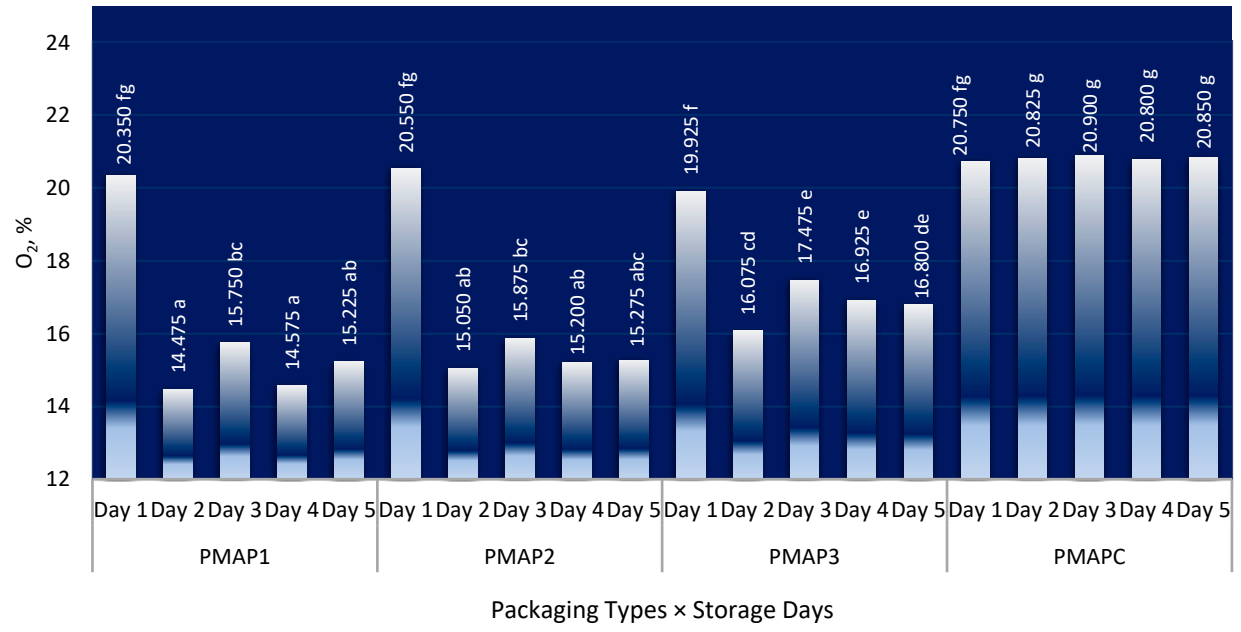
3.1. Headspace Gas Composition during the Storage Days

The results of the analysis of variance showed that packaging type, storage days, and the double interaction effects of storage days × packaging type had significant effects ($p < 0.0001$) on the changes in CO_2 and O_2 content inside the packages (Table 1). The mean values of the main effects and the results showed that the concentration of CO_2 from day 2 to day 5 within the packaging system increased significantly in PMAP1, PMAP2, and PMAP3 compared to PMAPC as shown in Figure 5a. In the normal atmosphere, there are about 78% N_2, 21% O_2, 0.3% CO_2, and small amounts of other gases in a storage room. PMAP1 and PMAP2 had slightly higher CO_2 and lower O_2 compared to PMAP3 after 5 days of storage. The mushrooms packed in PMAP1, PMAP2, and PMAP3 showed an increase in CO_2 concentration with time reaching 6.3%, 6.6%, and 5.5% after 5 days, respectively, whereas the CO_2 concentrations did not change significantly (0.85%–0.4%) over 5 days in the control packages (PMAPC). Similarly, the concentrations of O_2 after Day 1 were significantly higher in PMAP3 compared to PMAP1 and PMAP2, but all were significantly

lower compared to the control PMAPC (Figure 5b). In PMAPC, the conventional packages' respiration rate of mushrooms was higher, as the entry rate of O_2 was high through the micro perforated packaging film (Figure 5b). There was no significant effect of preharvest treatment with silicon on CO_2 or O_2 concentration (Table 1).



(**a**)



(**b**)

Figure 5. The interaction effects of packaging type and storage days on (**a**) CO_2 concentration; and (**b**) O_2 concentration of mushrooms. PMAP1: one hole of perforation size of 1.1 mm on monoPET lidding film; PMAP2: two holes of perforation size of 0.53 mm on monoPET lidding film; PMAP3: three holes of perforation size of 0.53 mm on monoPET lidding film; PMAPC: OMNI-PW film micro perforated conventional packaging system. 'a–g' means within each figures (**a**,**b**) with different superscripts are different ($p < 0.05$) when analyzed using analysis of variance.

Table 1. Analysis of variance of the effect of preharvest treatments (PHTs), storage days (SD), and packaging type (PT) on the gas composition and quality of mushrooms, Pr > F values.

Category	Model	SD	PHT	PT	SD×PHT	SD×PT	PHT×PT	SD×PHT×PT
CO_2	<0.0001	<0.0001	0.731	<0.0001	0.996	<0.0001	0.896	1.000
O_2	<0.0001	<0.0001	0.650	<0.0001	0.992	<0.0001	0.838	1.000
*L**	<0.0001	<0.0001	0.471	<0.0001	0.855	<0.0001	0.958	0.179
*a**	<0.0001	<0.0001	0.126	<0.0001	0.094	<0.0001	0.688	0.637
*b**	<0.0001	<0.0001	0.023	<0.0001	0.928	0.258	0.553	0.999
Δ*E*	<0.0001	<0.0001	0.104	<0.0001	0.954	<0.0001	0.622	1.000
EL	<0.0001	0.005	0.135	<0.0001	0.147	0.240	0.016	0.130
Density	<0.0001	0.001	0.072	<0.0001	0.008	0.002	0.004	0.001
TSS	<0.0001	<0.0001	0.531	<0.0001	<0.0001	<0.0001	0.023	0.097

EL: Electrolyte Leakage; TSS: Total Soluble Solids.

Figure 5 presents the mean value of the changes in oxygen and carbon dioxide content during the storage days. The results show that with the passage of storage days, the amount of oxygen decreased and the amount of carbon dioxide increased due to the respiration of the product inside the package except for the control PMAPC. The changes in headspace O_2 and CO_2 concentrations varied as a function of the number of perforations in the lid. An inverse relationship (highly negative correlation, $r^2 = -0.9$) with the trends of CO_2 and O_2 concentrations was observed during the storage period. The difference in gas composition between the micro perforated OMNI-PW film package and the other treatments was due to the varying film permeability. The high oxygen permeability of the micro perforated OMNI-PW film enables gas exchange with the product inside, while the low permeability of the monoPET lidding film restricts gas transfer into the package. Consequently, the product's respiration causes a significant decrease in oxygen and an increase in carbon dioxide levels inside the packages.

The variability in the permeability of the films was found to be the main reason for the significant difference in the amount of oxygen and carbon dioxide in the PMAP packaging type, as it affects the entering and exiting of gases based on the number and sizes of perforations, as also noted by Gholami et al. [17]. Perforation-mediated MAP in thermoformed rPET tray and monoPET lidding film packaging systems should increase the shelf life of mushrooms by lowering the amount of reactive oxygen species, blocking microbial activity and respiratory metabolism. Research has indicated that MAP is considered an effective, simple, and relatively affordable packaging method for fresh mushrooms [9]. To establish a passive modified atmosphere, packaging bags, films that cover the tray, or trays with microporous materials are punctured directly [16,19], helping to preserve the mushrooms by regulating the quantity and composition of gases by creating a balanced gas exchange within the packaging systems.

The amount of oxygen in the PET packages had become almost stable after the second day until the end of the storage day, and the amount of oxygen remained in the range of 14.4%–17.4% in PET with different perforations compared to 20.8% in OMNI-PW film micro perforated packages, which was slightly above the range reported for mushrooms [37]. The optimum packaging conditions obtained for ~200 g of fresh mushrooms were PMAP1 and PMAP2 leading to an equilibrium exchange of O_2 and of CO_2 in the headspace gas composition, after 1 day of storage at 4 °C and 99.9% RH. In line with the results of our study, an equilibrium gas composition in the headspace was achieved after, approximately, 1 day for packages containing sliced mushrooms [16]. The amount of 20.8% oxygen and 0.4% carbon dioxide in OMNI-PW film micro perforated packages was found to be in agreement with the findings reported for mushrooms in PVC conventional packaging [15–17]. It is important to note that the two perforation-mediated MAP packaging systems (PMAP1 and PMAP2) effectively modified the exchange of oxygen and carbon dioxide between the package and the surrounding environment. In PMAP1 and PMAP2, an equilibrated gas exchange was reached after the first storage day conforming to and validating the results obtained from perforation-mediated MAP modelling for the mushrooms.

3.2. Colour Changes during the Storage Period

The results of the analysis of variance showed that there was a significant main effect of storage days and packaging type on all the colour parameters, L^*, a^*, b^*, and ΔE ($p < 0.001$). All the parameters showed deterioration with increased storage days, compared to Day 1, as might be expected (Supplementary Table S1). Packaging types PMAP1, PMAP2, and PMAP3 had better L^*, a^*, b^*, and ΔE characteristics compared to the control PMAPC. There was no significant effect of preharvest treatment as a main effect on the colour parameters, except for b^* value ($p < 0.05$). The results showed a significantly lower b^* value (whiter mushrooms) for silicon-treated mushrooms (12.0) compared to the control (12.4).

There was a significant interaction effect between storage days × packaging type for all the colour parameters ($p < 0.0001$) (Table 1, Figure 6), but not for b^* (Supplementary Table S2). The results showed a significantly lower ΔE value (whiter mushrooms) for PMAP1, PMAP2, and PMAP3 after the 5 days of storage compared to mushrooms under the OMNI-PW film micro perforated conventional packaging system. This finding probably reflects the impact of the PMAP design in reducing the rate of respiration (Figure 4) while keeping the cellular structure and integrity of the mushrooms. For example, controlling respiratory metabolism could help to minimize the oxidative damage to nutrients and leakage of electrolytes, ultimately limiting the availability of substrates for browning enzymes such as tyrosinase and polyphenol oxidase thereby inhibiting their activities [38–40].

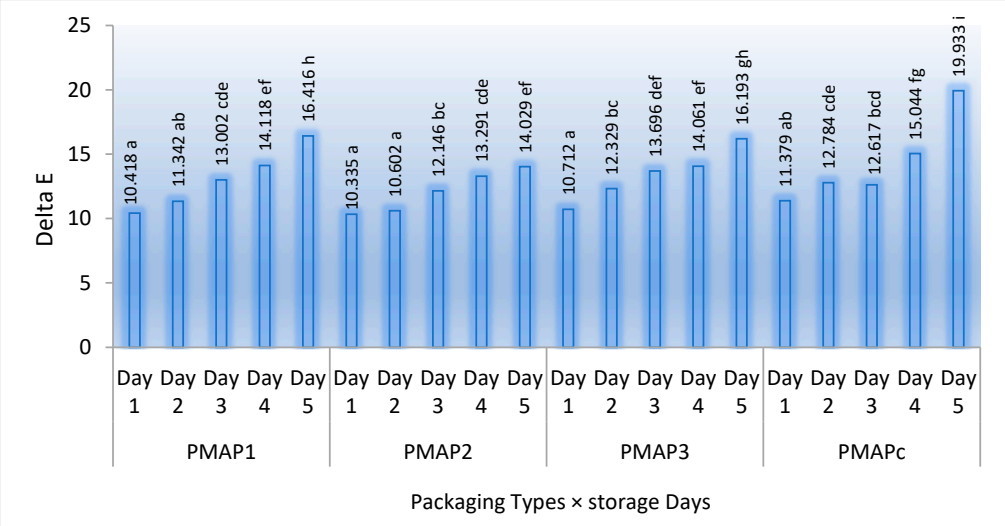


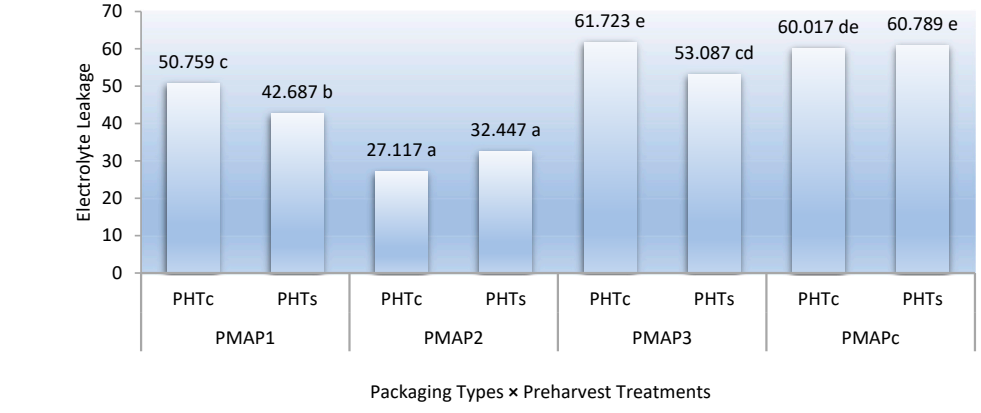
Figure 6. The interaction effects of packaging type and storage days on ΔE of fresh mushrooms. PMAP1: one hole of perforation size of 1.1 mm on PET lidding film; PMAP2: two holes of perforation size of 0.53 mm on PET lidding film; PMAP3: three holes of perforation size of 0.53 mm on PET lidding film; PMAPC: conventional packaging system. 'a–i' means within a figure with different superscripts are different ($p < 0.05$) when analyzed using analysis of variance.

Overall, an increasing trend in ΔE was observed due to a decrease in L^* combined with an increase in a^* and b^*, with the increase in storage days. This may be linked to the absence of a cuticle and the bruising during harvesting and packaging which increases the metabolic rate of tyrosinase and polyphenol oxidase enzymes leading to enzymatic browning reactions [41–45]. Research has indicated that utilizing a combination of 10% O_2 and 5% CO_2 MAP packaging, along with treatment involving calcium chloride and citric acid, led to a significant decrease in respiration rate, an increase in radical scavenging activity, a reduction in browning, and the retention of quality in *Pleurotus florida* mushrooms for up to 25 days [16]. The use of a polyvinyl chloride polyethylene–silicon window resulted in a lowered respiratory rate, delayed texture and flavour changes, reduced browning and weight loss, and delayed senescence in pine mushrooms [46]. Moreover, applying a 2% alginate coating with 100% O_2 led to a reduction in microbial count, inhibited the activity

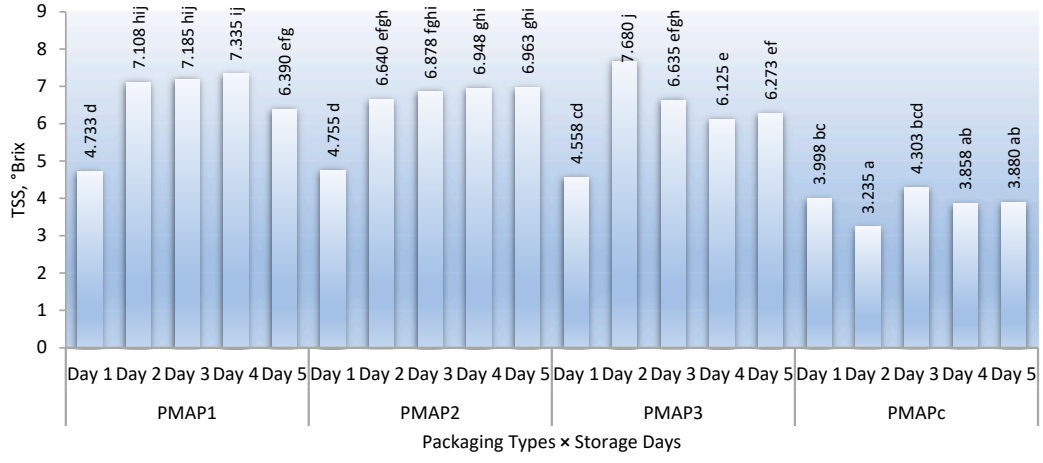
of polyphenol oxidase, maintained firmness, and delayed browning, cap opening, and changes in soluble solids, total sugars, and ascorbic acid, extending the shelf life of *Lentinus edodes* mushrooms to 16 days [47]. Furthermore, a higher respiration rate was achieved by using a combination of 20% CO_2 and 15% O_2 at a temperature of 4 °C and a relative humidity of 95%, leading to an increase in the total phenolic content and a decrease in browning, thereby prolonging the shelf life of *Pleurotus eryngii* mushrooms to 10 days [48].

3.3. Electrolyte Leakage, TSS, and Density during the Storage Period

Besides colour, electrolyte leakage, density, and TSS are very important indices related to the quality and metabolism of mushrooms. The results of the analysis of variance showed that the storage time ($p < 0.001$), packaging type ($p < 0.0001$), and the double interaction effects of preharvest treatment × packaging type had significant effects ($p < 0.05$) on the changes in electrolyte leakage (Table 1). The PMAP2 packaging type resulted in the least electrolyte leakage of all packaging types, while preharvest treatment with silicon reduced the electrolyte leakage significantly in PMAP1 and PMAP3 while it had no significant effect in PMAP2 or PMAPC (Figure 7a). There were significant differences in TSS levels for the main effects of storage days and packaging type as well as a two-way interaction between these two parameters ($p < 0.001$). The control PMAPC had lower TSS levels across storage days, while for PMAP1, PMAP2, and PMAP3 packaging types, the TSS levels increased during storage (Figure 7b). Preharvest treatment with silicon had a minimal impact on TSS levels. For density, ANOVA (Table 1) indicated significant effects of storage time ($p< 0.001$), packaging type ($p < 0.0001$), and the double interaction effects of storage days × packaging type ($p < 0.002$). Other interaction effects were more variable. The density was lowest with PMAPC packaging, and it continued to decrease with increased storage time compared to the other packaging types (Figure 7c).

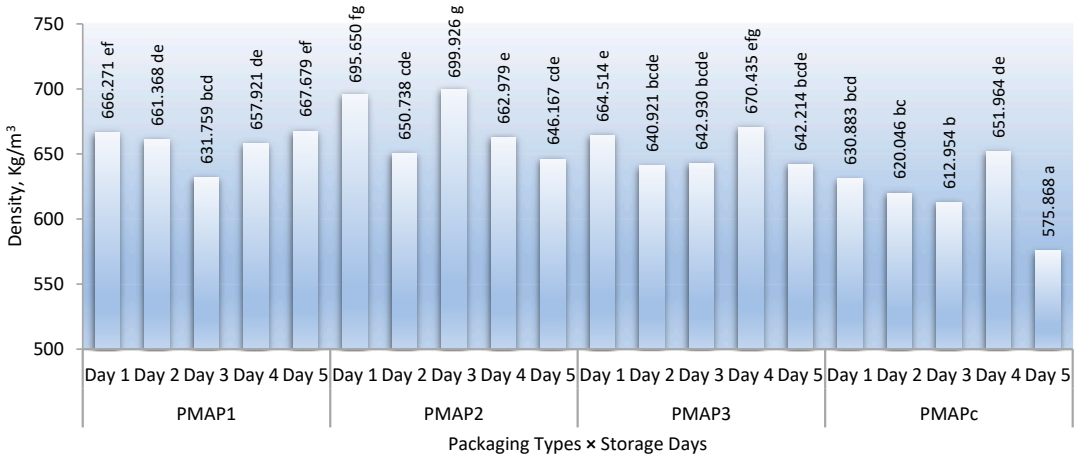


(**a**)



(**b**)

Figure 7. *Cont.*



(**c**)

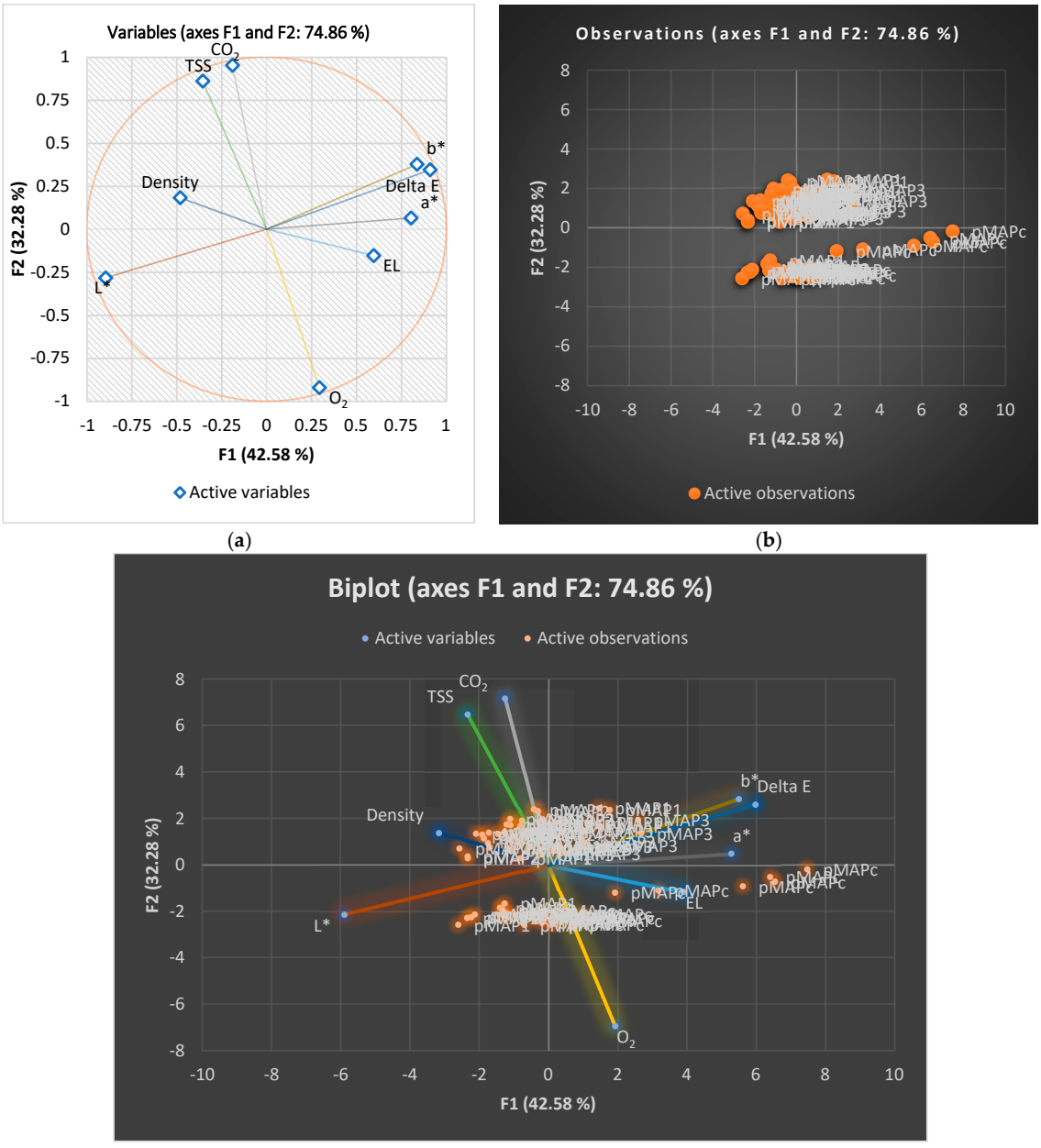
Figure 7. The interaction effects of packaging type and storage days on electrolyte leakage (**a**), TSS (**b**), and density (**c**) of mushrooms. PHTC: control preharvest treatment; PHTS: silicon preharvest treatment; PMAP1: one hole of perforation size of 1.1 mm on PET lidding film; PMAP2: two holes of perforation size of 0.53 mm on PET lidding film; PMAP3: three holes of perforation size of 0.53 mm on PET lidding film; PMAPC: conventional packaging system. 'a–j' means within each figures (**a**–**c**) with different superscripts are different ($p < 0.05$) when analyzed using analysis of variance.

Overall, the mushrooms packaged in PMAPC had significantly lower TSS and density compared to the other packaging types, and higher EL compared to PMAP1 and PMAP2. An increase in electrolyte leakage could potentially be linked to a loss of TSS and density leading to the senescence or quality deterioration of the mushrooms with a low negative correlation between *EL* and TSS (r = −0.307) and density (r = −0.281). This can also be clearly viewed form the principal component analysis chart in Figure 8a,b. Figure 8 presents a summarized view of the interaction effects of preharvest treatments with packaging types where the first two principal components (F1 and F2) explained 74.86% of the total variability in gas composition and quality parameters of the mushrooms. F1 differentiates mushroom colour *L**, TSS, density, and CO_2 on the left-hand side from Δ*E*, *b**, *a**, *EL*, and O_2 on the right-hand side (Figure 8a).

The PCA biplot chart clearly shows the highest EL and O_2 for the PMAPC conventional mushroom packaging system indicating the potential for faster decay with this packaging system (Figure 8b). On the other hand, the highest CO_2, *L**, density, and TSS of fresh mushrooms were recorded for PMAP2 (two holes of a perforation size of 0.53 mm) compared to the other packaging treatments, revealing that the low respiration rate could be linked with better quality maintenance.

The results presented in Figure 8 show that the respiratory rate in terms of oxygen concentration is positively correlated with electrolyte leakage (r = 0.31 and *a** (r = 0.30), and highly negatively correlated with TSS (r = −0.91) which might also be linked to the variation in the gaseous permeability rate of the different types of perforations on PMAP systems. This result can be explained by the fact that PMAP1 and PMAP2 were more effective in blocking the passage of gas (CO_2) from inside the package to the outside, which is in agreement with the findings of other research on perforated MAP conditions. The findings support previous research demonstrating that micro perforated films may decrease the respiration rate of strawberries and mushrooms [49,50]. The results above suggest that micro perforations can reduce the rate of mushroom spoilage by potentially creating an altered internal atmosphere, which limits the exchange of gases between the environment and the mushrooms. In relation to the presence of CO_2, this could not only lead to an increase in the respiration rate but also promote the growth of specific microorganisms, such as aerobic bacteria, yeast, and moulds. Additionally, the researchers examined the impact of various

initial gas components (ranging from as low as 3% to as high as 100% of O_2 content) on the nutrient components (polysaccharides, total phenols, and free amino acids) of fresh *Lentinula edodes* mushrooms [51,52]. The authors suggest that packaging with high O_2 levels (exceeding 50%) could increase the umami amino acid content and prevent the formation of ethanol and electrolyte leakage. Moreover, modified atmosphere packaging using a combination of polyethylene/polyamide, calcium chloride, and citric acid with 10% O_2 and 5% CO_2 resulted in a significant decrease in the respiration rate, increased the radical scavenging activity, maintained quality, received higher sensory ratings, reduced changes in weight, pH, and TSS, lowered the total polyphenol content, and extended the storage life of *Pleurotus florida* mushrooms to 25 days [19].



(**a**) (**b**)

(**c**)

Figure 8. PCA (principle component analysis) of variables (**a**), observations (**b**), and biplot (**c**) of the effects of packaging type on quality of mushrooms (mean values). PMAP1: one hole of perforation size of 1.1 mm size on PET lidding film; PMAP2: two holes of perforation size of 0.53 mm on PET lidding film; PMAP3: three holes of perforation size of 0.53 mm on PET lidding film; PMAPC: conventional packaging system.

Mushrooms have a high respiration rate; therefore, creating an equilibrium gas composition such as using perforation-mediated MAP techniques may be a useful tool to maintain the quality and extend the shelf life. In this study, after 1 day of storage for packaging with perforations in monoPET films, O_2 decreased to 16.1%, 16.4%, and 17.4% in the packages with one hole of 1.1 mm, two holes of 0.53 mm, and three holes of 0.53 mm sizes, respectively, compared to 20.9% in the OMNI-PW film micro perforated conventional packaging system. Mushrooms in perforation-mediated MAP maintained significantly higher TSS, density, and CO_2 concentration along with lower O_2 concentration and electrolyte leakage with the increase in storage days compared to PMAPC conventionally packaged mushrooms. Research indicates that reducing the oxygen level to a minimum lowers the respiration rate and slows down the cap development, decreases aerobic spoilage, minimizes weight loss, and reduces tyrosinase activity, which in turn decreases enzymatic browning [16,53]. Despite the numerous advantages of low oxygen levels, an oxygen level lower than 2% might lead to the growth of anaerobic microbes like *Clostridium botulinum* and *Staphylococcus aureus* [54]. Moreover, the concentration of CO_2 must not exceed 12% as excessive CO_2 accumulation can cause physiological damage to mushrooms.

4. Conclusions

Fresh mushrooms are characterized by the lack of a cuticle, high respiratory and metabolic rates, moisture content, and enzyme activity. In response to these intrinsic factors and the atmosphere surrounding them, the product has a very short shelf life, typically 3 days under ambient conditions and 5 to 8 days in a cold storage system. This study presented a novel approach to enhance the shelf life through the combined application of silicon preharvest treatment and perforation-mediated passively modified atmosphere packaging (PMAP) to minimize the physiological and morphological quality degradation in mushrooms. This work also identified an optimized passive MAP design, with respect to the number and size of perforations in the film, in order to achieve the best headspace gas composition over time, for maintaining the quality and extending the shelf life of fresh edible mushrooms. The simulated MAP system based on a macroscopic perforation of a 1 mm hole size in monoPET lidding films regulated the O_2 flow into the package and the flow of CO_2 out while minimizing the changes in the quality parameters of fresh mushrooms. Good-quality mushrooms generally have characteristics such as low ΔE, high L^*, high TSS, high density, and low *EL*. The optimum packaging types that maintained the quality of 200 g of fresh mushrooms were PMAP1 with a single hole of 1.1 mm and PMAP2 with two holes of 0.53 mm size in a monoPET lidding rPET tray thermoformed package size of 149 mm × 149 mm × 60 mm, leading to equilibrated conditions for the flow of O_2 and CO_2, after the first day of storage at 4 °C. The concentration of CO_2 within the packaging system, TSS, and density of the mushrooms were significantly higher in PMAP1 and PMAP2 compared with the PMAP3 and PMAPC packaging formats. Notably, mushrooms in all passive MAP showed significantly ($p < 0.001$) lower ΔE value, with the lowest recorded in PMAP1 and PMAP2, suggesting that a passive MAP design can successfully help to control or regulate the respiratory metabolism thereby minimizing the oxidative damage to nutrients and leakage of electrolytes, ultimately inhibiting browning enzymes' activities in fresh mushrooms. However, silicon treatment had little effect overall and did not affect the ΔE values where respiration was reduced in PMAP1, PMAP2, and PMAP3. Further work is required to understand how reduced respiration affects mushrooms' colour characteristics. Electrolyte leakage was significantly lower in PMAP2 indicating the potential extension of the shelf life of fresh mushrooms with a micro perforated optimized passive MAP design in a thermoformed recycled PET packaging system. Therefore, micro perforation using laser technology involving a simulated optimized MAP design is recommended for validating the results in industrial scalable systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/coatings14060754/s1, Table S1: The main effects of packaging types

and storage days, and preharvest treatments on colour values of mushrooms; Table S2: The double interaction effects storage days x packaging types on colour values of mushrooms.

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