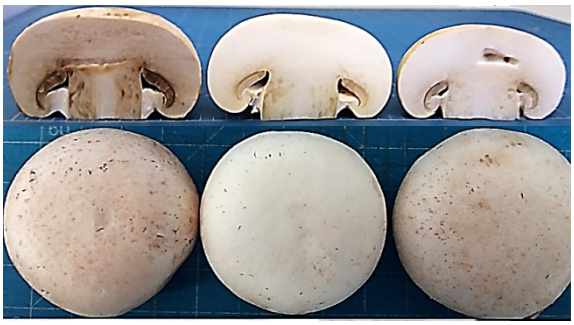
Highlights

- Shelf life extension of button mushrooms was evaluated by a combined approach
- Temperature, packaging material, and modified atmosphere (MA) were investigated
- Tissue softening is avoided for high CO_2 concentration (up to 20%)
- The best performance was obtained for the ‘nano’ packaging with MA at 4°C

Graphical Abstract

After 22 days at 4°C



CONTROL | 'NANO' PACKAGING + MAP | 'NANO' PACKAGING

Shelf life extension of white mushrooms (*Agaricus bisporus*) by low temperatures conditioning, modified atmosphere, and nanocomposite packaging material

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Abstract

In this work, we have explored a new integrated approach for the shelf life extension of button mushrooms (*Agaricus bisporus*). The effect of temperature (4°C and 25°C), packaging configuration (PET/coating/LLDPE oxygen barrier material over conventional PVC stretchable film), and modified atmosphere (15% O_2/5% CO_2/80% N_2 over air) were monitored during 10 days of storage. The influence of a chitosan coating deposited on the cap surface was also investigated. Temperature was the most important factor in preserving the quality attributes of mushrooms over time. The test material had a positive impact on weight loss, cap opening percentage, and firmness of mushrooms compared with the control film (~ 1.0% versus ~ 7.1%; ~ 55% versus ~ 65%; and ~ 10.3 N versus ~ 7.6 N, respectively), which was ascribed to the excellent and good oxygen and water vapor barrier properties of the new material, respectively. Mushrooms packaged under the modified atmosphere behaved decidedly better after a prolonged storage time of 22 days at 4°C. Impressively, after this extended temporal window, the mushrooms looked freshly packed by fully recovering their original color. We explained this striking observation in consideration of the oxygen that permeated the package during these additional 12 days of storage, which would have promoted a gradual resumption of respiratory activity in the overall metabolism of the mushrooms after the "freezing" effect of the rich-CO_2 atmosphere inside the package.

Keywords: button mushroom; modified atmosphere packaging (MAP); nanocomposite coating; PVC; shelf life.

1 Introduction

High nutritional value, sensory properties, medicinal attributes, ease of harvesting, and lower price compared to other mushrooms are the main reasons for the widespread cultivation of *Agaricus bisporus* (also interchangeably known as button mushrooms, white mushroom, and champignon) in many parts of the world, insomuch as it is currently the most cultivated edible mushroom worldwide (Meng et al., 2017; Qin et al., 2015). However, the commercial potential of this type of mushroom is somehow relented by its very short shelf life, which is ~ 3–4 days at room temperature and ~ 8 days under refrigerated conditions (Jiang, 2013). This short shelf life, especially if compared with other fresh vegetables, has a main structural reason: button mushrooms have no cuticle to act as a physical barrier against mechanical damage, water loss, or microbial attack. A high respiration rate and high moisture content contribute to the rapid senescence of button mushrooms, promoting microbial attack and enzymatic browning (Aguirre, Frias, Ryan, & Grogan, 2008). Eventually, color changes, tissue rotting, loss of turgor, off-flavors, and microbial spoilage become the most important quality attributes affecting postharvest storage, marketability at retail stores, and consumers' acceptance.

Different postharvest approaches have been proposed to control (and possibly delay) the rapid quality decay of button mushrooms. First, storage in refrigerated conditions relents overall metabolism, although it has been pointed out that this can also have detrimental effects on product quality, particularly during prolonged storage periods (Lagnika, Zhang, & Mothibe, 2013). Chemical pretreatment of button mushrooms using citric acid, ethylenediaminetetraacetic acid (EDTA), hydrogen peroxide, or sodium hypochlorite has been proposed by several authors, although undesirable changes in the appearance and general quality of the final product may occur (Lagnika, Zhang, & Mothibe, 2013).

Unconventional approaches have also been proposed to slow down the postharvest decay of button mushrooms, such as γ-irradiation (Benoit, D'Aprano, & Lacroix, 2000), ultrasound and high-pressure argon (Lagnika, Zhang, & Mothibe, 2013), pulsed light (Oliu, Aguayo, Belloso, & Fortuny, 2010), UV-c (Wu et al., 2016), gaseous ozone treatments (Akata, Torlak, & Erci, 2015), the use of edible coatings (Jiang, 2013), and antimicrobial and moisture-absorbing active packaging (Qin et al., 2015; Mahajan, Rodrigues, Motel, & Leonhard, 2008).

Packaging plays a crucial role in the control of the rate of mushrooms' senescence. Modified atmosphere packaging (MAP) in particular is a powerful tool to control both microbial growth and physiological effects in mushrooms (Li et al., 2014). In this regard, high CO_2 concentrations have to be discouraged because anaerobic conditions can lead to metabolic disorders and undesirable fermentation resulting in off flavors (Jacxsens, Devlieghere, & Debevere, 2002). Nevertheless, high CO_2 concentrations (95–100%) in combination with ventilation using a new packaging method have been very recently proposed (Lin et al., 2017). Other authors have agreed on optimal recommended atmosphere with low O_2 content (less than 10%) and limited CO_2 content (5% maximum). However, combinations of O_2 and CO_2 are rather difficult to maintain over time because high gas permeability values and high perm-selectivity of packaging materials (i.e., a high ratio between CO_2 and O_2 permeability) would be needed (Guillaume, Schwab, Gastaldi, & Gontard, 2010). High O_2 atmospheres have also been tested for button mushrooms. Liu, Wang, Zhu, & Wang (2010) reported that a high oxygen atmosphere, especially 100% O_2, was a suitable method of storage for button mushrooms, whereas Liu & Wang (2012) demonstrated that mushrooms exposed to high oxygen concentration (80% O_2) had a higher whiteness index and a lower increase in relative electrolyte leakage rate, lipid peroxidation, and ROS ($O_2^{\bullet -}$ and H_2O_2) production indicating less membrane damage.

Both packaging technology and the selection of packaging material can have a dramatic impact on quality. Different materials can be selected in relation to storage conditions (refrigerated or room temperature), type of presentation (whole or sliced), and packaging technology (with or without MAP, type of MAP). Button mushrooms are conventionally packaged in rigid plastic (e.g., polyethylene terephthalate, PET) punnets or foam trays (e.g., expanded polystyrene, EPS) wrapped with PVC film or other stretchable films. However, alternatives have been proposed, such as the use of PET with different degrees of perforation (Taghizadeh, Gowen, Ward, & O'Donnell, 2010), biaxially oriented polypropylene (BOPP) (Xing, Wang, Feng, & Tan, 2008), and materials obtained from renewable resources such as poly(lactic acid) (PLA)/poly(ε-caprolactone) (PCL) blend films (Qin et al., 2015) and wheat gluten-coated paper (Guillaume et al., 2010).

In this work, we have investigated the combined effect of temperature, MAP, and packaging material on the shelf life extension of whole button mushrooms. In particular, we have selected an innovative bio-hybrid packaging material based on a "nano" technology with super oxygen barrier properties, but permeable to CO_2 to a certain extent, in both dry and refrigerated conditions. We have also decided to test a relatively high O_2 concentration (three times higher than CO_2). The rationale underlying this approach is that such configuration would allow mushrooms to preserve their original quality attributes for a long time due to a twofold effect: at the beginning, the high oxygen concentration inside the package would act as a reservoir for the metabolism of the mushrooms; in a second step, as soon as the metabolism of the mushrooms decreases, the CO_2 accumulation inside the package would act as a preservative against the detrimental decay reactions that would otherwise impair mushrooms' marketability. Nonetheless, due to the increasing demand for replacing chlorine-based materials (such as PVC) with less impacting materials (PVC poses serious concerns due to the production of dioxin during incineration), the

93 use of an alternative packaging configuration can also be seen in terms of environmental and
94 consumers' health impact.
95 The effect of the deposition of a chitosan coating on the mushrooms' surface was also
96 investigated. To the best of our knowledge, a similar approach has never been reported.
97
98 **2 Materials and methods**
99 2.1 Materials
100 Button mushrooms were kindly supplied by Fungorobica srl (Cenate Sotto, Italy). Mushrooms
101 from second flush and at the closed cap stage were carefully selected according to a uniform shape
102 and size (cap size of 40–50 mm diameter). Samples were then stored at 4 °C and 75 ± 2% RH for
103 24 h before analyses.
104 A PET/coating/LLDPE film was used as a test packaging material. It was obtained by first
105 depositing an oxygen barrier coating (0.5 µm thick) onto the 12 µm thick corona-treated PET film
106 (Metalvuoto spa, Roncello, Italy), and then laminating the coated PET with the 60 µm LLDPE
107 layer (Metalvuoto spa, Roncello, Italy) by means of a double-component polyurethane adhesive
108 (AD 737, Novachem Industriale, Legnano, Italy). The oxygen barrier coating has been obtained
109 according to the procedure reported in detail in our previous work (Introzzi et al., 2012). Briefly,
110 it consists in a bionanocomposite coating made of a main biopolymer phase (the exopolysaccharide
111 pullulan) that intercalates an inorganic filler (natural cloisite). Pouches 30 cm × 20 cm (Figure 1a)
112 were prepared using a thermal heat sealer Polikrimper TX/08 (Alipack, Pontecurone, Italy),
113 provided by smooth bars at 130 °C for 0.5 s and 4.5 bar pressure. PVC (11 µm thick) stretchable
114 film and EPS trays (Fungorobica srl, Cenate Sotto, Italy) were used as a control packaging
115 configuration (Figure 1b). Permeability properties of both materials against oxygen, carbon

dioxide, and water vapor, expressed as oxygen transmission rate (OTR), carbon dioxide transmission rate (CO_2TR), and water vapor transmission rate (WVTR), respectively, are reported in the Table S1 of Supporting Information.

Chitosan powder from crab shells (degree of deacetylation: 75–85%; molar mass distribution: 190,000–310,000; viscosity range: 200-800 cP, 1 wt. % in 1% acetic acid at 25 °C by Brookfield method) was purchased from Sigma Aldrich (Milano, Italy) and used without further purification.

2.2 Methods

2.2.1 Modified atmosphere packaging (MAP) samples.

Mushrooms under MAP were packaged using a tabletop vacuum packaging machine E100 (Tecnovac srl, Grassobbio, Italy), fitted with a gas mixer MAP Mix 9001 (Mocon Dansensor srl, Segrate, Italia), with the following gas composition: 15% O_2, 5% CO_2, 80% N_2. The evolution over time of the gas composition inside the package was monitored every two days using a Hewlett Packard 5890 Series gas chromatograph mounting a single TCD detector.

2.2.2 Coating preparation

A master solution was prepared by dissolving 5 g of chitosan in 495 g distilled water at pH 4. Mushrooms were dipped in the chitosan solution for 2 min and then placed at room temperature for 2 h on grid trays to allow excess solution to drip off and the coating to form.

2.2.3 Experimental plan

We packaged 100 g of both coated and uncoated mushrooms in three different packaging configurations: (i) control (EPS trays and stretchable PVC film); (ii) test film (PET/bionanocomposite coating/LLDPE); and (iii) test film under MAP. All packages were stored at both 4°C and 25°C in climatic chambers. Samples were analyzed every two days for a time span of 10 days.

2.2.4 Analyses

2.2.4.1 Weight loss, pH, and TSS.

Weight loss was determined by weighing the mushrooms before and during the storage period and calculating the percentage loss according to the following equation:

$$Weight\ Loss\ (\%) = \frac{W_0 - W_t}{W_0} \times 100 \quad (1)$$

where W_0 is the initial weight of the mushroom and W_t the weight at time t (with t = 2, 4, 6, 8, and 10 days).

For the pH and total soluble solids (TSS) determination, mushrooms were homogenized for 10 s using a DI 25 Basic homogenizer (Ika-Werke, Stanfen, Germany) at a speed of 8000 rpm and squeezed with a hand press. The resulting juice was filtered using Whatman® quantitative circle (Ø 125 mm) grade 40 ashless filter paper (Sigma-Aldrich, Milano, Italy). The pH and TSS were determined at 25°C using a pH-meter (mod. Basic 20+, Crison, Barcelona, Spain) and a digital refractometer (Atago-PLA1, Tokyo, Japan), respectively.

2.2.4.2 Thermal imaging.

The thermal profile of mushrooms during storage was obtained by an infrared thermograph system made of a thermal imaging camera FLIR T420 (Biofotonica srl, Roma, Italy) with the following

161 characteristics: field of view (FOV): 25° × 19°; thermal sensitivity: < 0.045°C at 30°C; frame rate:
162 60 Hz; detector: focal plane array (FPA) with spectral range of 7.5 µm–13 µm and 320 × 240 pixel
163 resolution; display LCD 3.5 inches. For consistency, thermal images of the cap surface of whole
164 mushrooms were always taken in the afternoon at 3 p.m., in the same laboratory at 25 ± 0.5°C and
165 using the same setup (i.e., light exposure/background, stage, and operator). The emissivity of
166 mushrooms was set at 0.98 like other biological products (Buera, Lozano, & Petriella, 1986). Three
167 replicates were observed for each storage time. For each replicate, the surface temperature was
168 recorded at three different random locations.
169
170 2.2.4.3 Color.
171 The surface color of mushroom caps was measured with a UV/VIS spectrophotometer (Lambda
172 650, PerkinElmer, Waltham, USA) coupled with an integrative sphere (Ø = 150 mm). The
173 reflectance spectra of the mushrooms were collected between 800 nm and 380 nm and the CIE
174 $L^*a^*b^*$ coordinates were eventually obtained using the software Color v5 (PerkinElmer, Waltham,
175 USA). The L^* (light/dark), a^* (red/green), and b^* (yellow/blue) values of each mushroom cap
176 were monitored throughout 10 days so that the color change (ΔE) and browning index (BI) were
177 evaluated by the following equations (Jiang, 2013):

178 $$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (2)$$

179 $$BI = \frac{100(x - 0.31)}{0.172} \quad (3)$$

180 where:

181 $$x = \frac{a + 1.75L}{5.645L + a - 3.012b} \quad (4)$$

The browning index represents the purity of brown color and is reported as an important parameter in processes where enzymatic or nonenzymatic browning takes place (Palou, Malo, Canovas, Chanes, & Swanson, 1999).

2.2.4.4 Cap opening percentage.

According to Jiang (2013), criteria for judging the percentage of open caps were based on the development of umbrella-like shape of the cap followed by failure of the veil. The open caps percentage was determined according to the following relationship:

$$Open\ caps\ (\%) = \frac{N_{oc}}{N_t} \times 100 \quad (5)$$

where N_{oc} = number of open capped mushrooms and N_t = total number of mushrooms.

2.2.4.5 Mechanical properties.

A penetration test was carried out on cylindrical specimens (20 mm height) cored from the mushroom caps by a spoon soil auger (Ø = 25 mm) according to the so-called puncture test method using a large deformation analysis dynamometer (mod. Z005, Zwick Roell, Ulm, Germany) fitted with a 4 mm diameter cylindrical probe. Specimens were punctured up to 5 mm in depth at a cross-head speed of 5 mm s^{-1} using a 100 N cell load. "Force versus time" plots were recorded and firmness was gathered as the first maximum force peak. The software TestXpert V10.11 Master was used for data analysis.

2.2.4.6 Statistical analysis.

The influence of the independent variables (type of material and presence of the coating) on each parameter monitored over 10 days was statistically assessed by one-way analysis of variance

205 (ANOVA) using SPSS software (IBM SPSS Statistics for Windows, Version 19.0., Armonk, NY).
206 The mean values, where appropriate, were compared by Duncan's test with significance level (p)
207 < 0.05.
208
209 **3 Results and discussion**
210 3.1 Effect of temperature
211 The effect of temperature was investigated by storing the different sample batches (namely, under
212 MAP and without MAP, using conventional packaging configuration and the test one, coated and
213 uncoated) at 25°C and 4°C. Remarkably, not all the samples stored at the higher temperature
214 exceeded the fourth day of shelf life, irrespective of the specific technology adopted (MAP,
215 material, coating). At the fourth day of storage, color changes, texture failures, and beginning of
216 microbial spoilage were so evident that any attempt to prolong the experimentation would have
217 been useless. Because the strategies used did not bring any remarkable advantage over
218 conventional packaging conditions, we decided to focus on the part of the work related to the
219 samples stored at 4°C.
220
221 3.2 Evolution of the headspace gas composition
222 Changes in O_2 and CO_2 concentrations inside control and test packaging solutions are displayed
223 in Figure 2. At the beginning (day 0) control and test (no MAP) configurations exhibited the typical
224 ambient atmosphere composition, whereas the test configuration (MAP) showed 15% O_2, 5% CO_2,
225 and 80% N_2, indicating successful packaging operation (Figure 2a,b). Yet after one day of storage,
226 O_2 concentration decreased in all three packages due to the mushrooms' respiration (Figure 2a).
227 However, the extent of such decreases varied according to both the specific packaging material

and inner atmosphere. More specifically, O_2 decreased slightly in the control packaging because O_2 consumption was partly counterbalanced by the O_2 transfer inside the package due to the poor oxygen barrier properties of the PVC stretchable film. In both test packaging configurations without and with MAP, the O_2 consumption was not observably counterbalanced by any permeation phenomena owing to the excellent O_2 barrier properties of the PET/nano coating/LLDPE film. The difference seen between these two samples can be explained in light of the initial amount of oxygen inside the packages (21% and 15% without and with MAP, respectively). As also observed by Iqbal, Rodrigues, Mahajan, & Kerry (2009), equilibrium was apparently reached in the three configurations after only 2 days of storage, which is typical when gas diffusion through the film exactly compensates O_2 consumption and CO_2 production by mushrooms (Floros & Matsos, 2005). At equilibrium, O_2 concentration was 16–18% in the control package, 1–1.5% in the test (no MAP) configuration, and no detectable O_2 in the MAP test configuration. Here, we have noticed a subtle increase at the 8th and 10th day of storage, which could be a first sign of oxygen permeation through the package not yet consumed by the mushrooms.

In contrast to O_2, CO_2 concentration in the control packaging increased slightly during the 10 days' storage time due to the combined effect of permeation and respiration, achieving ~ 5% at the 10th day (Figure 2b). The increase was remarkably higher in the test packaging material, again due to the good barrier properties of this material toward CO_2, which is produced during respiration and cannot escape the package. Noticeably, the difference in the CO_2 concentrations in the test packaging configurations without and with MAP tended to decrease until almost resetting at the end of the storage time, reflecting the decrease in the intensity of respiration as time went by.

From a statistical point of view, both storage time and type of packaging configuration affected significantly the gas composition (O_2 and CO_2 concentrations), while the deposition of the chitosan coating did not (Table S2).

3.3 Weight loss, pH, and TSS

Loss of water during storage is one of the primary factors of degradation in mushrooms, causing detrimental effects such as tissue shrinkage resulting in excessive weight loss (Lagnika, Zhang, & Mothibe, 2013). As shown by other authors (Guillame et al., 2010), weight loss occurred continuously with time regardless of the type of packaging material employed, though to different extents for the three packaging configurations (Figure 3a). After 10 days, the highest weight loss occurred in the mushrooms packaged in the EPS trays wrapped with PVC film, with the coated mushrooms losing the most moisture (~ 9.3% compared to 7.1% for the uncoated samples). The test film behaved decidedly better, with no statistical difference between mushrooms packaged in air (~ 1.0%) or with a modified atmosphere (~ 0.84%).

These values were below the limit of acceptance of 5% found by Mahajan, Oliveira, Montanez, & Frias (2007). The results were confirmed by the thermal images of mushrooms during storage. After 10 days, the lowest average surface temperature was recorded for the mushrooms packaged using the PVC (control) film, whereas the highest one pertained to the samples packaged in the PET/nano coating/LLDPE film (12.23 ± 0.26 °C and 13.91 ± 0.34 °C, respectively) (Figure 4). As reported by Veraverbeke et al. (2006), lower surface temperature of fruits and vegetables with initial high moisture content is explained by the higher moisture losses and transpiration rates at the surface. Because weight loss is associated with both loss of water from the package to the surrounding atmosphere and to the loss of carbon upon formation of CO_2

during respiration (Kim, Ko, Lee, Park, & Hanna, 2006), the superior performance of the PET/nano coating/LLDPE film over the PVC film is plausibly due to the lower WVTR and CO_2TR (see Table S1 of Supporting Information), which contributed to reduce the vapor and gas pressure difference across the packaging film.

Changes in pH and TSS in button mushrooms are shown in Figures 3b and 3c, respectively. Both parameters increased slightly in all packaging materials, similarly to Jiang (2013) and Tao, Zhang, Yu, & Sun, (2006). The highest pH percent increase over the 10 days of analysis was recorded for the samples packaged in the conventional configuration (~ 9% increase), whereas the increase recorded for the mushrooms packaged with the nano film was ~ 3%, irrespective of the modified atmosphere. Statistical analysis confirmed the effect of the package in preserving the original pH of mushrooms, whereas neither the internal atmosphere nor the presence of the chitosan coating affected significantly this parameter. Similar results were obtained for the TSS analysis. Here, however, it should be noted that no statistical difference was observed in the TSS value between the first and the last day of analysis (i.e., after 10 days) for the mushrooms stored using the test packaging material in the presence of the modified atmosphere. This relevant result can be explained in consideration of both decreased respiration rates (which relent the synthesis and use of metabolites resulting in lower TSS due to the slower hydrolysis of carbohydrates to sugars) and less pronounced senescence (the solubilization of the cell wall polysaccharide and hemicelluloses is higher in senescent mushrooms) (Jiang, 2013).

3.4 Color

Changes in color and browning are primary postharvest issues for mushrooms' commercialization because these parameters most affect consumers' acceptance (Liu & Wang, 2012; Khan et al.,

296 2014). For this reason, measurement of total color variation (ΔE), lightness (L^*), and browning
297 index (BI) is crucial to predict the potential suitability of new preservation strategies for marketing
298 purposes. At first glance, the negative effect of the chitosan coating on the overall color properties
299 of mushrooms can be noted (Figure 5). Indeed, the presence of the coating led to an increase in
300 ΔE, a decrease in L^*, and an increase in BI, which can be ascribed to the inherent yellowish color
301 of chitosan.

302 Referring to the uncoated samples, an increase in ΔE, a decrease in L^*, and an increase in
303 BI were observed during the 10 days of storage for the mushrooms packaged according to the three
304 different configurations. There was no significant difference between samples concerning both ΔE
305 (14.57 ± 3.89, 12.21 ± 4.67, 14.49 ± 3.50 for control and test samples without and with MAP,
306 respectively) and L^* (85.67 ± 1.80, 89.38 ± 2.96, 89.96 ± 3.39). Based on the L^* values, and
307 according to the classification proposed by Gormley (1975), the quality of mushrooms at the end
308 of the storage time can be deemed good ($L^* > 86$) and fair ($80 < L^* < 85$).

309 Surprisingly, there was a significant difference between control and test packaging materials
310 (regardless of the presence of a modified atmosphere) as far as the BI was concerned, with the
311 highest values recorded for the mushrooms packaged with the test material. The reason for this
312 result can be found in the internal atmosphere, as the CO_2 concentration was much higher
313 compared with the control sample soon after the first day of storage. The deleterious effect of CO_2
314 was already reported by Lin et al. (2017), who concluded that high CO_2 concentrations could cause
315 damage to the mushroom cap surface tissue, resulting in high BI values. However, because the
316 enzymatic browning occurs in the presence of oxygen (Jiang, 2013), it is plausible that the
317 increased BI observed for the samples packaged with the nano film had nothing to do (at least
318 directly) with the enzymatic browning. The same authors (Lin et al., 2017) observed an opposite

319 effect as the time went by; namely, the BI was lower for the samples treated with high CO_2
320 concentrations compared with the control. The mushrooms used in this work experienced
321 somehow the same phenomenon. Indeed, at the end of the 10th day we decided to keep the
322 packaged mushrooms in the refrigerated chamber. Surprisingly, after 12 additional days (i.e., 22
323 days of storage total) mushrooms packaged using the test material were unequivocally better than
324 those packaged using the PVC film, with the best performance apparently belonging to the MAP
325 samples, which seemed to recover completely their original color. This can be clearly observed in
326 Figure 6. We hypothesize that after 22 days the amount of oxygen accumulated inside the package
327 was sufficiently high to prompt a renewed respiratory activity in the mushrooms. A similar effect
328 was reported by Briones et al. (1992) when white mushrooms were placed again in normal air after
329 exposure to CO_2 concentrations higher than 5% at 10°C. Although further investigation is
330 necessary to confirm these results, it is the first time that a shelf life of 22 days at 4°C has been
331 reported for button mushrooms. A shelf life of up to 8 days at 4°C has been reported for the button
332 mushroom (Borchert et al., 2014).
333
334 3.5 Cap opening percentage
335 The opening of the cap during storage can be considered as a maturity/freshness index of
336 mushrooms and is due to the loss of internal moisture. Consequently, the higher the water loss, the
337 drier become the tissues and more rapidly the caps and veil will lose their original integrity. The
338 percentage of cap opened during storage increased in all the treatments and was higher in
339 mushrooms packaged in the control film (PVC). In particular, after 10 days of storage the cap
340 opening percentage for uncoated samples was approximately 65% in the control mushrooms, 58%
341 in the test samples under air, and 51% in the test samples packaged using a modified atmosphere

342 (see Table S3). These results are in line with those of the weight loss discussed above, thus
343 confirming the positive impact of the test packaging material in terms of barrier properties
344 (WVTR, in this case). However, the high CO_2 concentration in the test packages could also have
345 played a role. As reported by Briones et al. (1992), high CO_2 concentrations are necessary to slow
346 down cap opening.
347
348 3.6 Mechanical properties
349 Button mushroom texture is one of the most important attributes contributing to consumer
350 satisfaction (Khan et al., 2014). The textural properties of mushrooms during storage are subjected
351 to a depletion driven by both enzymatic activity and water loss. Therefore, both softening and loss
352 of turgor of the tissues are widely reported by other authors, irrespective of the packaging and
353 storage conditions (Qin et al., 2015; Liu et al., 2010; Guillame et al., 2010; Khan et al., 2014).
354 Firmness (expressed as maximum force in a typical penetration test) is the most widely used
355 attribute to define the quality of button mushrooms. That is, the higher the firmness, the better the
356 textural quality of mushrooms.
357 As expected, firmness decreased after 10 days in mushrooms packaged using the control
358 material, from ~ 7.3 N to ~ 4.2 N (Figure 7). In contrast, mushrooms packaged with the test
359 material did not experience any significant loss in firmness (i.e., their original textural attributes
360 were almost unaltered).
361 In particular, firmness varied from 7.62 ± 1.32 N to 9.12 ± 1.12 N in mushrooms packaged
362 in air, and from 10.32 ± 0.71 to 10.37 ± 0.74 in mushrooms packaged under modified atmosphere.
363 To our knowledge, this is the first time that a similar result was achieved by only the use of
364 packaging technologies. Although surprising, it was not totally unexpected. Based on previous

365 works, high CO_2 concentrations seem to play a key role in preserving the textural properties of
366 mushrooms (Briones, et al., 1992; Briones, Varoquaux, Bureau, & Pascat, 1993; Fandos, Olarte,
367 Gimenez, Sanz, & Simon, 2001; Simon, Fandos, & Tobar, 2005). Moreover, the better water vapor
368 barrier properties of the test material over the control film most likely played a role, by reducing
369 moisture loss over time and, in essence, slowing down mushrooms' aging.
370
371 **4 Conclusions**
372 Shelf life extension of button mushrooms has been achieved by simultaneous use of low
373 temperatures, an innovative packaging material, and a modified atmosphere. While the use of an
374 oxygen barrier material with good permeability properties against CO_2 and low permeability to
375 water vapor showed much better performance over the conventional PVC film, the use of MAP
376 (15% O_2 and 5% CO_2) provided an extra benefit especially in terms of quality decay (e.g., in terms
377 of overall appearance and weight loss).
378 The approach presented in this study represent a promising alternative to conventional
379 storage of white mushrooms. However, to confirm the importance of these results, additional tests
380 will follow this first set of experiments. In particular, quantification of the respiration rate, enzyme
381 assay (for the analysis of enzyme activity), malondialdehyde (MDA) content analysis (MDA is the
382 main product of membrane lipid peroxidation), polyphenoloxidase (PPO) and peroxidase (POD)
383 activity, antioxidant potential, and total phenolic content (all of them influencing the rate of
384 enzymatic browning in the mushrooms) would be of help to unravel the basic mechanisms
385 underlying the combined effect of temperature/packaging/MAP. Microbiological and sensory tests
386 will instead provide the necessary information on safety and consumers' perception.
387

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Captions to Illustrations

Figure 1. Button mushrooms packaged in the bionanocomposite-based laminate/EPS tray (a) and in the conventional PVC stretchable/EPS tray configuration (b) tested in this study.

Figure 2. Oxygen (a) and CO_2 (b) percentage evolution inside the control (PVC stretch film – EPS tray) and test (PET/coating/LLDPE film – EPS tray) packaging configurations with and without MAP of coated and uncoated mushrooms stored at 4°C for 10 days.

Figure 3. Weight loss (a), pH (b), and total soluble solids (TSS) (c) of mushrooms uncoated and coated with chitosan biopolymer film, packaged using the control (PVC stretch film – EPS tray) and test (PET/coating/LLDPE film – EPS tray) configurations, with and without MAP, at 4°C for 10 days.

Figure 4. Examples of thermal images captured on mushrooms packaged with the test (PET/coating/LLDPE) material (a) and the control (PVC) film at the 10^{th} day of storage at 4°C.

Figure 5. Color changes (ΔE) (a), lightness (L^*) (b), and browning index (BI) (c) of mushrooms uncoated and coated with chitosan biopolymer film, packaged using the control (PVC stretch film – EPS tray) and test (PET/coating/LLDPE film – EPS tray) configurations, with and without MAP, at 4°C for 10 days.

Figure 6. Surface (up) and cross-section (down) digital camera images of uncoated mushrooms packaged using the control film (PVC stretch film) (a), the test film (PET/coating/LLDPE film – EPS tray) with (b) and without (c) MAP after 22 days of storage at 4° C.

Figure 7. Maximum force (F_{max}) of mushrooms uncoated and coated with chitosan biopolymer film, packaged using the control (PVC stretch film – EPS tray) and test (PET/coating/LLDPE film – EPS tray) configurations, with and without MAP, at 4°C for 10 days.

Supplementary Material

Table S1. Oxygen, carbon dioxide, and water vapor transmission rate of control and test films at different experimental conditions.

Material	Analysis				
	OTR (cm^3 STP/m^2 24h)		CO_2TR (cm^3 STP /m^2 24h)		WVTR (g/m^2 24h)
	23°C 0% RH	23°C 65% RH	23°C 0% RH	23°C 65% RH	23°C 90% RH
Control[a]	4.85	> 7,500	11.12	> 18,000	148.47
Test[b]	< 0.05	0.302 ± 0.115	5.69	144.67	1.27

[a] PVC 11 μm thickness

[b] PET/coating/LLDPE 72.5 μm thickness

OTR and CO_2TR tests were conducted at 1033 mbar ambient pressure and at 1 atm oxygen partial pressure difference on the two sides of the specimen, with and carrier gas (N_2) flux of 70 mL/min using a MultiPerm permeability analyzer (Permtech Srl, Lucca, Italy) equipped with an electrochemical sensor.

Table S2. Mean values for the ten days period of O_2 and CO_2 atmospheric composition, weight loss, pH, total soluble solids (TSS), lightness (L^*), color variation (ΔE), browning index (BI), cap opening (%), and maximum force (F_{max}) of button mushrooms for different packaging configurations.

Treatment	O_2 (%)	CO_2 (%)	Weight loss (%)	pH	TSS (%)	L^*	ΔE	BI	Cap opening (%)	F_{max} (N)
Control	17.30 ± 0.16^{a}	3.81 ± 0.19^{a}	4.34 ± 0.53^{a}	6.82 ± 0.09^{a}	5.18 ± 0.25^{a}	88.49 ± 2.72^{a}	8.70 ± 1.93^{a}	14.98 ± 3.23^{a}	27.50 ± 2.15^{a}	5.71 ± 0.82^{a}
Nano	1.88 ± 0.14^{b}	18.85 ± 0.23^{b}	0.45 ± 0.15^{b}	6.71 ± 0.04^{b}	5.61 ± 0.18^{b}	88.78 ± 2.19^{a}	7.15 ± 1.85^{b}	19.77 ± 3.39^{b}	26.25 ± 1.20^{a}	9.45 ± 1.16^{b}
Nano MAP	0.07 ± 0.01^{c}	21.40 ± 0.28^{c}	0.30 ± 0.24^{b}	6.35 ± 0.05^{c}	5.65 ± 0.10^{b}	86.95 ± 2.16^{b}	6.24 ± 1.74^{b}	26.25 ± 4.54^{c}	23.88 ± 3.65^{b}	10.25 ± 1.27^{c}
Uncoated	6.62 ± 0.12^{A}	14.56 ± 0.27^{A}	1.53 ± 0.31^{A}	6.64 ± 0.09^{A}	5.65 ± 0.17^{A}	91.80 ± 1.57^{A}	6.72 ± 1.80^{A}	12.52 ± 2.71^{A}	26.51 ± 3.45^{A}	8.25 ± 1.22^{A}
Coated	6.30 ± 0.08^{A}	14.83 ± 0.19^{A}	1.87 ± 0.33^{B}	6.63 ± 0.04^{A}	5.46 ± 0.20^{B}	82.58 ± 3.03^{B}	8.00 ± 1.98^{B}	28.08 ± 4.92^{B}	25.16 ±3.33^{A}	8.52 ± 0.98^{A}

Different superscripts within a group (i.e., within each column) denote a statistically significant difference at $p \leq 0.05$ (or 95% confidence interval). Results are expressed as mean values ± standard deviation.

Table S3. Values of O_2 and CO_2 atmospheric composition, weight loss, pH, total soluble solids (TSS), lightness (L^*), color variation (ΔE), browning index (BI), cap opening (%), and maximum force (F_{max}) of button mushrooms for each day of analysis within the ten days period for different packaging configurations.

	Day 1		Day 2		Day 4		Day 6		Day 8		Day 10	
O_2 (%)	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
Control	19.60 ± 0.26	19.30 ± 0.10	18.37 ±0.25	18.04 ±0.05	17.94 ±0.04	17.03 ±0.06	17.26 ±0.21	16.72 ±0.29	16.77 ±0.06	16.07 ±0.11	16.31 ±0.25	15.70 ±0.26
Nano	6.11 ± 0.12	4.30 ± 0.18	1.70 ±0.49	1.06 ±0.02	1.29 0.18	1.11 ±0.01	1.09 ±0.03	1.11 ±0.01	1.07 ±0.02	1.12 ±0.05	1.23 ±0.21	1.43 ±.35
Nano+MAP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.44 ± 0.01	0.35 ± 0.01	0.04 ± 0.03	0.08 ± 0.01
CO_2 (%)	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
Control	2.54 ±0.10	2.80 ±0.10	3.20 ±0.26	3.70 ±0.10	3.17 ±0.29	3.61 ±0.09	3.60 ±0.10	4.04 ±0.15	4.47 ±0.45	5.23 ±.32	4.73 ±0.21	4.94 ±0.06
Nano	10.45 ±0.26	12.10 ±0.11	16.89 ±0.36	16.77 ±0.19	17.60 ±0.41	18.60 ±0.12	20.12 ±0.38	20.30 ±0.17	23.10 ±0.28	23.01 ±0.08	23.41 ±0.10	23.86 ±0.26
Nano+MAP	17.60 ±0.34	17.81 ±0.04	19.53 ±0.39	19.72 ±0.55	21.33 ±0.15	20.10 ±0.12	21.65 ±0.11	21.46 ±0.45	23.64 ±0.12	24.10 ±0.24	25.13 ±0.57	24.77 ±0.23

Table S3 (continued)

	Day 0		Day 2		Day 4		Day 6		Day 8		Day 10	
Weight loss (%)	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
Control	N.D	N.D	1.39 ±0.55	2.11 ±0.20	3.52 ±1.22	3.65 ±1.30	4.49 ±0.25	6.45 ±0.77	6.01 ±0.78	7.97 ±0.51	7.12 ±0.76	9.36 ±0.57
Nano	N.D	N.D	0.18 ±0.01	0.38 ±0.02	0.77 ±0.31	0.07 ±0.04	0.80 ±0.14	0.21 ±0.01	0.77 ±0.10	0.24 ±0.02	1.00 ±0.24	1.02 ±0.18
Nano+MAP	N.D	N.D	0.11 ±0.07	0.07 ±0.03	0.21 ±0.17	0.35 ±0.04	0.27 ±0.20	0.38 ±0.04	0.34 ±0.27	0.64 ±0.42	0.46 ±0.37	0.83 ±0.49
pH	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
Control	6.66 ±0.11	6.73 ±0.01	6.74 ±0.44	6.90 ±0.01	6.74 ±0.38	6.94 ±0.04	6.74 ±0.04	6.87 ±0.02	6.91 ±0.04	6.56 ±0.04	7.10 ±0.05	7.20 ±0.07
Nano	6.49 ±0.02	6.44 ±0.03	6.70 ±0.10	6.79 ±0.10	6.78 ±0.70	6.05 ±0.04	6.58 ±0.03	6.55 ±0.02	6.69 ±0.05	6.72 ±0.01	6.85 ±0.08	6.80 ±0.02
Nano+MAP	6.49 ±0.01	6.48 ±0.03	5.6 ±0.01	5.23 ±0.20	6.58 ±0.05	6.50 ±0.02	6.52 ±0.01	6.51 ±0.01	6.54 ±0.02	6.65 ±0.04	6.73 ±0.04	6.57 ±0.02
TSS (%)	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
Control	5.13 ±0.55	4.97 ±0.78	4.96 ±0.37	4.73 ±0.15	5.36 ±0.37	4.97 ±0.20	5.23 ±0.15	5.80 ±0.10	5.10 ±0.10	5.23 ±0.20	5.50 ±0.10	5.56 ±0.20
Nano	6.15 ±0.20	5.20 ±0.10	5.90 ±0.10	5.10 ±0.10	5.30 ±0.10	5.57 ±0.20	5.27 ±0.15	5.50 ±0.10	5.57 ±0.30	5.77 ±0.40	6.35 ±0.10	5.70 ±0.38
Nano+MAP	5.68 ±0.07	5.43 ±0.11	6.95 ±0.04	6.78 ±0.02	6.03 ±0.05	5.3 ±0.10	5.96 ±0.11	5.66 ±0.11	5.90 ±0.15	5.91 ±0.15	5.46 ±0.15	5.16 ±0.15

Table S3 (continued)

	Day 0		Day 2		Day 4		Day 6		Day 8		Day 10	
Δ*E*	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
Control	N.D	N.D	2.46 ±1.56	10.83 ±6.24	6.06 ±2.66	8.64 ±1.52	5.07 ±0.88	9.61 ±2.13	12.54 ±2.35	17.78 ±3.10	14.57 ±3.89	17.32 ±0.77
Nano	N.D	N.D	5.68 ±1.40	7.12 ±3.69	6.21 ±0.59	7.51 ±0.77	7.60 ±0.77	7.03 ±2.59	8.09 ±1.95	6.64 ±1.38	12.21 ±4.67	17.69 ±4.38
Nano+MAP	N.D	N.D	2.31 ±1.95	4.55 ±2.32	5.36 ±1.73	9.48 ±2.55	3.64 ±1.61	3.81 ±1.12	9.60 ±2.97	4.89 ±1.64	14.49 ±3.50	10.61 ±1.46
*L**	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
Control	96.15 ±1.41	92.49 ±1.77	95.66 ±1.91	85.64 ±9.48	93.69 ±3.00	85.88 ±1.59	91.59 ±1.38	84.23 ±1.04	88.86 ±2.39	81.49 ±2.35	85.67 ±1.80	80.47 ±1.36
Nano	94.70 ±2.05	89.51 ±4.13	93.98 ±0.84	85.31 ±4.05	93.62 ±0.44	86.92 ±1.52	92.61 ±0.29	85.89 ±2.86	92.63 ±1.96	85.21 ±0.85	89.38 ±2.95	75.65 ±4.31
Nano+MAP	94.53 ±0.52	80.56 ±0.48	94.65 ±1.62	81.34 ±1.93	92.76 ±0.53	81.08 ±10.29	93.48 ±1.10	77.50 ±1.09	90.39 ±0.71	76.70 ±3.08	89.96 ±3.39	70.61 ±2.32
BI	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
Control	3.87 ±0.75	11.32 ±1.03	3.24 ±1.94	20.12 ±1.14	5.12 ±1.08	18.58 ±5.47	3.58 ±1.86	18.67 ±3.29	15.36 ±2.79	32.32 ±5.02	16.05 ±6.12	30.74 ±1.19
Nano	6.62 ±2.54	16.58 ±4.75	11.71 ±2.10	24.79 ±5.96	12.46 ±0.70	24.86 ±1.49	14.63 ±0.67	24.54 ±4.40	15.29 ±2.64	24.50 ±2.23	21.41 ±7.00	40.18 ±6.26
Nano+MAP	7.96 ±0.68	35.59 ±8.04	9.03 ±3.22	39.84 ±3.99	14.09 ±2.02	33.12 ±16.17	11.90 ±2.20	32.34 ±1.51	20.45 ±5.90	39.42 ±2.02	28.34 ±4.53	41.10 ±4.26

Table S3 (continued)

	Day 0		Day 2		Day 4		Day 6		Day 8		Day 10	
Cap opening (%)	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
Control	N.D	N.D	5.0 ±2.18	1.67 ±0.88	18.33 ±5.16	13.33 ±3.43	36.67 ±11.47	33.33 ±10.12	48.33 ±14.09	46.67 ±13.89	65.0 ±16.87	60.0 ±15.78
Nano	N.D	N.D	8.33 ±3.40	6.67 ±1.49	18.33 ±4.26	16.67 ±2.42	31.67 ±9.16	30.0 ±9.17	45.0 ±15.79	43.33 ±12.36	58.33 ±14.02	56.67 ±13.05
Nano+MAP	N.D	N.D	3.33 ±1.05	6.67 ±1.98	15.0 ±3.47	18.33 ±4.38	31.67 ±10.08	28.33 ±10.06	41.67 ±13.27	40.0 ±12.07	51.67 ±11.46	50.0 ±12.14
F_{max} (N)	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
Control	6.94 ±1.85	7.57 ±1.14	5.91 ±1.28	6.33 ±0.68	5.48 ±1.46	5.94 ±0.73	5.12 ±0.46	7.14 ±0.11	4.60 ±0.47	4.93 ±0.69	4.07 ±0.48	4.49 ±1.24
Nano	7.61 ±1.32	8.95 ±1.31	10.13 ±1.30	10.14 ±0.69	9.84 ±1.16	9.54 ±0.47	9.37 ±0.76	11.17 ±0.87	9.79 ±2.59	9.18 ±2.04	9.12 ±1.12	5.50 ±0.25
Nano+MAP	10.32 ±0.71	11.78 ±0.56	10.67 ±2.02	10.41 ±1.88	10.29 ±1.08	10.32 ±0.31	9.11 ±1.68	10.67 ±1.56	9.67 ±1.53	8.46 ±1.87	10.37 ±0.74	10.92 ±1.29

Results are expressed as mean values ± standard deviation.

Figure 1. Button mushrooms packaged in the bionanocomposite-based laminate/EPS tray (a) and in the conventional PVC stretchable/EPS tray configuration (b) tested in this study.

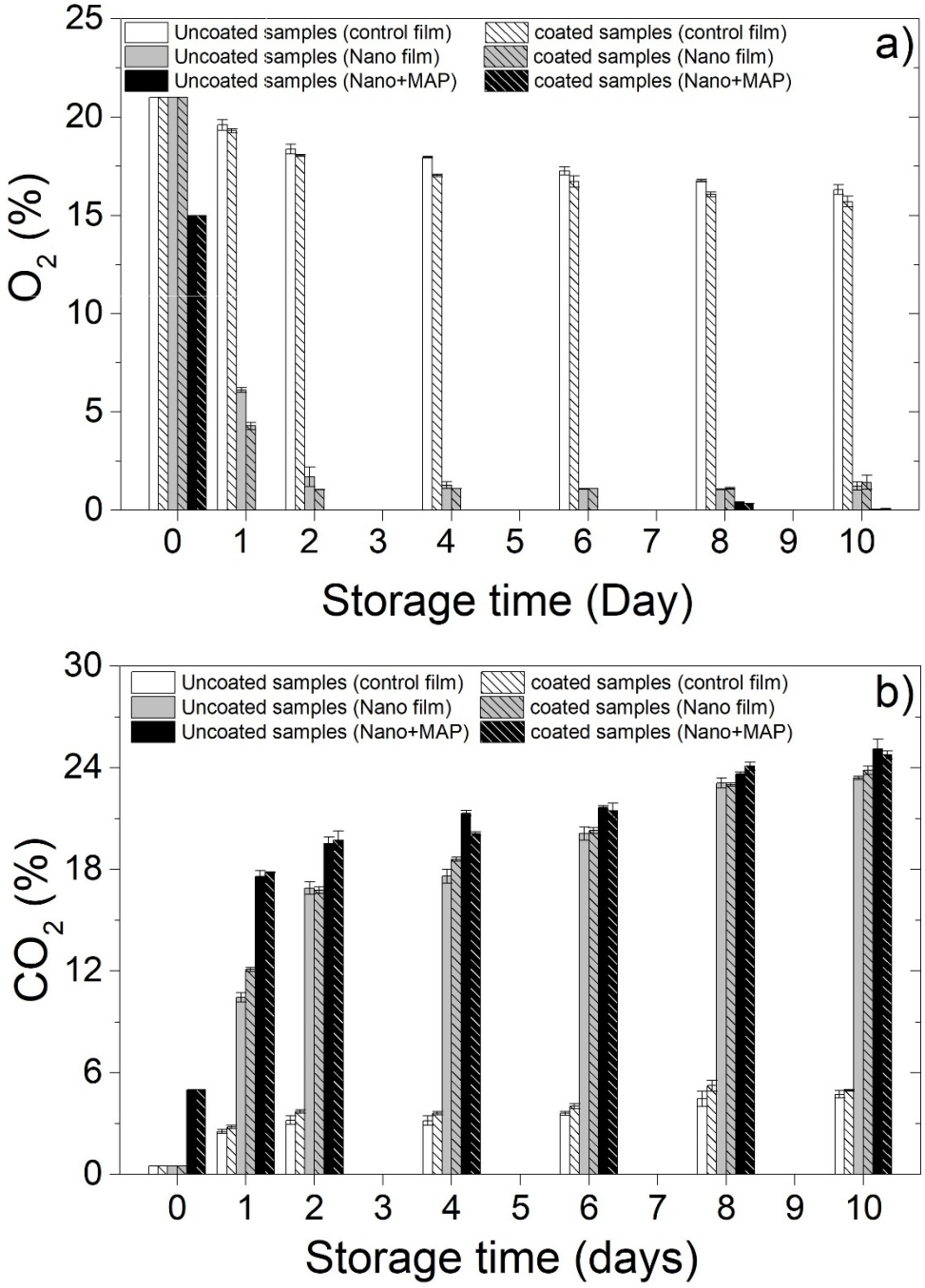

Figure 2. Oxygen (a) and CO_2 (b) percentage evolution inside the control (PVC stretch film – EPS tray) and test (PET/coating/LLDPE film – EPS tray) packaging configurations with and without MAP of coated and uncoated mushrooms stored at 4°C for 10 days.

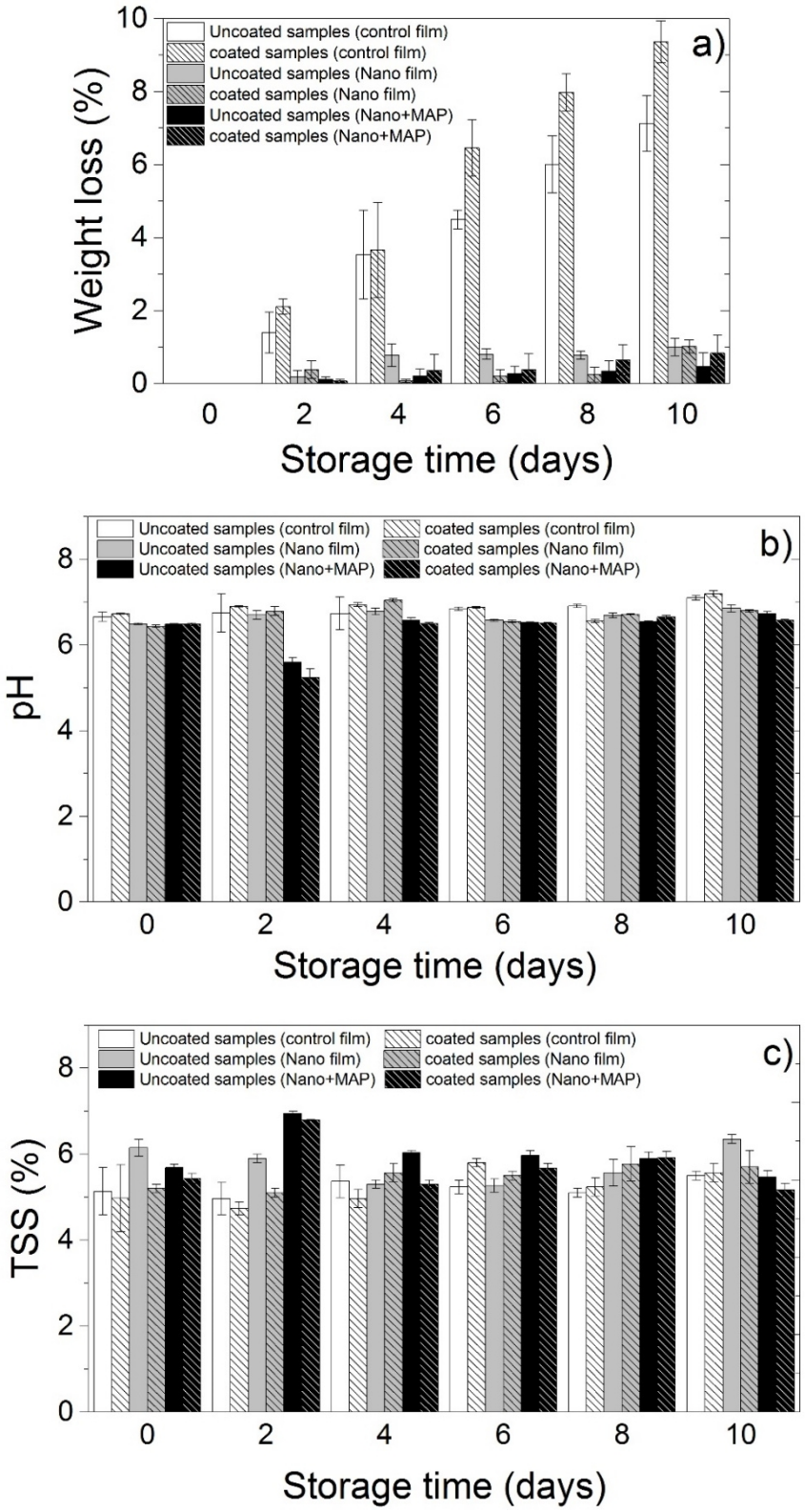

Figure 3. Weight loss (a), pH (b), and total soluble solids (TSS) (c) of mushrooms uncoated and coated with the chitosan biopolymer film, packaged using the control (PVC stretch film – EPS tray) and test (PET/coating/LLDPE film – EPS tray) configurations, with and without MAP, at 4°C for 10 days.

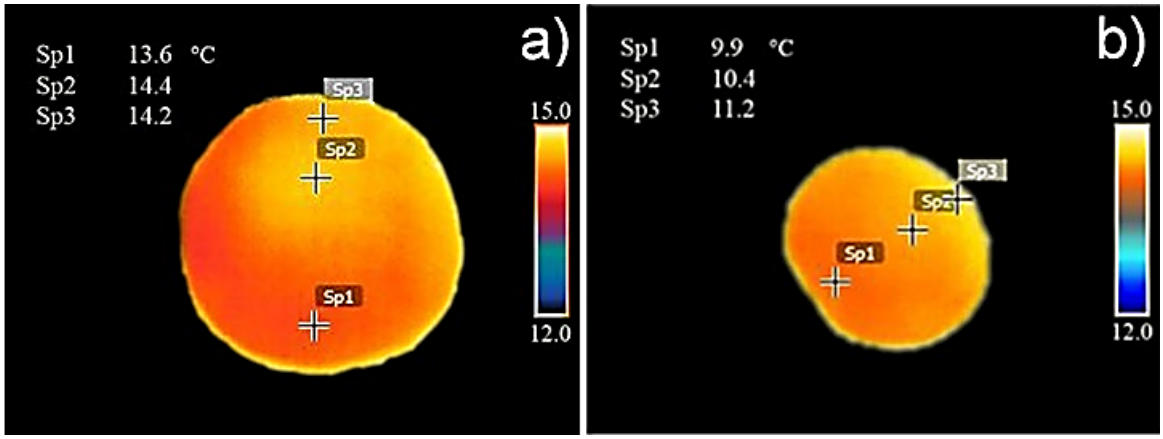

Figure 4. Examples of thermal images captured on mushrooms packaged with the control (PVC) film (a) and the test (PET/coating/LLDPE) material (b) after 10 days of storage at 4°C.

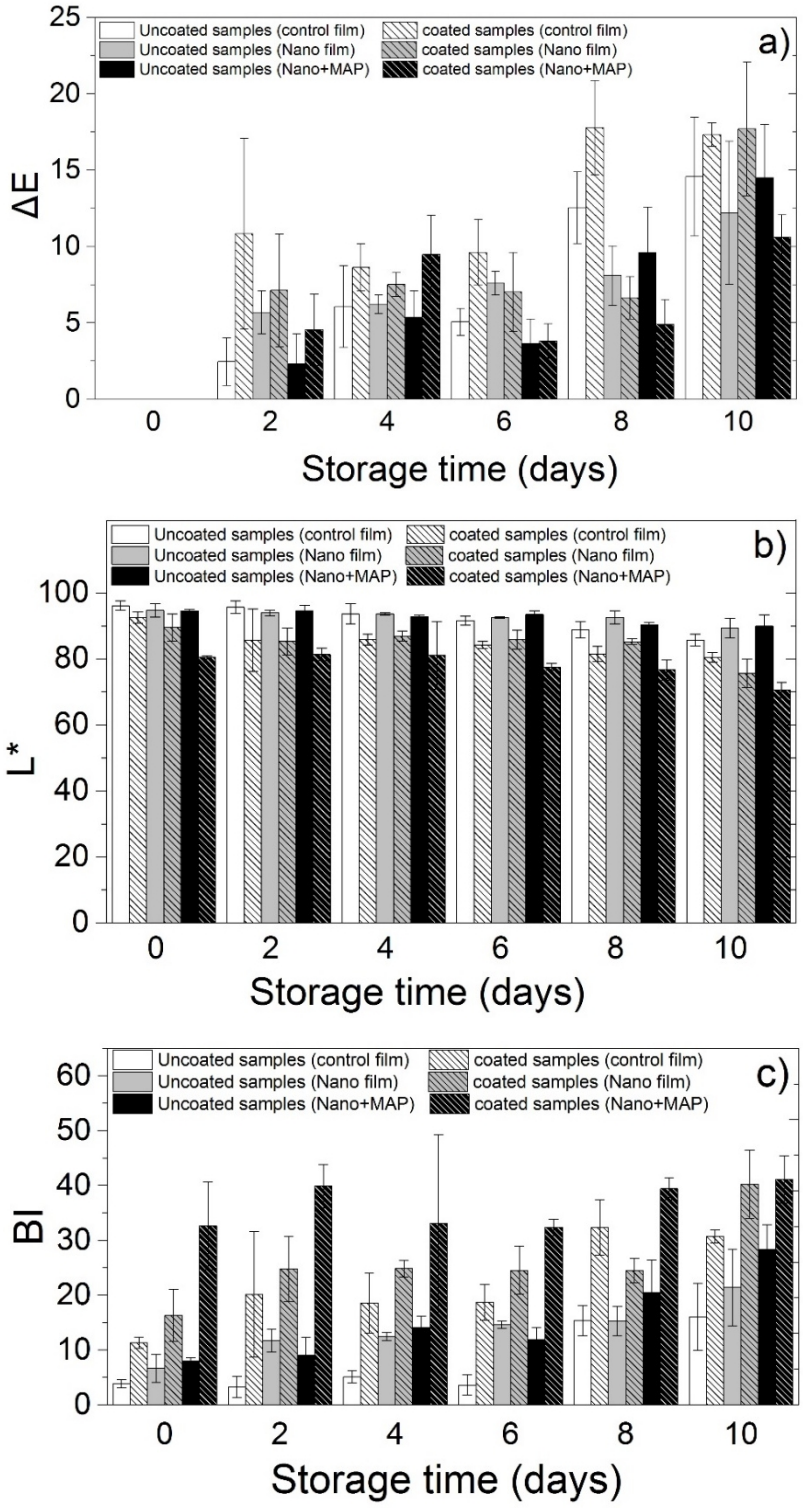

Figure 5. Color changes (ΔE) (a), lightness (L^*) (b), and browning index (BI) (c) of mushrooms uncoated and coated with chitosan biopolymer film, packaged using the control (PVC stretch film – EPS tray) and test (PET/coating/LLDPE film – EPS tray) configurations, with and without MAP, at 4°C for 10 days.

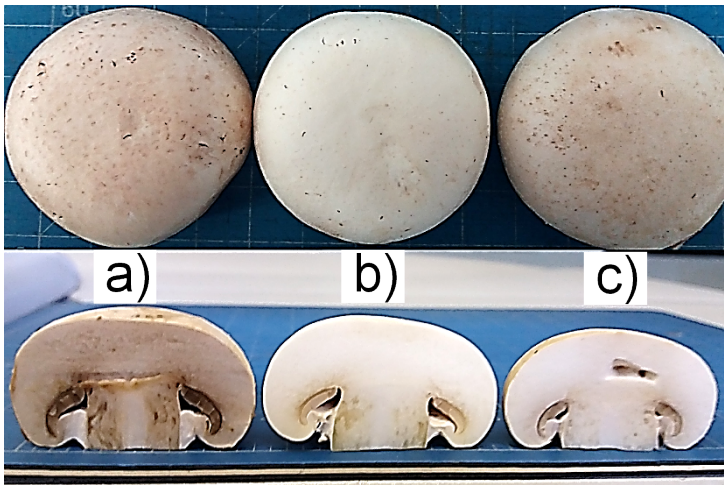

Figure 6. Surface (up) and cross-section (down) digital camera images of uncoated mushrooms packaged using the control film (PVC stretch film) (a), the test film (PET/coating/LLDPE film – EPS tray) with (b) and without (c) MAP after 22 days of storage at 4° C.

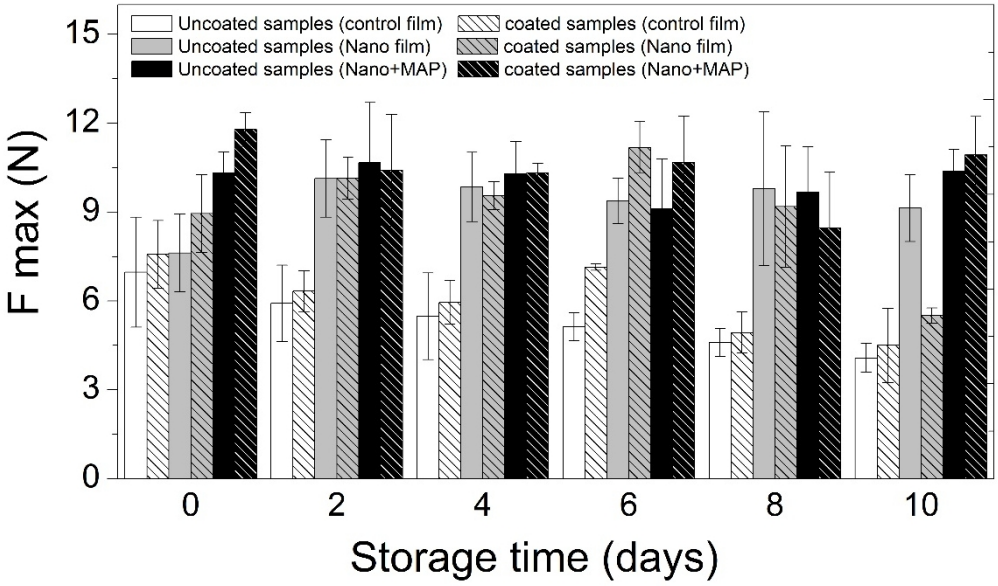

Figure 7. Maximum force (F_{max}) of mushrooms uncoated and coated with chitosan biopolymer film, packaged using the control (PVC stretch film – EPS tray) and test (PET/coating/LLDPE film – EPS tray) configurations, with and without MAP, at 4°C for 10 days.