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Packaging in a High O₂ or Air Atmospheres and in Microperforated Films Effects on Quality of Button Mushrooms Stored at Room Temperature

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Abstract: Cold conditions are obligatory for mushrooms storage. However, in most cases, the cold chain is interrupted at various stages. Thus, it is of great importance to propose a packaging system capable of inhibiting the detrimental effect of high temperature on mushrooms' quality. The study evaluates the effect of high oxygen atmosphere (80% O₂) in conjunction with films of different levels of microperforations (polysulfon (PSF) films, low: PSF_1000, PSF_2000; medium: PSF_3500; and high: PSF_7000) on antioxidant capacity, volatile compounds profile, sensory acceptance, and quality of mushrooms stored at 20 °C. Results showed that high O₂ atmosphere inhibits the respiration rate of mushrooms. Application of high O₂ atmosphere and film of high level of microperforations preserved desired color and profile of volatile compounds, ensured consumers color and overall acceptance. In turn, the single effect of the perforation level of the applied film was observed for antioxidant capacity, weight loss, vitamin C, malonyldialdehyde (MDA), and phenolics content. Packaging in low microperforated films led to the least amount of phenolics, highest MDA content, and poor antioxidant capacity in mushrooms. In turn, packaging with films of a medium level of perforation contributed to the highest vitamin C and phenolic content. There was no effect of treatment on texture, maturity index, protein content, and percentage of open capped mushrooms.

Keywords: high oxygen; microperforations; ambient temperature; volatile compounds; antioxidant activity

1. Introduction

White button mushrooms (*Agaricus bisporus*) are a rich source of nutrients, vitamins, minerals, and bioactive compounds [1]. *Agaricus bisporus* exhibit also medicinal attributes, unique sensory properties, and low caloric value. Moreover, in comparison to other mushrooms, they are relatively easy and inexpensive to harvest. Therefore, its production accounts for 30% of total mushroom production in the world [2]. However, high respiration and transpiration rates, high moisture content (over 90%), and lack of cuticle makes button mushrooms very perishable [3]. The shelf-life of *Agaricus bisporus* at ambient conditions (25 °C, 70% relative humidity RH) is estimated around 1–2 days and at cold conditions (2 °C), around 3–5 days. Short durability causes problems with distribution and sales [4].

Modified atmosphere packaging (MAP) is proposed by many researchers as the most effective way to maintain freshness of mushrooms for a longer time. Most of those studies advocate for mushroom packaging in low CO₂ (2.5–5%) and low O₂ (5–10%) atmospheres [5,6]. Carbon dioxide in small amounts is essential to inhibit microbial growth, while oxygen inhibits growth of anaerobic

bacteria. In general, there is no trend to introduce high amounts of oxygen to a modified atmosphere for mushroom packaging. Higher O₂ concentration in packages leads to oxidation of substrates and acceleration of mushrooms' respiration rate [7]. However, there are studies documenting the positive effect of mushroom packaging in high oxygen atmospheres [8,9]. Moreover, it was proven that super atmospheric O₂ gas composition changes respiration pathways of plants. High oxygen content in packages may keep high energy level in cells. An increased level of ATP allows to preserve integrity of plants membrane. In turn, stability of the membrane counteracts the acceleration of browning processes [10]. Nonetheless, modified atmosphere packaging preserves mushrooms from deterioration only to some extent. The high respiration rate favors high relative humidity inside the package, which in turn leads to water condensation and discoloration of mushrooms [11].

The key is to propose packaging film with water permeability on an appropriate level. A low level of humidity inside the package is a reason for high-water loss and shriveling, but excessive humidity causes fast microbial growth and spoilage. Most polymeric films presented in studies have lower water permeability, thus films with perforations were proposed to increase the water vapor transition rate. According to some researchers, microperforated films seem to be the best solution for excessive RH [4,12].

However, most of the mentioned studies were performed at low temperatures (2–5 °C) during the whole storage period. It is commonly known that cold conditions are crucial to preserve quality of mushrooms at an acceptable level. Even though low temperature is important, it also has limitations. Cold conditions may delay the peak of browning but do not have the capability to break it completely. In order to inhibit mushrooms, spoilage is recommended to introduce simultaneously other preservation treatments. Additionally, temperature fluctuations during transportation is a fact [13]. Distribution and sale are performed in most cases at room temperature as well. Thus, it seems to be of great importance to verify the impact of the combined effect of high oxygen atmosphere, microperforated film packaging, and storage at higher temperature on the quality of white button mushrooms. To the best of our knowledge, there is no study on the topic. Thus, we designed the study to verify the impact of the mentioned factors on physico-chemical and sensorial properties of white button mushrooms stored for 5 days at ambient temperature (20 °C).

2. Materials and Methods

2.1. Sample Preparation

White button mushrooms (*Agaricus bisporus*) were grown at a local cultivation farm in Skórzec, Poland. Fruiting bodies at a mature state were harvested, precooled at 4 °C, and transported at the same day to the laboratory (4 °C). Uniform mushrooms were selected randomly from boxes and divided into 5 batches of 225 each. Batches differed in the type of film used for packaging. Film types tested in the study were: non perforated plasticized polyvinyl chloride (PVC) film and four polysulfone films (PSF_1000; PSF_2000; PSF_3500; PSF_7000) of 25 µm thickness, differed by level of microperforations. Polysulfone films differ in number of holes (PSF_1000-6 holes, PSF_2000-12 holes, PSF_3500-23 holes, PSF_7000-25 holes), in hole sizes (PSF_1000-90 µm, PSF_2000-91 µm, PSF_3500-90 µm, PSF_7000-143 µm), and in O₂ transmission rate (PSF_1000-1000 cm³ day⁻¹ 0.1 MPa⁻¹, PSF_2000-2000 cm³ day⁻¹ 0.1 MPa⁻¹, PSF_3500-3500 cm³ day⁻¹ 0.1 MPa⁻¹, PSF_7000-7000 cm³ day⁻¹ 0.1 MPa⁻¹). Six mushrooms were packed per one PET tray and wrapped with plasticized PVC film or packed with PSF films in a high oxygen modified atmosphere (80% O₂, 5% CO₂, 15% N₂) using Sealpac Traysealer M3 (Sealpac, Oldenburg, Germany). Three trays were packed for each batch, on each storage day (3 × 5 × 5). After packaging, mushrooms were kept at room temperature (20 °C) for 5 days (0, 2nd, 3rd, 4th, 5th). Analysis was performed on each storage day.

2.2. Total Soluble Solids (TSS) and pH

Fresh mushrooms after storage were cut into small pieces with a diameter of 2 mm. Then, the extract was obtained manually by squeezing. The obtained juice was used to determine the TSS and pH as proposed by Jafri et al. [14]. Total soluble solids were measured instrumentally using a refractometer (Digital Refractometer PAL-1/ATAGO CO.LTD., Tokyo, Japan). Obtained values were expressed as Brix (°Brx) degrees. The pH was measured using a digital pH meter (FiveEasy F20, Mettler Toledo, Warsaw, Poland).

2.3. Headspace Gas Composition

Gas composition (O₂ and CO₂) inside the sealed packages were detected by gas analyzer (Witt-Gasetechnik, Witten, Germany). Three packages of mushrooms from each group were tested on each storage day by needle insertion directly into a package.

2.4. Total Phenolic Compounds Assay

Dried mushrooms (1 g) (dried in hot air dryer for 12 h at 45 °C) were homogenized (IKA T18 digital, ULTRA TURRAX) in 9 mL of methanol for 30 s at 13,000 RPM. Then, extraction with ultrasound (Elma Schmidbauer GmbH, Elmasonic S 180 H, Singen, Germany) was carried out for 30 min at 40 °C. Obtained extract was shaken with a rotary shaker (MX-RL_PRO, Chemland, Stargard Szczeciński, Poland) for 15 min, and centrifuged (320R, Hettich Universal, Tuttlingen, Germany) at 9000 RPM for 10 min (4 °C). Supernatant was stored at 4 °C until analysis. Total phenolic compounds (TPC) in *Agaricus bisporus* extracts was measured according to the method presented by Singleton and Rossi [15]. Briefly, Folin-Ciocalteu's reagent, 7% Na₂CO₃ and H₂O were added to the extract and shaken. The mixture was kept in the dark for 30 min. After that, the absorbance was measured at 750 nm wavelength using UV-VIS spectrophotometer (UV-1800, Shimadzu Corp., Tokyo, Japan) against the blank. TPC was expressed as mg of gallic acid equivalent per gram of dry weight.

2.5. Vitamin C Content

The extraction process was carried out in accordance to the following methodology of Balogh and Szarka [16]. Dried mushrooms (2 g) were homogenized (IKA T18 digital, ULTRA TURRAX, Warsaw, Poland) in 20 mL of 5% acetic acid for 30 s at 13,000 RPM, then shaken (MX-RL_PRO, Chemland, Stargard Szczeciński, Poland) for 30 min. Afterwards, extract was centrifuged (320R, Hettich Universal, Germany) for 10 min at 9000 RPM (4 °C). The analysis of vitamin C content was performed according to Balogh and Szarka [16]. Briefly, 85% orthophosphoric acid, 1% 2,2'-bipyridyl, 0.1% FeCl₃ was added to the mushroom extract. Then, the absorbance of the mixture was measured at 525 nm against blank using UV-VIS spectrophotometer. Results were expressed as mg of ascorbic acid equivalent per gram of dry weight.

2.6. Antioxidant Capacity

2.6.1. DPPH Analysis

Dried mushrooms (1 g) (dried in hot air dryer for 12 h at 45 °C) were homogenized (IKA T18 digital, ULTRA TURRAX, Warsaw, Poland) in 9 mL of methanol for 30 s at 13,000 RPM. Then, extraction with an ultrasound (Elma Schmidbauer GmbH, Elmasonic S 180 H, Singen, Germany) was carried out for 30 min at 40 °C. The obtained extract was shaken with a rotary shaker (MX-RL_PRO, Chemland, Stargard Szczeciński, Poland) for 15 min, and centrifuged at 9000 RPM for 10 min (4 °C). The supernatant was stored at 4 °C until analysis. The analysis of the free radical-scavenging effect of the antioxidants present in the mushroom extract on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was measured according to the procedure described by Shimada et al. [17]. Firstly, DPPH solution was added to the mushrooms extracts. Then, the reaction mixture was shaken and incubated in the dark

for 30 min at room temperature after that the absorbance was determined at 520 nm with UV-VIS spectrophotometer. Results were expressed as mg of ascorbic acid equivalent per gram of dry weight.

2.6.2. FRAP Analysis

Dried mushrooms (1 g) (dried in hot air dryer for 12 h at 45 °C) were homogenized (IKA T18 digital, ULTRA TURRAX) in 9 mL of methanol for 30 s at 13,000 RPM. Then, extraction with ultrasound was carried out for 30 min at 40 °C. Obtained extract was shaken with a rotary shaker (MX-RL_PRO, Chemland, Stargard Szczeciński, Poland) for 15 min, and centrifuged (320R, Hettich Universal, Tuttlingen Germany) at 9000 RPM for 10 min (4 °C). Supernatant was stored at 4 °C until analysis. FRAP analysis was performed according to the method described by Belwal et al. [18]. FRAP solution was prepared by mixing ferric chloride (20 mM), 2,4,6-Tri(2-pyridyl)-s-triazine (10 mM in 40 mM HCl), and sodium acetate buffer (300 mM, pH 3.6) in a ratio 1:1:10. The mushroom extract was mixed with FRAP solution and incubated in the dark for 15 min. The absorbance was measured at 593 nm against blank using UV-VIS spectrophotometer. Results were expressed as mg of ascorbic acid equivalent per gram of dry weight.

2.6.3. Malonyldialdehyd (MDA) Content

Extraction was conducted according to Shah et al. [19]. Fresh mushrooms tissue (1 g) was homogenized (IKA T18 digital, ULTRA TURRAX) in 10 mL of 0.5% TBA in 10% TCA for 30 s at 13,000 RPM. Then, the mixture was shaken with a rotary shaker (MX-RL_PRO, Chemland, Stargard Szczeciński, Poland) for 15 min. Further, the extract was centrifuged at 9000 RPM for 20 min (4 °C). The collected supernatant was stored at cold conditions (4 °C) until analysis. The extract was incubated in a water bath at 100 °C for 20 min and then quickly cooled in an ice bath. Afterwards, the absorbance was measured at 450, 532, 600 nm against blank using UV-VIS. Results were calculated according to the formula of Wang et al. [20] and were expressed as μmol of MDA equivalent per gram of fresh weight.

2.7. Protein Content

The extraction was conducted according to Kimatu et al. [21]. Briefly, 1 g of dried mushrooms were shaken (MX-RL_PRO, Chemland, Poland) for 90 min in 15 mL of 5% acetic acid in 0.1% β -mercaptoethanol. Further, the mixture was centrifuged for 10 min at 9000 RPM (4 °C). Then, the supernatant was saturated with $(\text{NH}_4)_2\text{SO}_4$ (1:1) and left overnight at 4 °C. Lastly, the precipitated proteins were collected by centrifugation for 20 min at 9000 RPM (4 °C). The protein content was measured according to the method of Bradford [22]. The Bradford solution (195 μL) was added to 30 μL of extract and 795 μL of H_2O , then the absorbance was measured at 595 nm against blank using UV-VIS spectrophotometer. Results were expressed as mg of BSA (bovine serum albumin) equivalent per gram of dry weight.

2.8. Physiological Weight Loss

Weight loss was expressed as a percentage drop of initial weight of packed mushrooms. In order to calculate weight loss, packages with mushrooms were weighed before and after storage. Obtained results were calculated based on the following equation:

$$\text{Weight loss (\%)} = \frac{W_0 - W_s}{W_0} \times 100, \quad (1)$$

where W_0 is the weight of mushrooms before storage and W_s is the weight of mushrooms after the storage period.

Presented results are averages of six replicates.

2.9. Maturity Analysis

2.9.1. Maturity Index

The maturity index was determined visually, based on a 7-point scale described by Guthrie [23]. Eighteen mushrooms (six mushrooms from each of three packages) from each group were evaluated according to following points: (1) tight veil, (2) stretched veil, (3) veil broken but no more than in half, (4) veil broken in more than half, (5) completely broken veil, (6) gills exposed, (7) flat surface of gills. Visual assessment of mushrooms was carried out on each storage day.

2.9.2. Percentage of Open Capped Mushrooms

The percentage of open capped mushrooms were judged visually based on two criteria: formation of umbrella-like shape in mushrooms caps and veil detachment. The following equation was used to calculate the percentage of open capped mushrooms:

$$\% \text{ Open caps} = \frac{N_o}{N_t} \times 100, \quad (2)$$

where N_o is a number of open capped mushrooms and N_t is a total number of mushrooms in the package.

2.10. Color Change Analysis (L^* , ΔE , BI)

The surface color of the mushroom caps was measured in CIE $L^*a^*b^*$ system using the Konica Minolta chromameter (CR400, Konica Minolta Inc., Tokyo, Japan), calibrated before measurements on a white standard plate. The area of single measurement (eight-millimeter-wide) was illuminated by D65 light source. Eighteen measurements were taken in each group. Obtained results of lightness (L^*), a^* (redness), and b^* (yellowness) were used to calculate the browning index (BI), an indicator of brown color intensity and ΔE , a total color difference between mushrooms from the same group analyzed on different storage days. BI and ΔE were calculated according to the following formulas:

$$\Delta E = \sqrt{(L_s^* - L_0^*)^2 + (a_s^* - a_0^*)^2 + (b_s^* - b_0^*)^2} \quad (3)$$

$$BI = \frac{100 - (x - 0.31)}{0.17}, \quad (4)$$

where $x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}$,

L_0^* , a_0^* , b_0^* color parameters on the first day and L_s^* , a_s^* , b_s^* color parameters measured at the last day of storage.

2.11. Texture Analysis

2.11.1. Penetration Test

The firmness of mushrooms was evaluated by the penetration test performed with the usage of the Instron 5965 Universal Testing Machine (Instron, Norwood, MA, USA) equipped with a 5-mm diameter cylindrical probe. Penetration was conducted at 2 mm s^{-1} speed during the pretest and puncturing to a depth of 8 mm. The maximum force recorded was defined as firmness. The penetration test was performed on twelve mushroom samples from each group on each storage day.

2.11.2. Compression Test

The toughness of the tested samples was analyzed using the Instron 5965 Universal Testing Machine (Instron, Norwood, MA, USA). The test was conducted on uniform probes of 12 mm diameter and 10 mm height, dissected from pilei and compressed by a 4-cm diameter cylinder probe. Each probe was compressed with 5 N contact force of 5 mm s^{-1} speed to a 50% of original disk height. Time between

two compressions lasted 5 s. Springiness was evaluated and defined as the physical capacity of the product to spring back to the primal form. The compression test was performed on twelve mushroom samples from each group on each storage day.

2.12. Sensory Analysis

Sensory analysis was performed by an 8-member panel consisting of employees of the Department of Technique and Food Product Development. Determination of three basic sensory attributes: color, odor, and overall acceptability were assessed based on a 9-point descriptive scale. Color of the mushrooms represented the degree of browning (1—lack of browning, 5—moderate browning, 9—intense browning), odor similarity with typical mushroom scent (9—full characteristic mushroom odor, 5—slightly perceptible, 0—no typical mushroom odor), and overall acceptability with visual acceptance of the mushrooms (9—highly accepted, 5—moderately accepted, 0—unacceptable). Mushrooms were judged at room temperature within 2 hours after package opening, in order to save typical odor. Samples were served on odorless paper plates, coded with three-digit random numbers. Analysis was performed on each storage day.

2.13. Electronic Nose Analysis

The volatile compounds profile of mushrooms was analyzed by Heracles II Electronic Nose (Alpha M.O.S., Toulouse, France), equipped with two columns of different polarity (MXT-5 non polar and MXT-1701 polar). Kovats retention indexes were used to detect main volatile compounds in the samples. The parameters of analysis were set as follows: injector at 200 °C, oven temperature program 60 °C for 2 s, a 3 °C s⁻¹ ramp to 270 °C, isotherm for 30 s at 270 °C, and flame ionization detectors (FID) at 270 °C. Briefly, 1.5 g of cut mushrooms sample was put into 20 mL glass vial and locked with teflon-silicon rubber cap. Each sample was incubated at 45 °C for 10 min (under agitation 8.33 Hz), after that, the autosampler injected 3500 µL of gas from the samples (headspace) to the columns with a rate of 125 mL s⁻¹. Nine samples from each group were analyzed on each storage day.

2.14. Statistical Analysis

In order to determine normality of data distribution, the Shapiro–Wilk test was conducted. Factorial analysis of variance (ANOVA) was performed in case of TPC, DPPH, FRAP, vitamin C, MDA, protein, pH, TSS, color, texture, and weight loss followed by Tukey HSD test. The data of maturity index, percentage of open capped mushrooms, and sensory analysis were subjected to Kruskal–Wallis ANOVA, followed by multiple comparisons of mean ranks. For multifactorial analysis, 95%, 99%, and 99.9% confidence intervals were established, in other cases 95%. Data analysis was conducted using STATISTICA software version 13.3 (StatSoft, Tulsa, OK, USA). AlphaSoft Version 8.0 software was used for principal component analysis (PCA).

3. Results and Discussion

3.1. pH and TSS Analysis

Results of pH and total soluble solids (TSS) of white button mushrooms are presented in Table 1.

As shown, the pH in mushrooms decreased gradually during the entire storage period in all treatment groups. The highest pH drop after storage was noted for the group packed with high oxygen atmosphere and with the film of the highest perforation level (PSF_7000) and for PVC groups. In turn, the least pH decrease was observed for the group packed with a high oxygen atmosphere and film of the smallest level of perforation (PSF_1000). Thus, it can be concluded that the perforation level has an impact on the pH of stored mushrooms. Initial pH of fresh mushrooms was 6.39, similar to previously reported data [12]. However, the pH drop in the presented studies was much more pronounced. Oliveira et al. [12] noted a pH decrease of about 0.3 after 3 days of storage, while in the presented studies, it was 0.7. The difference occurred probably from three factors: high initial oxygen atmosphere,

high level of perforation, and high storage temperature. Elevated oxygen content inside packages favors growth of aerobic bacteria, which produces organic acids. A high perforation level maintains a constant supply of oxygen, while a high temperature favors rapid microbial growth. In the case of TSS, there was no increase observed in any group (Table 1). Moreover, in PSF_1000, PSF_7000, and PVC, a slight decrease was noted. The high content of TSS means that mushrooms overripe. The older the mushrooms are, the more soluble polysaccharides of mushroom tissue are. In turn, higher content of soluble polysaccharides elevates overall content of soluble sugars in analyzed samples (higher TSS values) [24]. In the presented studies, the TSS values were low and did not increase in time. An explanation for this might be a short storage time applied in the experiment.

Table 1. Multivariate analysis. Level of significance of film type and storage time on the parameters: O₂ (oxygen content), CO₂ (carbon dioxide content), WL (weight loss), MI (maturity index), OC (percentage of open caps), L* (lightness, color parameter), BI (browning index), ΔE (color change), F (firmness), S (springiness), SC (color evaluated sensorially), SO (odor evaluated sensorially), SOA (overall acceptability evaluated sensorially).

	Factor		
	Film Type	Storage Time	Film Type × Storage Time
O ₂	ns	***	ns
CO ₂	ns	***	ns
WL	***	***	***
MI	***	***	**
OC	**	*	ns
L*	***	***	***
BI	***	***	***
ΔE	***	***	***
F	ns	***	ns
S	ns	***	ns
SC	***	***	***
SO	*	**	ns
SOA	***	***	ns

Ns-not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

3.2. Gas Composition

The oxygen and carbon dioxide level in each group on each storage day was presented in Figure 1.

A rapid decrease of oxygen inside the packages was observed in all groups after one day of storage at ambient temperature. Oxygen concentration drop from 80% on day 0 to 11% for PSF_7000 and 10% for PCV on day 2. After 5 days of storage, the highest oxygen concentration was noted for mushrooms packed with film of the highest perforation level (PSF_7000). In the PCV group, a high level of O₂ on the last day of storage was also noted. Contrary, in packages sealed with films of a lower perforation level (PSF_1000, PSF_2000, PSF_3500), a small oxygen content was observed throughout the whole storage period (less than 1%). A low oxygen concentration below 2% is dangerous for consumers because of the possible growth of *Clostridium botulinum* [25]. Thus, it can be concluded that permeation rates of gases in these films were not sufficient to balance the dynamics of O₂ consumption and CO₂ production. Obtained results for the PSF_7000 group are in line with these presented by Sun et al. [26]. Additionally, is worth noting that in the mentioned studies, a similar level of oxygen was detected in high oxygen permeable packages but in a much lower temperature (5 °C). According to Barker and Mapson [27], oxygen in high concentration blocks citrate-α-ketoglutarate cycle. Thus, it might be concluded that high oxygen atmosphere conjugated with highly perforated films in packages could inhibit the respiration rate of mushrooms.

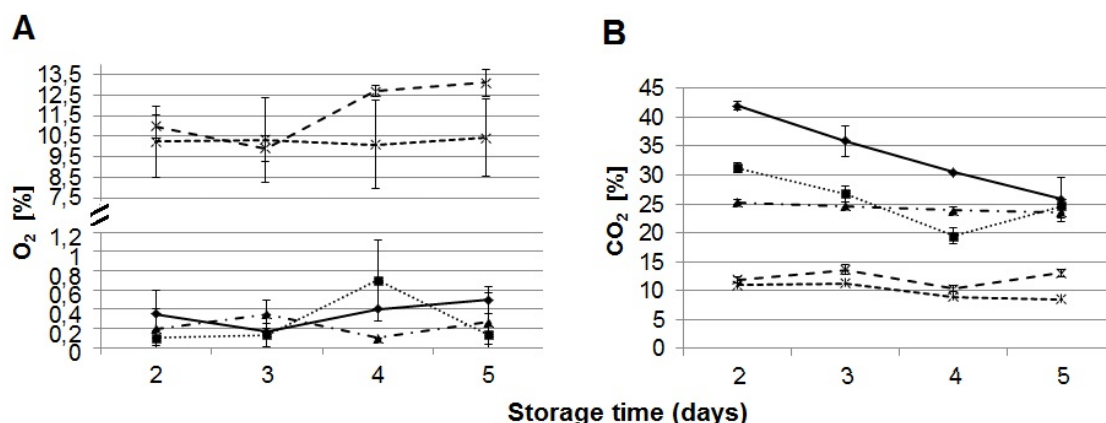


Figure 1. Oxygen (A) and carbon dioxide (B) concentrations in mushrooms packed with PVC (polivynyl chloride) film and PSF (polysulfone) films of different perforation level, stored at 20 °C for 5 days. Standard error is presented in a form of vertical bars. The results of each treatment group are differentiated with the usage of signs as follows: ♦ PSF_1000, ■ PSF_2000, ▲ PSF_3500, ● PSF_7000, × PVC.

3.3. Total Phenolics and Antioxidant Capacity

Phenolic compounds content through the storage in each treatment group was presented in Figure 2. In each group, there was a same upward trend observed in phenolic accumulation in mushrooms. On the last day of storage, a significant difference in TPC was noted between groups. The highest phenolic content was observed for group packed with high oxygen atmosphere and film of moderate level of perforations (PSF_3500). In turn, mushrooms packed with a high oxygen atmosphere and film of the smallest level of perforation contained the least polyphenols.

Perforation promoted accumulation of phenolic compounds. In most studies, an increase of polyphenols in the first storage period is observed, but usually after three to five days, the rapid drop is reported [28]. In our study, there were no decrease observed. The lack of polyphenol loss in mushroom tissue after 5 days of storage could have been caused by the high storage temperature. Zhang et al. [29] noted that after a mild heat treatment, a total content of polyphenols increased, and polyphenol oxidase enzyme decreased.

DPPH and FRAP methods are commonly utilized to evaluate the antioxidant capacity of plant extracts. Figure 2 shows changes in antioxidant capacity (AC) of fresh white button mushrooms. The initial value of DPPH in mushrooms was 7.42 mg of ascorbic acid (AA) equivalent per gram of dry weight and FRAP was 5.46 mg AA g⁻¹ dw. Application of packaging with a high oxygen modified atmosphere had no effect on antioxidant capacity of mushrooms. Contrary, the effect of film type used for mushroom packaging on its antioxidant capacity was observed. On the last day, mushrooms packed with a high oxygen atmosphere and film of the lowest level of perforation was characterized by the lowest antioxidant capacity measured both by FRAP (4.18 mg g⁻¹ dw) and DPPH (6.48 mg g⁻¹ dw) methods. Other groups had similar results of AC after storage.

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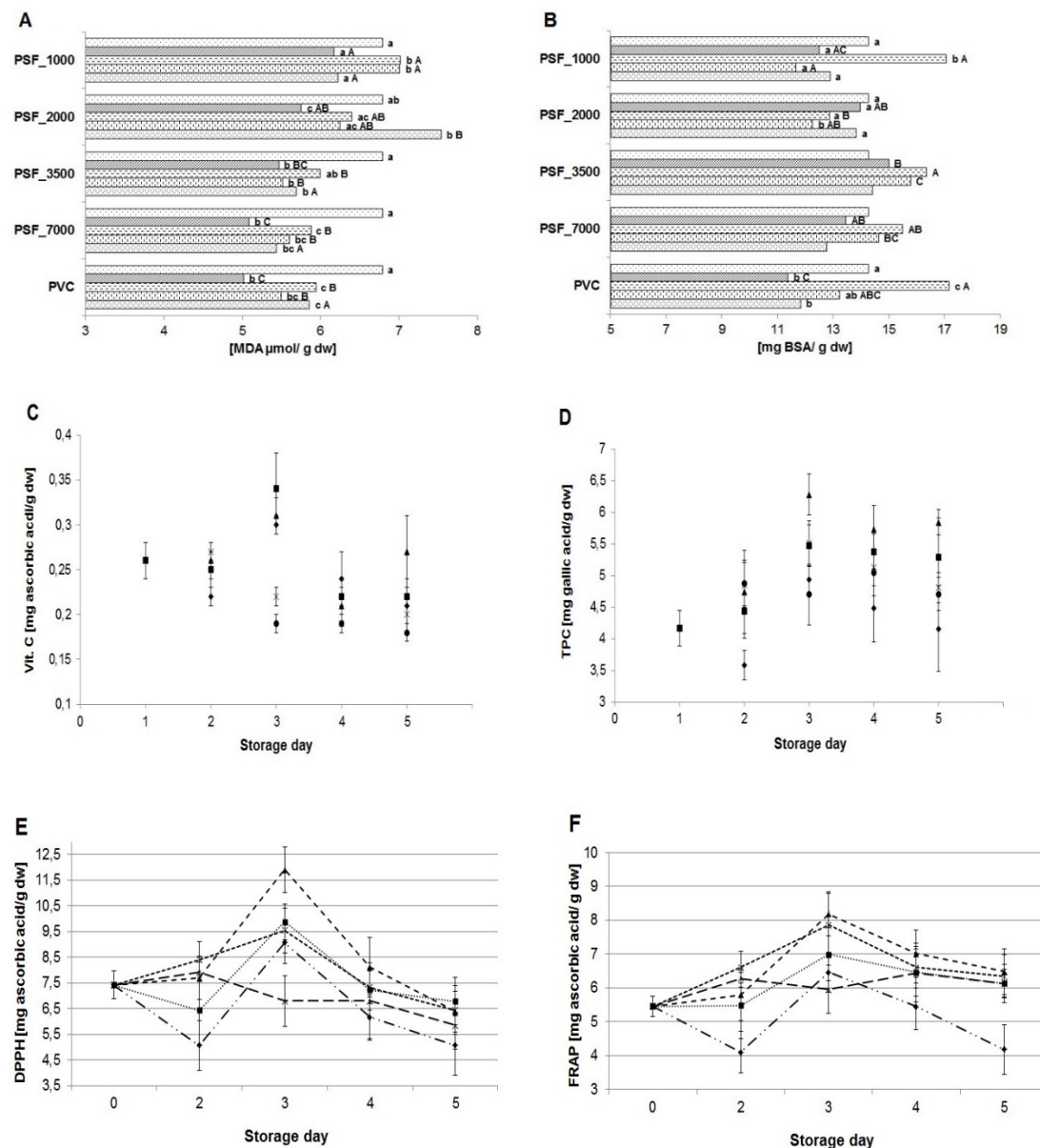


Figure 2. Protein, malondialdehyde, vitamin C, total polyphenols content, and antioxidant capacity (DPPH, FRAP) measured in mushrooms packed with PVC film and PSF films of different perforation levels, stored at 20 °C for 5 days. Standard error is presented in a form of vertical bars. For (A,B) diagrams, bars filled with different patterns differentiate the results according to the day of storage as follows: □ d0, ▒ d2, ▒ d3, ▒ d4, ▒ d5. For (C–F), diagrams results of each treatment group are differentiated with the usage of signs as follows: ♦ PSF_1000, ■ PSF_2000, ▲ PSF_3500, ● PSF_7000, × PVC. a,b,c—small letters mark differences between the days of storage within the studied group, A,B,C—big letters mark differences between treatment groups within the same storage day.

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lowest antioxidant capacity measured both by FRAP ($4.18 \text{ mg g}^{-1} \text{ dw}$) and DPPH ($6.48 \text{ mg g}^{-1} \text{ dw}$) methods. Other groups had similar results of AC after storage.

3.4. Vitamin C and Protein Content

Ascorbic acid content in white mushrooms during the storage in each treatment group is depicted in Figure 2. In all groups, with the passage of time, a decrease of vitamin C content was observed with the exception of mushrooms packed with a high oxygen atmosphere and film of a moderate level of perforation (PSF_3500). In turn, the smallest amount of vitamin C on the last day was detected in mushrooms packed with a high oxygen atmosphere and film of the highest level of perforation (PSF_7000). Thus, it can be concluded that constant exposure of mushrooms to a high oxygen atmosphere in packages is detrimental for vitamin C content. Nonetheless, similar low amounts of vitamin C content were detected in mushrooms packed with film of a low level of perforation (PSF_1000). Even though studies document the adverse effect of high concentration of carbon dioxide on vitamin C maintenance in mushroom tissues [30], and in the PSF_1000 group, a high level of this gas was detected in packages throughout the storage. The same high level of CO_2 was shown for PSF_3500. Thus, the perforation seems to be crucial for preservation of a high vitamin C level in mushrooms stored at high temperature. Obtained results showed that both exposure to a high oxygen atmosphere, as well as a low level of perforation in packages, are factors that are responsible for vitamin C decrease in stored mushrooms. The reason for the vitamin C drop in both of those groups was probably that a high oxygen atmosphere and low level of perforation in packaging films contributed to acceleration of oxidative processes which decreased the content of this antioxidant. The results are of great importance given that most of the studies show a decrease of vitamin C with the time of storage, regardless of the applied treatments [31–33]. Even more important is the fact that most of those studies in contrast to our research were carried out at low temperatures. It is commonly established that vitamin C is thermally sensitive, the higher the temperature is, the more vitamin C is decomposed [34].

Both treatment and time did not affect the protein content in mushrooms (Figure 2). Results are in contrast to those showed by Li and Zhang [35] who reported the effect of perforation sizes in packaging films on protein content measured after storage. Similar results were shown by Dhalsamant et al. [36] who noted the effect of perforation on protein content in *Volvariella volvacea* mushrooms. The difference might be caused by the duration of the storage period. It is possible that the time in the presented study was too short to observe any differences.

3.5. Malondialdehyde (MDA) Content

Content of malondialdehyde is commonly utilized as an index of membrane lipid peroxidation. The application of a high oxygen atmosphere while packaging did not have the impact on the MDA level fluctuations between groups, only the effect of perforation level was observed (Figure 2). There was no increase in MDA content at the last storage day in all groups except for the PSF_2000 group. Simultaneously, after five days of storage, the least MDA was detected in the PSF_7000 group. However, worth noting is that in each group, after 2 days of storage, a decrease in the MDA level was observed. This rapid drop of MDA content was probably dictated by the mobilization of the antioxidant system in mushrooms, caused by the shock effect of a high storage temperature. Obtained data are partially in line with those presented by Jiang et al. [37] who found the preservative effect of high CO_2 content inside packages on membrane lipid peroxidation expressed as MDA content. In our study, also MDA content was low in each group until day 2. After that time, the MDA level rapidly increased in groups packed with the films of low level of perforation (PSF_1000 and PSF_2000). This change resulted probably from the mechanical damage of cell membranes, caused by water drops formed inside low perforated packages due to high saturation.

3.6. Weight Loss and Maturity Index

Water loss was observed in each group and advanced linearly with the time of storage (data not shown). The highest percentage of water decrease was observed for the PSF_7000 group on each storage day. In turn, the PVC group was characterized by the lowest drop of water content. Nonetheless, the water loss on the last day was not higher than 1.2% in each group. Thus, obtained results were at an acceptable level, taking into consideration the upper limit of weight loss set by Mahajan et al. [38] at 5%. Decrease of water content above this value causes marked quality deterioration of mushrooms. However, small decrease of water content in each group, especially in the PVC group is determined by the low water transmission rate of the film. In higher temperatures, the respiration rate of mushrooms rapidly increases [11]. It results in excessive water production, saturated conditions, and water condensation inside the packages. This process was observed in the presented study and shown in Figure 3. Water condensation might be eliminated by mushroom packaging with perforated films. Obtained results are in line with those presented by Simón et al. [5].

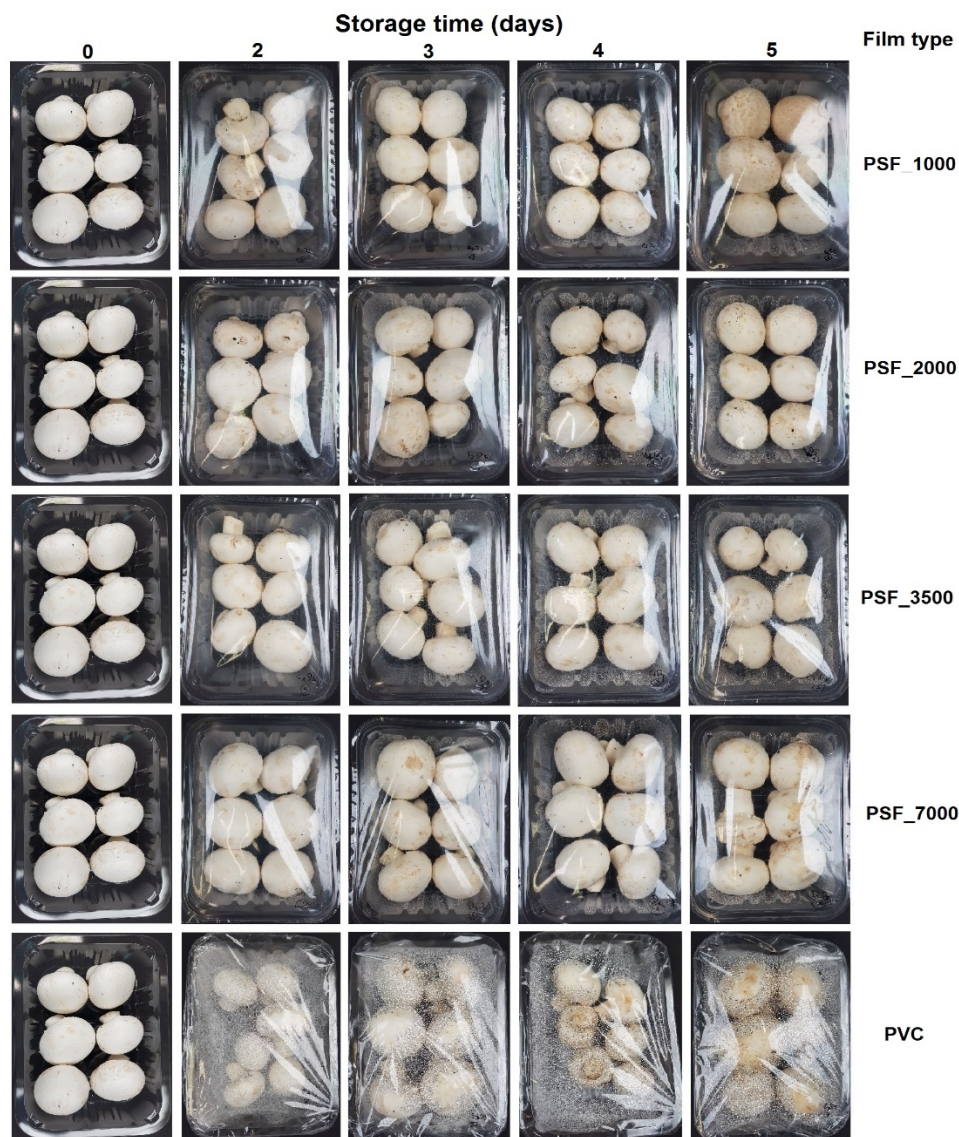


Figure 3. Pictures of mushrooms packed with PVC film and PSF films of different perforation levels, stored at 20 °C. Pictures were taken at each day out of 5 days of storage.

The maturity index (MI) increased slightly during the whole storage period (data not shown). There was no difference in MI values among groups in each storage day. After 5 days of storage, the maturity index in all groups reached 2 points. Similarly, there were no differences in the percentage of open capped mushrooms between groups. Obtained results are comparable to those of González-Fandos et al. [39]. Low maturity indexes and low percentage of open capped mushrooms resulted probably from high CO₂ content [40] lower than atmospheric O₂ pressure [36] and high relative humidity inside packages [41]. Carbon dioxide in high concentration inhibits the mushroom veil opening. In turn, high relative humidity protects mushroom from excessive weight loss and preserves cohesive forces responsible for veil integrity intact.

3.7. Color Change Analysis

Analysis of variance showed a significant impact of film type, storage time, and interaction between those factors on lightness (L*), browning index (BI), and total color difference (ΔE) of white button mushrooms stored at ambient temperature (Table 1). Lightness of mushrooms decreased over storage time in all groups (Table 2).

Table 2. The effect of time x film type on parameters reflecting color change of mushrooms: lightness (L*), browning index (BI), and total color difference (ΔE). Results presented as LS means and SEM.

Film Type	Storage Time (days)	L*	BI	ΔE
NPM*	0	94.32 ^a	-	-
PSF_1000	2	93.54 ^{a,b,c,d}	10.84 ^a	2.98 ^{a,b}
	3	92.57 ^{b,c,d,e}	12.69 ^{a,b}	3.87 ^{a,b,c}
	4	90.61 ^{g,h,i}	17.23 ^{c,d,f}	7.09 ^{d,e,f}
	5	89.4 ⁱ	22.1 ^g	10.67 ^g
PSF_2000	2	93.81 ^{a,b,c}	10.53 ^a	2.05 ^a
	3	92.05 ^{d,e,f,g}	13.08 ^{a,b}	4.21 ^{a,b,c}
	4	90.29 ^{h,i}	17.75 ^{c,d,f}	7.6 ^{e,f}
	5	90.45 ^{g,h,i}	18.89 ^{f,g}	8.49 ^{f,g}
PSF_3500	2	94.08 ^{a,b}	10.87 ^a	2.38 ^{a,b}
	3	92.34 ^{c,d,e,f}	12.54 ^{a,b}	3.78 ^{a,b,c}
	4	91.68 ^{e,f,g,h}	15.3 ^{b,c,d,f}	5.8 ^{c,d,e,f}
	5	89.98 ⁱ	18.28 ^{d,f}	8.35 ^{f,g}
PSF_7000	2	93.48 ^{a,b,c,d}	10.48 ^a	2.57 ^{a,b}
	3	93.35 ^{a,b,c,d}	10.53 ^a	2.33 ^a
	4	92.57 ^{b,c,d,e}	12.36 ^{a,b}	3.48 ^{a,b,c}
	5	92.04 ^{d,e,f,g}	13.99 ^{a,b,c}	4.74 ^{a,b,c,d}
PVC	2	94.33 ^a	10.48 ^a	1.96 ^a
	3	93.38 ^{a,b,c,d}	12.07 ^{a,b}	3.22 ^{a,b,c}
	4	92.41 ^{c,d,e}	14.8 ^{b,c,d}	5.17 ^{b,c,d,e}
	5	90.75 ^{f,g,h,i}	18.31 ^{d,f}	7.98 ^{f,g}
SEM		0.29	0.75	0.56

NPM*—not packed mushrooms (fresh mushrooms analyzed at day 0); ^{a,b,c,d,e,f,g,h,i}—values marked with different small superscript letters within column differ significantly; $p \leq 0.05$.

Consequently, browning index and total color difference have followed the opposite trend and increased. The highest value of L* color parameter on the last day of storage was observed for PSF_7000 group and the lowest for PSF_1000. At the same time in PSF_7000 group was observed much slower browning process than in other groups, especially in PSF_1000 group. Similarly, PSF_7000 was characterized by the smallest ΔE value and PSF_1000 by the highest. Based on obtained results it might be concluded that the level of perforation in film have critical significance for color of mushrooms. Higher film permeability was associated with higher lightness of mushrooms. Those findings are

in line with conclusions made by Gantner et al. [42], who observed whiter mushrooms packed with thinner, more permeable film. All tested groups were characterized by lightness value higher than 80 in each storage day what is a wholesaler's threshold of consumers acceptance [43] and even more than 86 what classifies all groups as of good quality [44]. Nonetheless the highest value for PSF_7000 group means that mushrooms from this group would be chosen most often by consumers, as the white color is most decisive factor for consumers purchase decisions. Similar results were presented by Li et al. [8] who applied high oxygen atmosphere packaging of mushrooms and observed delay in browning process. In our study the effect is even more noticeable referring to the fact that experiment was carried out at room temperature. Low temperature slows down enzymatic activity responsible at ambient temperature for browning of mushrooms.

3.8. Texture Analysis

Texture changes are a sign of *Agaricus bisporus* deterioration [45]. Firmness and springiness of button mushrooms had tendency to decrease over time in all groups, as shown in Table 3.

Table 3. Texture parameters (firmness and gumminess) measured in mushrooms packed with PVC film and PSF films of different perforation level, stored for 5 day at 20 °C. Results presented as LS means and S.E.M.

Film Type	Storage Time (days)	Firmness [N]	Springiness [-]
NPM*	0	14.45 ^d	0.34 ^{a,b,c}
PSF_1000	2	13.13 ^{c,d}	0.32 ^{a,b}
	3	11.45 ^{a,b,c,d}	0.31 ^{a,b,c}
	4	10.72 ^{a,b,c}	0.28 ^{a,b}
	5	10.96 ^{a,b,c}	0.27 ^a
PSF_2000	2	13.71 ^{c,d}	0.40 ^{b,c}
	3	11.55 ^{a,b,c,d}	0.31 ^{a,b,c}
	4	10.88 ^{a,b,c}	0.28 ^{a,b}
	5	9.61 ^a	0.34 ^{a,b,c}
PSF_3500	2	13.60 ^{c,d}	0.41 ^c
	3	11.55 ^{a,b,c,d}	0.35 ^{a,b,c}
	4	11.05 ^{a,b,c}	0.27 ^a
	5	9.60 ^a	0.25 ^a
PSF_7000	2	13.84 ^{c,d}	0.37 ^{a,b,c}
	3	12.99 ^{b,c,d}	0.36 ^{a,b,c}
	4	9.98 ^{a,b}	0.33 ^{a,b,c}
	5	9.96 ^{a,b}	0.31 ^{a,b,c}
PVC	2	12.25 ^{a,b,c,d}	0.36 ^{a,b,c}
	3	11.43 ^{a,b,c,d}	0.35 ^{a,b,c}
	4	11.07 ^{a,b,c}	0.36 ^{a,b,c}
	5	9.57 ^a	0.27 ^{a,b}
S.E.M		0.62	0.02

NPM*—not packed mushrooms (fresh mushrooms analyzed at day 0); ^{a,b,c,d}—values marked with different small superscript letters within column differ significantly; $p \leq 0.05$.

No differences were observed in firmness between groups on any of storage days. Similarly, González-Fandos et al. [25] found no effect of film perforation degree on the texture of stored mushrooms. In turn, Dhalsamant et al. [36] observed correlation between the amount of perforations and firmness of paddy straw mushrooms. Such meaningful differences might occur from conditions applied in each of those experiments: temperature, modified atmosphere composition and size, and number of wholes in packages. In turn, the gradual firmness loss in the presented study resulted probably from several co-occurring factors: inhibition of lignin formation in cell walls, high moisture saturation,

and excessive CO₂ content inside packages. It was proven that MAP treatment can inhibit lignin formation in mushrooms, which are responsible for rigidity of cell walls [46]. Even though carbon dioxide in concentration of about 10% is considered a protectant for mushroom texture, in higher concentration, it is detrimental for its tissue [47].

3.9. Sensory Analysis

Both color and overall acceptability were ranked highest by the panelists in the case of the PSF_7000 group (Figure 4).

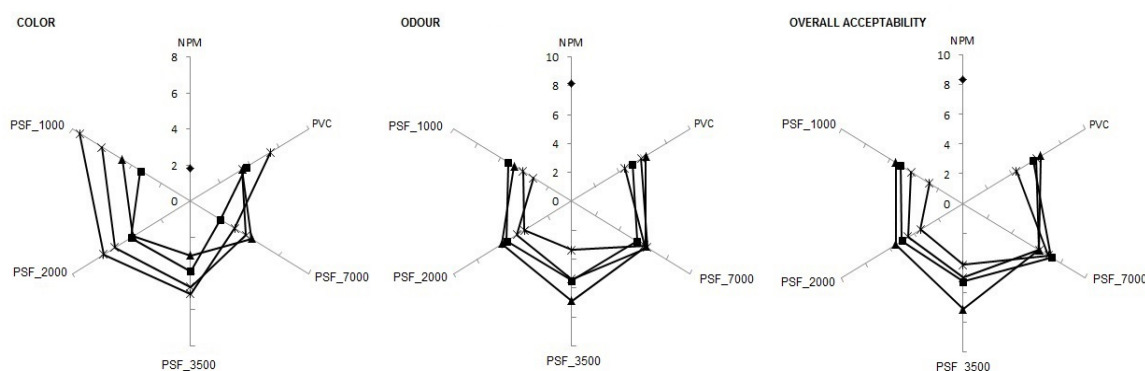


Figure 4. Sensory analysis of color, odor, and overall acceptance measured in mushrooms packed with PVC film and PSF films of different perforation levels, stored for 5 days at 20 °C. Results of each treatment group are differentiated with the usage of signs as follows: ♦ PSF_1000, ■ PSF_2000, ▲ PSF_3500, ● PSF_7000, × PVC.

However, those values were not significantly different from PSF_3500 and PVC groups. The overall acceptability after 5 days of storage in group PSF_1000 was evaluated at a very low level (2.75). In this group, the highest drop of overall acceptability was noted after the whole storage period as well. Differences in odor (in favor of PSF_7000 group) were perceived only on day 4, and they vanished on the last day of storage. Sensorially, the most important parameters for consumer assessment of mushrooms quality are color and appearance, rather than taste or odor [48]. Mushrooms of white, stainless caps are preferred by consumers while shopping [49]. Thus, based on consumer ranks, mushrooms packed with film of the highest perforation level were of best probability to be purchased. Perforations protected mushrooms from color deterioration and kept its high consumer acceptance, which is in line with findings made by Simón et al. [5]. Furthermore, similar results showed Que et al. [50], who noted that the highest O₂ concentration in packages maintained higher sensorial properties of mushrooms stored at a higher temperature. However, obtained results contrast with those of Simón et al. [5] and González-Fandos [25], who found the impact of the film type on the odor of mushrooms. In the mentioned studies, the unpleasant odor was caused by acetaldehyde and ethanol formed in anaerobic conditions.

3.10. Electronic Nose Analysis

A total of nineteen volatile compounds (VC) were identified in the tested samples (Table 4).

In fresh white button mushrooms (day 0), only six compounds were detected. After five days of storage at room temperature in PSF_7000 and PVC groups, a small amount of VC was identified. In turn, in PSF_1000, PSF_2000, and PSF_3500 groups, the amount of VC doubled. Most of the new compounds identified in those groups were aldehydes and alcohols. Visualization of analysis was presented as the PC1/PC2 score plot (Figure 5).

Table 4. Volatile compounds detected in mushrooms packed with PVC film and PSF films of different perforation levels based on comparison of Kovats indexes at first and last day of storage at ambient temperature.

Identified Volatile Compounds	Sensory Descriptors	Day of Storage					
		0	5				
		NPM*	PSF_1000	PSF_2000	PSF_3500	PSF_7000	PVC
1-Butanamine	ammoniacal		+	+			
1-Chloropentane	caramelized	+					
1-propanol	alcoholic	+	+	+	+	+	+
1-Propanol, 2-methyl-	alcoholic		+	+	+		
2-Decanone	citrus	+					
2-methylbutanal	almond		+	+			
2-methylpropanal	burnt	+	+	+	+	+	+
3-hexanone	etheral		+	+	+		
3-pentanol	fruity		+	+	+		
alpha-Pinene	camphor	+		+	+	+	+
Formic acid	acidic	+	+	+		+	+
Furfural	almond		+	+	+		
Methyl propanoate	etheral			+			
Phenol, 3-ethyl-	musty		+	+	+	+	+
Propan-2-one	fruity		+	+	+	+	+
Propanal	etheral		+	+	+		
trans-Carveol	caraway		+	+	+		
Trichloroethane	-		+	+			

NPM*—not packed mushrooms (fresh mushrooms analyzed at day 0).

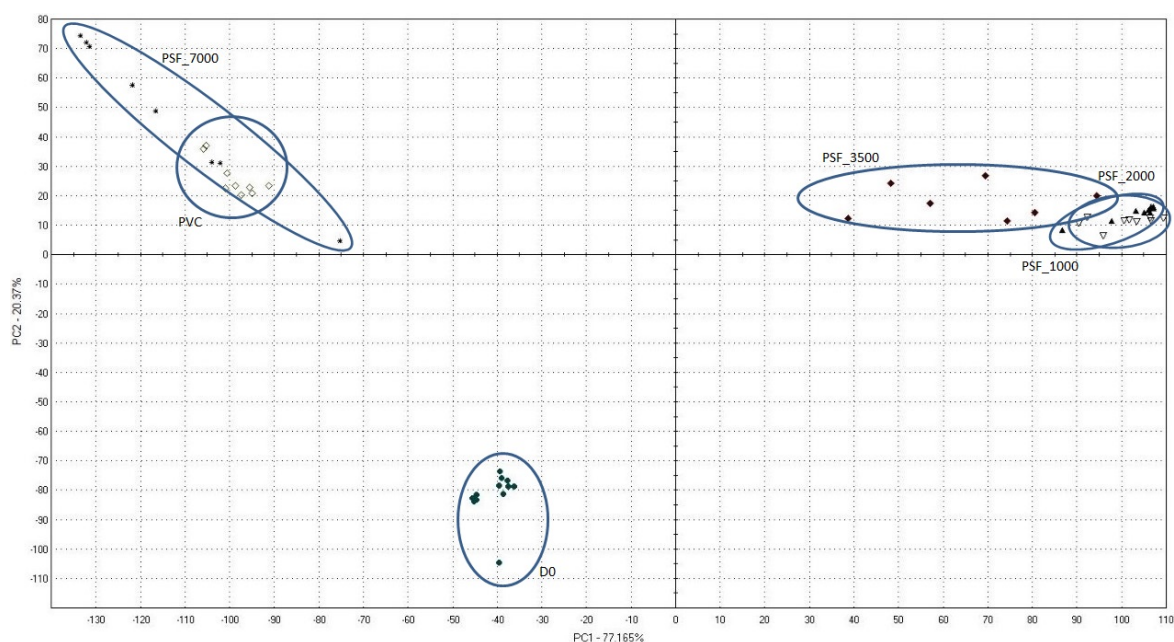


Figure 5. PCA (principle component analysis) of volatile compounds identified in mushrooms packed in PVC film and PSF films of different perforation levels, analyzed at day 0 and after 5 days of storage at 20 °C.

More than 77% of data variance was explained by vertical axis, while horizontal axis explained data in more than 20%. Groups separated by that axis were divided into 3 bigger groups similar in terms of identification of comparable volatile compounds. The first group consisted of fresh mushrooms tested at day 0, second of PVC and PSF_7000 groups, and third of PSF_1000, PSF_2000, and PSF_3500 groups subjected to analysis on the last day of storage. Fatty acids are main sources of volatiles in mushrooms [51]. In turn, aldehydes and alcohols are the products of lipid oxidation [52,53]. Thus, it can be concluded that packaging with film of a low level of perforation led to mushroom deterioration, manifested as an increased level of lipid oxidation products in the tested samples.

4. Conclusions

The aim of the research was to investigate whether a high oxygen atmosphere in conjunction with films of different levels of perforation is suitable to extend the shelf-life of mushrooms stored at ambient temperature. Based on the obtained data, it can be concluded that a high oxygen atmosphere inhibits respiration of mushrooms stored in higher temperatures. The integrative application of a high O₂ atmosphere and film of a high level of perforation maintain the white desirable color of mushrooms. The same effect was observed in sensory analysis. Moreover, the application of a high O₂ atmosphere and film of a high level of perforation inhibited water condensation inside mushroom packages and formation of unfavorable volatile compounds. However, the combination of those factors led to the most rapid pH drop. The single effect of the perforation level in packaging films was noted in the case of total phenolic content, antioxidant capacity, vitamin C content, MDA content, and weight loss of tested mushrooms. However, none of the tested factors exhibited effect on mushrooms' texture, maturity index, or protein content.

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