Research Article

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Improvement of shelf-life of cherry (*Prunus avium* L.) by combined application of modified-atmosphere packaging and antagonistic yeast for long-distance export

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Abstract

BACKGROUND: The last decade has seen a growing interest in reducing the use of chemical fungicides for postharvest decay control. In the research for new, safe alternatives, the combined application of biocontrol agents and passive modified-atmosphere packaging (MAP) has been shown to be a promising strategy to extend fruit quality. Therefore, the aim of this work was to evaluate the effect of the combined application of MAP and two antagonistic yeasts, *Metschnikowia pulcherrima* L672 and *Pichia kudriavzevii* PK18, on sweet cherry shelf life.

RESULTS: Microbiological, physico-chemical, and quality fruit analysis from batches treated with antagonistic yeast were compared with a control batch without yeast application and a batch to which fludioxonil (Scholar®) was applied. The composition of the atmosphere and physico-chemical traits showed similar values among the different batches during cold storage. However, interestingly, the combination of MAP with the antagonistic yeasts *M. pulcherrima* L672 and *P. kudriavzevii* PK18 increases the control of microbiological spoilage with results comparable to the application of fludioxonil. In addition, these batches experienced a slight decrease in volatile compounds associated with fresh fruit aroma, whereas in the control batch an increase of altered fruit aromas was observed. The same effect of control of spoilage was observed during the shelf life period.

CONCLUSION: These results showed the positive effect of the combination of antagonistic yeasts and MAP, obtaining similar results in terms of control of microbiological spoilage and physico-chemical quality compared with the application of fludioxonil.

Keywords: biocontrol; postharvest pathogens; MAP; supply chain; quality

INTRODUCTION

Cherry (*Prunus avium* L.) is a non-climacteric fruit highly appreciated for its flavour characteristics, colour, and richness in nutrients and phytochemicals.[1-4] At an optimal ripening stage, this fruit is characterised by a balance between soluble solid compounds and acidity, which together with colour have a decisive impact on consumer purchase and acceptance.[5]

Cultivar, harvesting time, handling, and packaging greatly affect the quality and shelf life (SL) of sweet cherry.[6] However, its high perishability after harvest is mainly due to its high respiration rate, physico-chemical changes (such as weight loss, softening, darkening, mechanical damage, or pitting and bruising, etc.),[7,8] and the development of spoilage microorganisms.[9] Among them, primarily diverse species of filamentous fungi cause relatively faster spoilage after harvesting. The most important spoilage fungi of sweet cherries are *Botrytis cinerea*, *Penicillium expansum*, and *Monilinia* spp.[10-13] Consequently, the SL of early cherry is limited

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to 7–10 days.[6,8] Therefore, strict storage conditions must be applied, alone or in combination with different postharvest strategies, to delay spoilage and maintain quality.

Fungal control during postharvest life is a major challenge for the fruit industry. Currently, fludioxonil (Scholar®, Syngenta) is authorised for postharvest use on stone fruit to prevent fungal spoilage during storage and transport.[14] However, there is growing evidence questioning its classification as 'reduced risk', and the scientific community is asking for its re-evaluation.[15] Therefore, fungicide chemical-free products are attracting growing consumer interest.[16,17] Different alternative strategies to synthetic chemicals have been studied to maintain quality and reduce microbial growth in cherry. Gamma irradiation treatments on '0900 Ziraat'[18] and ultraviolet irradiation on 'Takdaneh Mashhad'[19] and 'Van'[20] avoided quality loss as well as stimulated anthocyanin accumulation by promotion of phenylalanine ammonia lyase activity improving cherry colour. Edible coatings with *Aloe vera* gel on 'StarKing',[21] alginate on 'Sweetheart',[22] and chitosan on 'Ferrovia'[23] were effective in delaying increased respiration rate, weight loss, and fungal decay, and in preserving phytochemical compounds. Finally, postharvest applications of biocontrol agents (BCAs) have also been effective in controlling fungal decay of cherry.[11,24,25] Among them, one of the most useful strategies to extend the SL of cherries has been the application of passive modified-atmosphere packaging (MAP), in which low oxygen partial pressures, high carbon dioxide (CO_2) partial pressures, and high relative humidity are achieved.[26] MAP treatments reduced weight loss and decay and delayed ripening, and thus maintained quality characteristics such as composition, colour parameters, firmness, and sensory attributes.[27-30] Combination of MAP with other postharvest approaches has been shown to maintain sweet cherry quality. For example, its application along with Parka® biofilms improved firmness of cherries '0900 Ziraat',[30] or BCAs plus MAP has revealed a synergistic effect to control fungal pathogens. In this regard, the application of *Cryptococcus infirmo-miniatus* combined with a 50/50 gas mixture of CO_2 and nitrogen was effective in reducing brown rot decay on sweet cherry 'Lapins' and 'Lambert'.[31] Furthermore, *Metschnikowia pulcherrima* and *Hanseniaspora opuntiae* combined with MAP (11.2 kPa CO_2) were able to control *P. expansum* on 'Ambrunés'.[11] However, overall, the suitability of using MAP along with BCAs for industrial application has been scarcely explored to control fungal decay on sweet cherry.

Thus, the purpose of this work was to test the ability to extend the SL of early sweet cherry 'Burlat' by combining a passive modified atmosphere with two antagonistic yeast strains, *M. pulcherrima* L672[32] and *Pichia kudriavzevii* PK18,[33] in comparison with the current postharvest treatment based on fludioxonil treatment combined with MAP.

MATERIAL AND METHODS

Plant material and experimental design

'Burlat' cherries (*Prunus avium* L.) were supplied by the 'Agrupación de Cooperativas del Valle del Jerte' (Cáceres, Spain). In this study, the cherry packing process carried out in this packinghouse for exportation purposes was simulated. This process includes a pre-cooling and sanitisation with 50 mg L^{-1} of sodium hypochlorite for 3 min (5 °C) in a pilot hydrocooler (DS1000; Kronen GmbH, Willstätt, Germany). Subsequently, different batches were prepared. The application of the antifungal treatment was carried out after the pre-cooling and sorting process. The authorised fungicide treatment at postharvest stage involves an immersion in fludioxonil solution (Scholar 230SC (2.5 mL L^{-1}); Syngenta Crop Protection Inc., Greensboro, NC, USA). Thus, one batch was treated with fludioxonil for 5 min at 5 °C (fludioxonil batch). Additionally, one batch of cherries was immersed in water with *M. pulcherrima* L672 (MPL 672)[32] and another batch in water with *P. kudriavzevii* PK18 (PK18)[33] at cell densities of 10^8 mL^{-1} for 5 min at 5 °C, simulating the process of the antifungal application mentioned earlier herein. Finally, a control batch was obtained by immersion in cold water without antifungal agent (5 °C) for 5 min. All cherries were immediately air dried at room temperature for 30 min. After drying, cherries from the four batches were packaged in passive modified-atmosphere bags (Xtend 815-CH 98 for cherries, 2.5 kg; StePac, Migdal Tefen, Israel) with approximately 2.5 kg of fruits, closed with rubber bands, and placed in open cardboard boxes. All samples were identified and weighed.

SL simulation and sampling

Once cherries were packed under MAP, they were stored in a cold room (2 °C and 90–95% of relative humidity) for 40 days (cold storage: CS). Six bags per batch were randomly sampled at 5, 12, 25, and 40 days of storage in darkness. Three of them were opened and stored at 25 °C for 2 days to simulate the retail trade, considering this period as the SL. All determinations were performed on three different bags per sampling point.

Physico-chemical and microbiological analyses

Gas composition of MAP

Headspace gas composition in the MAP bags was measured using a CheckMate 3 headspace gas analyser (PBI Dansensor, Ringsted, Denmark). The gas analyser needle was inserted through a silicone rubber seal fixed to the plastic bag's external side. Results were expressed as kilopascals of CO_2 and kilopascals of oxygen (O_2).

Weight loss

Percentage weight loss during the cool storage period was recorded by comparing the weight recorded after batch preparation (w_0) and after sampling (w_t) at time "t" (days). Results were calculated using the following equation:

$$\text{Weight loss}\,(\%) = \frac{w_0 \times w_t}{w_0} \times 100$$

Microbial counts, and monitoring of inoculated yeast and percentage of decay

Twenty cherries per sample were randomly placed in a sterile plastic bag and diluted ten times with sterile peptone water (Condalab, Madrid, Spain). After homogenisation for 120 s in a stomacher instrument (Lab-Blender 400; Seward Lab., London, UK), serial tenfold dilutions were performed with sterile peptone water, and aliquots of 0.1 mL from each dilution were inoculated onto agar plates: plate count agar (Condalab) for enumeration of total aerobic mesophilic (TAM) microbes after incubation at 30 °C for 24 h; rose bengal chloramphenicol agar (Condalab) and potato dextrose agar (Condalab) acidified to pH 3.5 with a sterilised solution of tartaric acid (10%) for yeasts and mould enumeration after incubation at 25 °C for 4 days. Microbial counts were expressed in $\log_{10}$ CFU g^{-1} of cherry.

Inoculum monitoring was performed by isolating yeast colonies with morphologies compatible with the inoculated species. Once

isolated, species identification was performed by sequencing and comparison in the GeneBank database using the BLAST algorithm of the ITS1-5,8 S rRNA-ITS4 genomic fragment as described in Ruiz-Moyano *et al.*[34] In the control and fludioxonil batches, yeast colonies of the most representative morphologies were isolated and identified as explained earlier herein.

The presence of spoiled cherries was recorded and expressed as percentage of decay incidence. Fruit showing visual mycelium and symptoms of microbial spoilage (discolouring, softening, fermentations, etc.) were considered as decayed. To determine decay incidence percentage, 50 cherries were randomly selected and weighed (w_t). Then, the percentage of decay was determined by separating and weighing the spoiled cherries (w_d) and calculated using the following equation:

$$\text{Decay incidence\%} = \frac{w_d}{w_t} \times 100$$

Objective skin and flesh colour

Twenty cherries randomly selected per sample were analysed with a portable spectrophotometer CR-600d (Konica Minolta, Osaka, Japan). After skin colour determination, fruits were cut to analyse flesh colour. Then, CIELab space coordinates were obtained. The hue angle h^*, calculated as arctan(b^*/a^*), expresses the colour nuance, and values are defined as follows: red–purple: 0°; yellow: 90°; bluish green: 180°; blue: 270°. The chroma C^*, obtained as $(a^{*2} + b^{*2})^{1/2}$, is a measure of chromaticity, which defines the purity or saturation of the colour. Each datum represents the average of three measurements taken at equidistant points on the equatorial region of each fruit.

Firmness

Twenty fruits per sampling day per batch were randomly selected for firmness determination using a TA.XT2i texture analyser (Stable Micro Systems, Godalming, UK) as per Serradilla *et al.*[35]

pH, titratable acidity, and total soluble solids

Twenty-five fruits per sample were homogenised using an Omni Mixer homogeniser (Omni International, Marietta, GA, USA). Titratable acidity (TA) and pH were determined in 5 g aliquots diluted to 50 mL with deionised water from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Analyses were performed with an automatic 716 DMS Titrino titrator (Metrohm, Herisau, Switzerland). Samples were titrated with 0.1 mol L^{-1} sodium hydroxide up to pH 8.1. Results are expressed as grams of malic acid per kilogram of fresh weight.

Total soluble solids (TSS) values were measured using an automatic temperature-compensated DR101 digital refractometer (Optic Ivymen System, Barcelona, Spain). Results are expressed as degrees Brix (°Bx).

Volatile compounds

A total of 1 g of homogenate was weighed into a 10 mL headspace vial (Agilent Technologies, Santa Clara, CA, USA) and sealed with a polytetrafluoroethylene–butyl septum (Perkin-Elmer, Waltham, MA, USA) in an aluminium cap. Samples were stored at −80 °C until analyses. Extraction of volatile compounds by

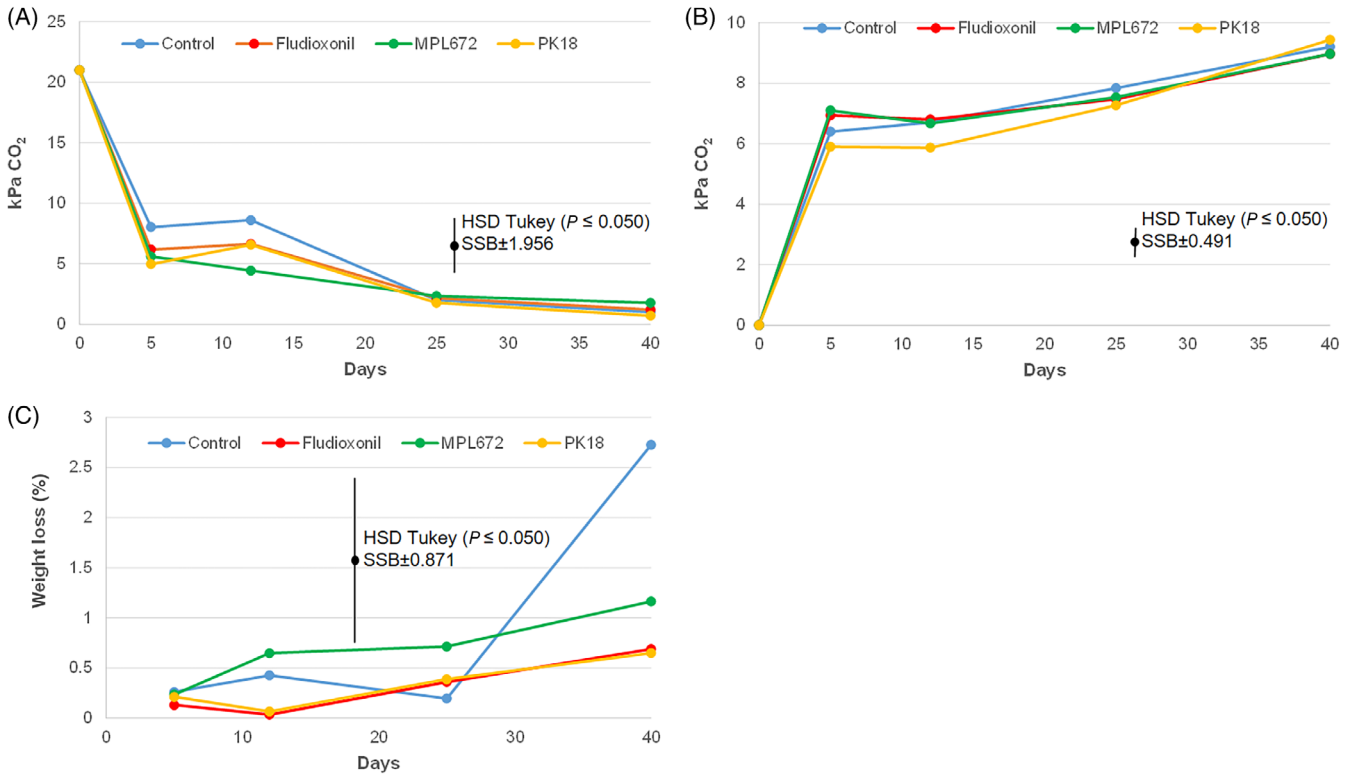

Figure 1. Partial pressures (A) kPa O_2 and (B) kPa CO_2 and (C) percentages of weight losses of 'Burlat' cherries packed in passive modified atmospheres at 2 °C and treated with *Metschnikowia pulcherrima* L672 (MPL672: in green), *Pichia kudriavzevii* PK18 (PK18: in yellow), fludioxonil (in red) and control (in blue). HSD Tukey: Tukey's honestly significant difference); SSB: statistical significance bar.

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solid-phase microextraction with a 10 mm long, 100 µm thick Carboxen™/polydimethylsiloxane-coated fibre (Supelco, Bellefonte, PA, USA), and gas chromatography–mass spectrometry analyses using the Agilent 6890 gas chromatograph–Agilent 5973 mass spectrometer system (Agilent Technologies) was carried out for the identification and semi-quantification of volatile compounds as described by Serradilla *et al.*[36]

Data analysis

Statistical analysis of the gas composition, percentage weight loss, microbiological counts, percentage microbial spoilage, and physico-chemical traits was carried out using a two-way analysis of variance. Data of percentage weight loss and percentage microbial spoilage were converted into Bliss angular values before analysis. The means were separated by Tukey's honestly significant difference test using SPSS for Windows, 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Impact of treatments on sweet cherry quality during CS

MAP batches experienced a pronounced decrease of O_2 at day 5, as shown in Fig. 1(A), although no statistical differences were found ($P > 0.050$). The mean values of the control batch were higher (at 8.03 ± 2.35 kPa) than the other batches, with the lowest mean values, 4.97 ± 2.05 kPa, in batch PK18. These results were maintained after 12 days. However, O_2 partial pressure decreased after 25 and 40 days of CS, with values ranging from 0.70 ± 0.17 kPa O_2 in batch PK18 to 1.76 ± 1.07 kPa O_2 in batch MPL672 ($P > 0.050$). The evolution of CO_2 concentration presented similar values in all batches ($P > 0.050$; Fig. 1(B)). At the beginning of CS, mean values from 5.90 ± 0.20 kPa CO_2 to 7.10 ± 0.17 kPa CO_2 were observed in batches PK18 and MPL672 respectively. On the contrary, at the end of CS, values from 8.97 ± 0.27 kPa CO_2 to 9.42 ± 0.25 kPa CO_2 were found in the fludioxonil and PK18 batches respectively.

The batches treated with antagonistic yeast and fludioxonil showed similar weight loss, with percentage loss of around 0.2% at 5 days and ranging from 0.64% to 1.16% after 40 days with no statistical differences. Nevertheless, the control showed a significant increase in weight loss ($P < 0.050$) after 25 days of storage (2.72 ± 2.36% of weight loss). Weight loss evolution was quite similar among batches ($P > 0.050$) up to 25 days of storage, with percentages below 0.75% (Fig. 1(C)). Afterwards, in the final storage phase, weight loss was significantly lower in the fludioxonil and PK18 batches ($P < 0.050$).

Microbial counts are shown in Fig. 2. TAM microbes showed differences at the beginning of storage, with lower counts ($P < 0.050$) in the control batch (values of 2.41 ± 0.36 $\log_{10}$ CFU g^{-1}) (Fig. 2(A)). Yeast-inoculated batches exhibited TAM counts around 3.3 $\log_{10}$ CFU g^{-1}. In general, TAM counts increased during storage to levels of approximately 5–6 $\log_{10}$ CFU g^{-1} at the end of storage, with no statistical differences between batches ($P > 0.050$) (Fig. 2(A)). Figure 2(B) shows yeast counts throughout storage. In general, the evolution of yeast counts was parallel to TAM counts, except at the end of storage. At 40 days, a decrease of yeast counts was observed in the control, fludioxonil, and PK18 batches, with final

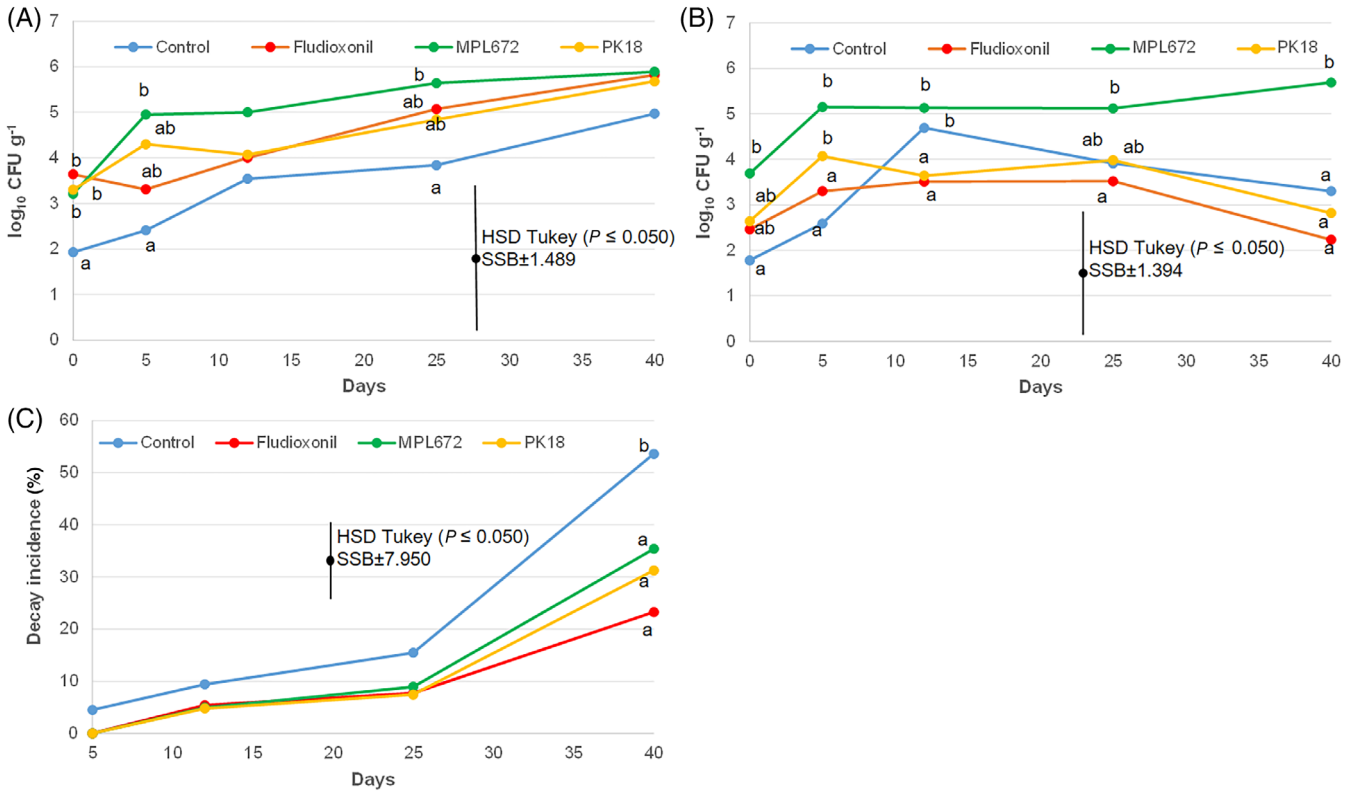

Figure 2. Counts of (A) total aerobic mesophilic microorganisms, (B) yeast, and (C) percentage decay incidence of 'Burlat' cherries packed in passive modified atmosphere at 2 °C and treated with *Metschnikowia pulcherrima* L672 (MPL672; in green), *Pichia kudriavzevii* PK18 (PK18; in yellow), fludioxonil (in red), and control (in blue). HSD Tukey: Tukey's honestly significant difference; SSB: statistical significance bar.

values of 3.30 ± 0.56 $\log_{10}$ CFU g^{-1}, 2.23 ± 0.68 $\log_{10}$ CFU g^{-1}, and 2.28 ± 0.41 $\log_{10}$ CFU g^{-1} respectively. By contrast, batch MPL672 maintained yeast counts similar to those of the TAM count after 40 days of CS. Finally, mould counts were below the threshold of detection (2 $\log_{10}$ CFU g^{-1}) for fludioxonil, PK18, and MPL672 batches during CS, and the control also failed to reach 2.00 ± 1.56 $\log_{10}$ CFU g^{-1} (data not shown).

Colony morphology analysis showed the predominance of inocula during CS. Genomic sequencing identification of isolates from yeast-inoculated batches confirmed the presence of *M. pulcherrima* and *P. kudriavzevii* species. In the non-inoculated batches, morphologies compatible with yeast-like mould species were mostly observed, which were molecularly identified as *Aureobasidium pullulans* and *Cystofilobasidium* spp.

The evolution of decay incidence is shown in Fig. 2(C). The control batch showed 4.50 ± 0.54% decay incidence at 5 days and increased up to 53.60 ± 16.32% at 40 days. Likewise, in the other batches, the decay incidence percentage increased during the first 25 days of storage in a similar way to the control. However, curiously, at 40 days it exhibited significantly ($P < 0.050$) lower mean values. Concretely, at 40 days, the decay incidence percentage was 23.36 ± 3.96%, 35.37 ± 3.57%, and 31.31 ± 6.98% for fludioxonil, MPL672 and PK18 batches respectively.

Regarding the physicochemical traits of the 'Burlat' cherries used in this study, they were 19.20 ± 0.06 °Bx, pH of 4.04 ± 0.04; TA of 6.31 ± 0.22 g malic acid kg^{-1} fresh weight, and 2.26 ± 0.41 N mm^{-1} at the beginning of storage. Overall, TSS were not affected during storage. However, batch PK18 showed higher ($P \leq 0.050$) TSS (mean values of 19.49 ± 1.10 °Bx) than fludioxonil (mean values of 18.90 ± 0.73 °Bx) and MPL672 (18.29 ± 1.06 °Bx). The pH values increased during storage, with differences among batches. Global pH values were significantly higher in the yeast-inoculated batches (4.21 ± 0.12 in PK18; 4.21 ± 0.15 in MPL672) than in the control batch (4.14 ± 0.14). Similarly, TA decreased during CS, with lower values in batch MPL672 (4.46 ± 0.61 g malic acid kg^{-1} fresh weight; $P \leq 0.050$). With respect to firmness, it was generally stable during the first 25 days of storage in all batches. However, a drastic decline was observed after 40 days. The yeast-inoculated batches showed higher mean values, especially PK18 with the highest statistical value ($P < 0.001$) of 3.06 ± 0.88 N mm^{-1}.

The colour parameters are showed in Fig. 3. Skin luminosity L^* increased at 5 days of storage in all batches, although the control

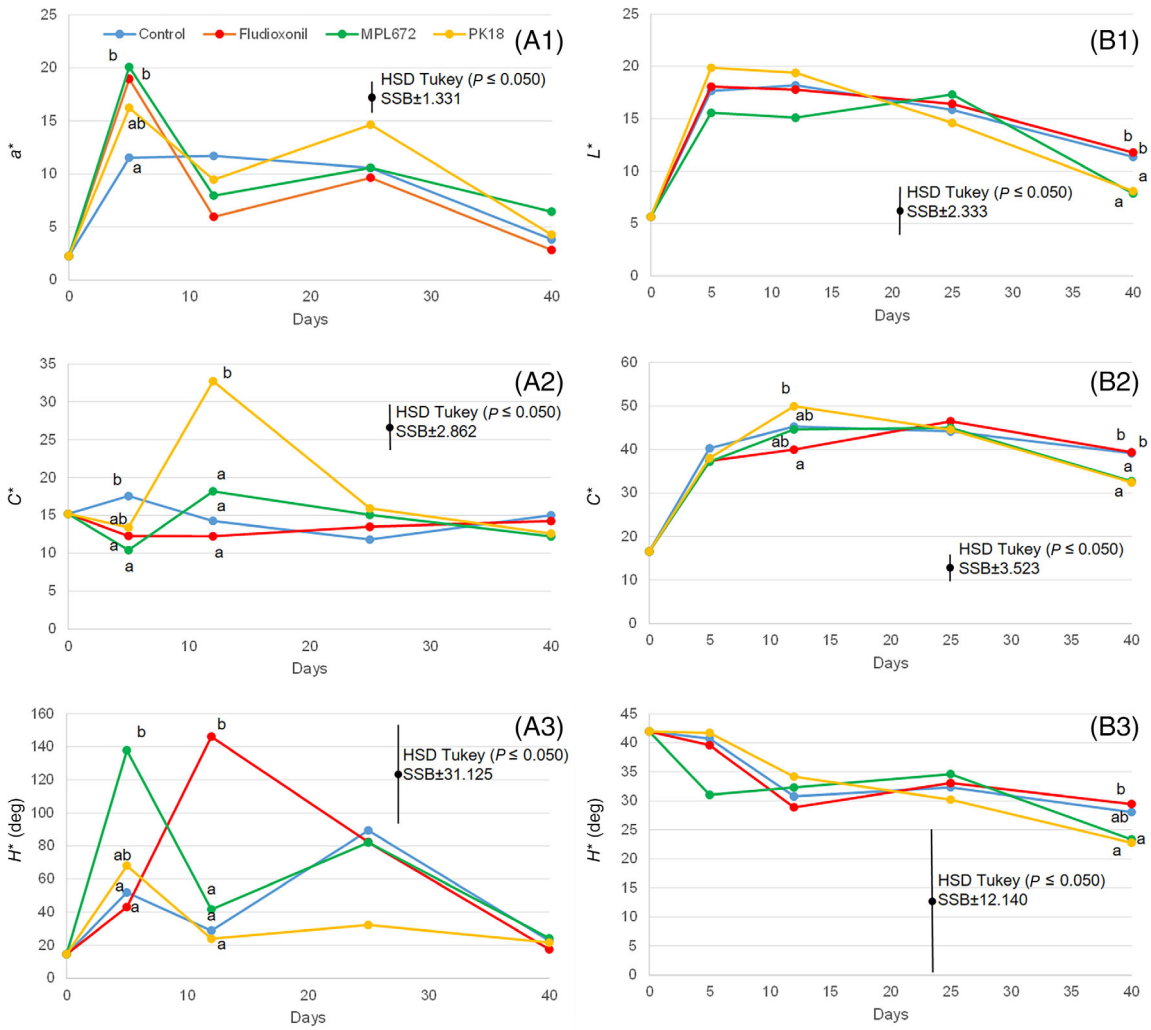

Figure 3. Colour parameters: (A) luminosity L^*, (B) chromaticity C^*, and (C) hue angle h^* of (1) skin and (2) flesh of 'Burlat' cherries packed in passive modified atmosphere at 2 °C and treated with *Metschnikowia pulcherrima* L672 (MPL672; in green), *Pichia kudriavzevii* PK18 (PK18; in yellow), fludioxonil (in red), and control (in blue). HSD Tukey: Tukey's honestly significant difference; SSB: statistical significance bar.

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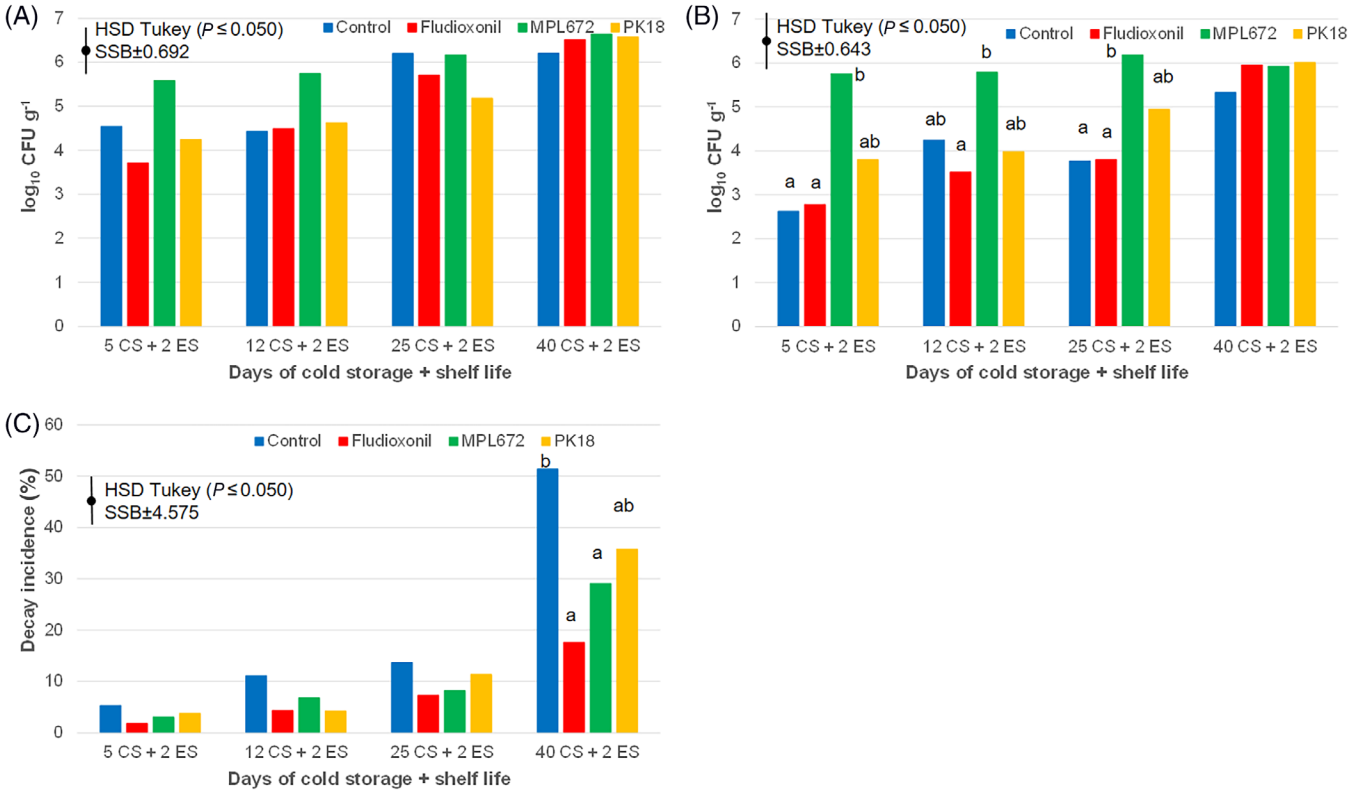

Figure 4. Counts of (A) total aerobic mesophilic microorganisms, (B) yeast, and (C) percentage decay incidence of 'Burlat' cherries packed in passive modified atmosphere and treated with *Metschnikowia pulcherrima* L672 (MPL672; in green), *Pichia kudriavzevii* PK18 (PK18; in yellow), fludioxonil (in red), and control (in blue). Cherries were stored at 2 °C and placed 2 days at 25 °C for shelf life simulation. HSD Tukey: Tukey's honestly significant difference; SSB: statistical significance bar.

batch showed the lowest values ($P < 0.050$). After that, L^* values decreased throughout storage to values up to ~5 units. Global analyses indicated that the skin of yeast-inoculated batches showed a higher brightness than the control ($P < 0.050$). Skin chromaticity C^* tended to be maintained throughout storage. However, a sharp increase was observed at 12 days of storage for batch PK18, reaching 32.72 ± 15.94 units ($P < 0.050$). h^* exhibited large fluctuations throughout storage and among batches. The highest values were observed at 5 days and 12 days for batches MPL672 and fludioxonil respectively. With respect to objective flesh colour during storage (Fig. 3(A2), (B2), (C2)), similar values were observed for L^*, C^*, and h^* in all batches ($P > 0.050$). L^* showed a slight decrease from 5 to 40 days; from values of 15–20 to values of 8–12 units. Flesh C^* was around 40 at 5 and 40 days of storage, showing a slight increase at intermediate sampling point. Finally, h^* decreased throughout storage from ~40º to 25–30º at 40 days of storage.

Treatments had no impact on the four selected marker volatile compounds (hexanal, 2-henexal, 1-hexanol, benzaldehyde) at 25 days of CS (Table 3). Additionally, at this sampling point, these compounds did not vary with respect to their content at the beginning of the experiment (day 0), although the mean values of benzaldehyde were much higher than those obtained at the beginning of the trial. At day 40, the occurrence of hexanal and [*E*]-2-hexenal decreased ($P < 0.050$) in the yeast- and fludioxonil-treated batches, and an increase of benzaldehyde was detected in the control batch with respect to the rest of the batches.

Impact of treatments on sweet cherry quality during SL

Figure 4 shows the impact of SL on microbial counts (2 days at 20 °C with MAP bags open) after CS. TAM mean counts progressively increased on each sampling day. However, no significant differences were found among batches ($P > 0.050$). Among batches, the mean counts of batch MPL672 were always the highest at all sampling points (Fig. 4A)). As for yeast counts, yeast-inoculated batches showed higher counts than non-inoculated cherries ($P < 0.050$), except at the final sampling point of SL (Fig. 4(B)). Batch MPL672 exhibited the highest mean counts with values ranging from 5.45 ± 0.46 $\log_{10}$ CFU g^{-1} at 5 CS + 2 SL days to 6.18 ± 0.95 $\log_{10}$ CFU g^{-1} at 25 CS + 2 SL days (Fig. 4(B)).

Figure 4(C) shows the percentage of microbial spoilage after the SL simulation. The control batch showed the highest mean values of spoilage at different sampling points of SL, whereas the fludioxonil batch showed the lowest. However, statistical differences were only found at the final sampling point, where fludioxonil (17.58 ± 4.58%) and MPL672 (29.06 ± 3.16%) batches reduced spoilage ($P < 0.050$) compared with the control batch (51.36 ± 8.35%).

Concerning physico-chemical analysis, no significant differences in TSS were found at the different sampling points of SL ($P > 0.050$), with mean values around 19–22 °Bx. Global mean values throughout the SL of batch PK18 showed higher values (21.07 °Bx; $P = 0.001$) than the rest of the batches. Overall, pH increased throughout SL from pH values around 4.2 to 4.5. In the case of batch MPL672, it showed the highest ($P = 0.048$) global mean pH values, reaching values of 4.71 ± 0.40 at 40 CS + 2 SL days, whereas the fludioxonil batch showed the lowest global mean pH value (4.23 ± 0.14). Contrary to pH values, TA decreased throughout the different sampling days, from values of 5.05–5.55 to 3.26–3.84 mg malic acid kg^{-1} fresh

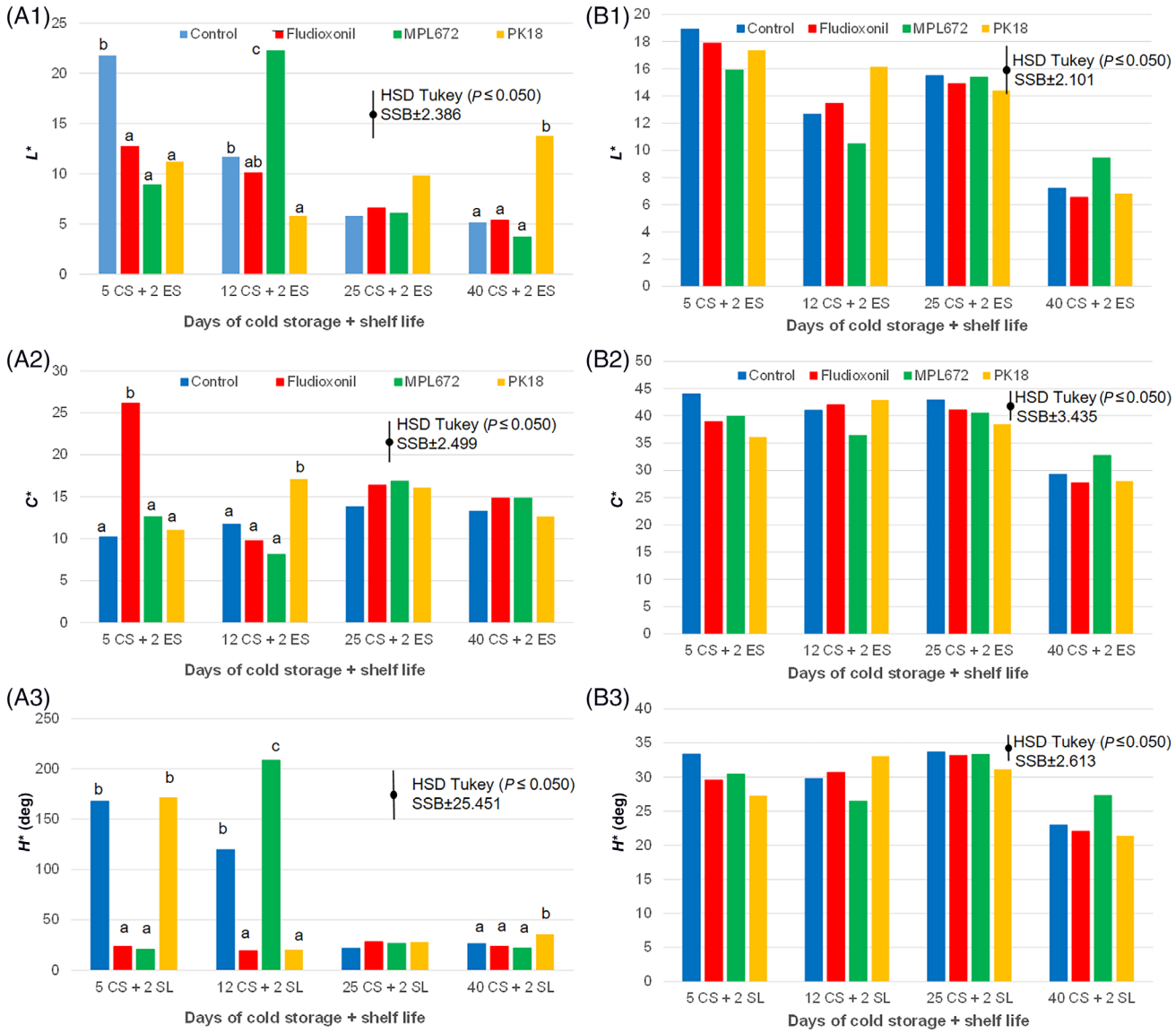

Figure 5. Colour parameters: (A) luminosity *L**, (B) chromaticity *C**, and (C) hue angle *h** of (1) skin and (2) flesh of 'Burlat' cherries packed in passive modified atmosphere and treated with *Metschnikowia pulcherrima* L672 (MPL672; in green), *Pichia kudriavzevii* PK18 (PK18; in yellow), fludioxonil (in red), and control (in blue). Cherries were stored at 2 °C and placed 2 days at 25 °C for shelf life simulation. HSD Tukey: Tukey's honestly significant difference; SSB: statistical significance bar.

weight. Batch PK18 exhibited the highest global mean TA (4.76 ± 0.83 mg malic acid kg^{-1} fresh weight; $P < 0.001$). Finally, treatments did not affect firmness over SL, with a decrease in firmness observed in all batches throughout SL simulation, from values around 1.90 N mm^{-1} at 5 CS + 2 SL days to 1.30 N mm^{-1} at 40 CS + 2 SL days.

In relation to skin colour of cherries after SL (Fig. 5(A1), (B1), (C1)), fluctuations and differences among batches were observed, mainly at 5 CS + 2 SL days and 12 CS + 2 SL sampling points. The control batch showed the highest *L** values ($P < 0.050$) at the first sampling point (21.78 ± 6.46 units); on the contrary, batch MPL672 had the highest brightness, whereas batch PK18 was the brightest at the final sampling point (13.67 ± 8.78 units). Differences in *C** were observed at the first and second sampling points of SL. Specifically, fludioxonil (26.15 ± 13.81 units) and PK18 (17.11 ± 11.22 units) batches exhibited the most intense colour ($P < 0.050$), respectively. The hue angle *h** was higher for control and PK18 batches at 5 CS + 2 SL days, whereas at 12 CS + 2 SL days the control and MPL672 batches showed the higher values, with values above 100°. After that, *h** was around 20–30° for all batches. Flesh colour throughout SL sampling points tended to decrease slightly, with no differences among batches (Fig. 5(A2), (B2), (C2)).

After SL, at sampling points 25 CS + 2 SL and 40 CS + 2 SL days, a decrease in the four marker volatile compounds was observed with no significant differences among batches ($P > 0.050$).

DISCUSSION

The effect of MAP on the preservation of cherry quality is widely documented.[8] The literature shows the effect of atmospheres with low O_2 concentrations and with partial pressures of 1–3 kPa using polyethylene films,[37,38] as well as high CO_2 reaching

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Table 1. Total soluble solids (TSS), pH, titratable acidity (TA), and firmness of treated cherries during cold storage (2 °C)

		Control		Fludioxonil		MPL672		PK18		
Parameter	Day	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*
TSS (°Bx)	5	18.17	1.37	19.55	0.82	19.20	0.75	19.75	0.54	
	12	18.72	0.71	19.22	0.39	18.87	1.21	18.10	0.80	
	25	20.63	0.25	18.67	0.05	17.78	0.56	19.72	0.96	
	40	19.77	1.66	18.17	0.56	17.33	0.27	20.42	0.45	
	Global	19.29^{bc}	1.28	18.90^{b}	0.73	18.29^{a}	1.06	19.49^{c}	1.10	<0.001
pH	5	4.16	0.06	4.16	0.02	4.21	0.04	4.24	0.03	
	12	4.08	0.07	4.16	0.02	4.17	0.06	4.24	0.07	
	25	4.22	0.05	4.21	0.06	4.26	0.01	4.30	0.04	
	40	4.34	0.08	4.32	0.10	4.36	0.14	4.29	0.04	
	Global	4.14^{a}	0.14	4.16^{ab}	0.14	4.21^{bc}	0.15	4.21^{c}	0.12	0.014
TA (g malic acid kg^{-1} fresh weight)	5	5.14	0.48	5.12	0.36	4.82	2.54	5.21	0.23	
	12	5.09	0.32	5.24	0.30	5.04	4.38	5.12	0.46	
	25	4.57	0.30	4.60	0.21	4.43	1.73	4.65	0.28	
	40	3.69	0.22	4.09	0.46	3.69	3.36	4.19	0.14	
	Global	4.62^{b}	0.91	4.76^{b}	0.49	4.49^{a}	6.10	4.71^{b}	0.50	0.003
Firmness (N mm^{-1})	5	2.52	0.50	2.45	0.42	3.04	0.52	3.49	0.60	
	12	2.81	0.43	2.92	0.49	2.97	0.54	3.31	0.62	
	25	2.75	0.89	2.63	0.67	2.79	0.65	3.48	0.82	
	40	2.04	0.62	1.96	0.91	1.72	0.63	2.00	0.41	
	Global	2.52^{a}	0.70	2.48^{a}	0.73	2.62^{a}	0.79	3.06^{b}	0.88	<0.001

[a] Mean values with different letters indicate statistical differences ($P \leq 0.050$).
Abbreviation: MPL672: *Metschnikowia pulcherrima* L672; PK18: *Pichia kudriavzevii* PK18.

CO_2 partial pressures of 10–45 kPa using biaxially oriented polypropylene film, cast polypropylene film, and polyethylene terephthalate–polyethylene films.[27] MAP generated using Xtend® mod. 815-CH 98 bags showed a rapid decrease in O_2 (5–8 kPa) and increase in CO_2 (~7 kPa) at 5 days of CS, reducing O_2 to ~1 kPa and increasing CO_2 to ~9 kPa after 40 days. A similar evolution

Table 2. Total soluble solids (TSS), pH, titratable acidity (TA), and firmness of treated cherries during shelf life

		Control		Fludioxonil		MPL672		PK18		*P*
Parameters	Day	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
TSS (°Bx)	5 + 2	18.98	0.80	20.00	0.86	19.45	0.39	21.55	1.62	
	12 + 2	21.00	0.73	20.67	2.34	22.12	1.78	20.80	1.84	
	25 + 2	18.93	0.65	19.20	1.23	18.27	0.91	20.90	4.22	
	40 + 2	19.96	1.16	18.18	0.65	18.05	0.50	21.07	0.22	
	Global	19.72^{a}	1.18	19.51^{a}	1.63	19.47^{a}	1.92	21.07^{b}	2.30	0.001
pH	5 + 2	4.21	0.04	4.17	0.02	4.25	0.03	4.22	0.05	
	12 + 2	4.13	0.10	4.11	0.04	4.11	0.03	4.11	0.04	
	25 + 2	4.25	0.05	4.22	0.02	4.26	0.04	4.26	0.06	
	40 + 2	4.46	0.13	4.46	0.09	4.71	0.40	4.50	0.08	
	Global	4.26^{ab}	0.15	4.23^{a}	0.14	4.33^{b}	0.30	4.27^{ab}	0.16	0.048
TA (g malic acid kg^{-1} fresh weight)	5 + 2	5.05	0.27	5.26	0.33	5.14	0.40	5.55	0.27	
	12 + 2	4.84	0.16	5.19	0.59	5.31	0.33	5.50	0.30	
	25 + 2	4.09	0.17	4.15	0.12	3.86	0.39	4.19	0.36	
	40 + 2	3.60	0.46	3.64	0.18	3.26	0.13	3.84	0.25	
	Global	4.39^{a}	0.59	4.56^{a}	0.79	4.39^{a}	0.90	4.76^{b}	0.83	<0.001
Firmness (N mm^{-1})	D5 + 2	1.95	0.39	1.95	0.46	1.97	0.34	1.81	0.40	
	12 + 2	1.61	0.38	1.80	0.36	1.71	0.39	1.50	0.37	
	25 + 2	1.40	0.36	1.42	0.38	1.53	0.47	1.41	0.24	
	40 + 2	1.29	0.67	1.38	0.81	1.21	0.31	1.34	0.46	
	Global	1.42	0.50	1.62	0.56	1.64	0.47	1.55	0.42	0.236

Abbreviation: MPL672: *Metschnikowia pulcherrima* L672; PK18: *Pichia kudriavzevii* PK18.

Table 3. Contents of volatile markers during cold storage and subsequent shelf life of MAP 'Burlat' cherries

		Mean ± SD (area arbitrary units)			
	Batch	Hexanal	[*E*]-2-Hexenal	1-Hexanol	Benzaldehyde
Day 0	C	617^{a} ± 187	1631^{bc} ± 450	605^{a} ± 240	847^{c} ± 1058
Storage					
25 days (2 °C)	C	403^{b} ± 36	1548^{bc} ± 472	580^{ab} ± 107	3062^{c} ± 1645
	MPL672	478^{ab} ± 41	1690^{bc} ± 603	564^{ab} ± 416	3608^{c} ± 1579
	PK18	453^{ab} ± 136	1880^{bc} ± 744	502^{ab} ± 153	3884^{bc} ± 1169
	Flud	399^{bc} ± 91	1367^{bc} ± 449	582^{ab} ± 210	2325^{c} ± 744
40 days (2 °C)	C	771^{a} ± 282	4010^{a} ± 2201	245^{bc} ± 23	13 624^{a} ± 3905
	MPL672	156^{c} ± 76	731^{c} ± 669	139^{bc} ± 120	4871^{bc} ± 2769
	PK18	398^{bc} ± 206	1702^{bc} ± 1116	180^{bc} ± 159	5244^{bc} ± 3247
	Flud	310^{bc} ± 69	2316^{b} ± 790	252^{bc} ± 62	7360^{b} ± 1852
Shelf life					
25 days (2 °C) + 2 days (25 °C)	C	193^{c} ± 82	712^{c} ± 540	119^{bc} ± 70	3679^{bc} ± 2203
	MPL672	178^{c} ± 207	1334^{bc} ± 873	332^{b} ± 188	3284^{c} ± 641
	PK18	147^{c} ± 20	809^{c} ± 425	214^{bc} ± 63	2900^{c} ± 1056
	Flud	69^{c} ± 67	234^{c} ± 220	78^{bc} ± 9	832^{c} ± 345
40 days (2 °C) + 2 days (25 °C)	C	34^{c} ± 32	125^{c} ± 63	52^{c} ± 59	839^{c} ± 210
	MPL672	0^{c} ± 0	0^{c} ± 0	140^{bc} ± 70	2461^{c} ± 2880
	PK18	66^{c} ± 94	717^{c} ± 1133	164^{bc} ± 127	2341^{c} ± 1538
	Flud	0^{c} ± 0	455^{c} ± 428	90^{bc} ± 46	5838^{bc} ± 7053

Abbreviation: C: control; MPL672: *Metschnikowia pulcherrima* L672; PK18: *Pichia kudriavzevii* PK18; Flud: fludioxonil.
[a] Mean values with different letters indicate statistical differences ($P < 0.050$) by column.

was reported by de Paiva *et al.*[11] using microperforated biaxially oriented polypropylene films.

One of the main advantages of MAP is the control of weight losses; and the results confirmed this statement with maximum losses of less than 3%. Our results agreed with Aglar *et al.*,[30] who reported values around 1% of weight loss after 21 days of MAP storage, and with Esturk *et al.*,[27] who also reported similar values after 42 days of MAP storage. The different treatments applied in this work had scarce effect on weight loss; however, overall analysis showed a better control of weight loss in treated cherries with respect to control cherries.

The microbial population of cherries during CS is dominated by mesophilic bacteria and yeasts.[39,40] In this respect, the evolution of TAM counts agreed with those reported by Serradilla *et al.*[9] in storage of 'Ambrunés' sweet cherries under controlled atmosphere with 3 kPa O_2 + 10 kPa CO_2. Their results showed that the dominant population of microbes (~5 $\log_{10}$ CFU g^{-1}) corresponded to mesophilic and psychrotrophic bacteria. In our case, yeast counts showed a similar evolution to TAM up to 12 days of CS; thereafter, a slight decrease was detected in the control, fludioxonil and PK18 batches towards the final storage. *P. kudriavzevii* PK18 was isolated from vineyards;[33] thus, low temperatures and environmental differences could hinder its implantation on cherries. Colony morphology and genomic identification confirmed the dominance of *P. kudriavzevii* throughout the CS period on PK18 cherries; however, the decrease in counts at the end of storage could be related to changes in atmosphere and/or cherry composition. The yeast population of non-inoculated cherries was dominated by Ascomycota yeast-like fungi, such as *Aureobasidium* spp., and *Cystofilobasidium* spp., in agreement with the identifications reported by Serradilla *et al.*[9] and Stanevičienė *et al.*[41] Cherries inoculated with MPL672 showed the highest counts and successful establishment and development throughout CS. Previous studies demonstrated that *M. pulcherrima* is able to compete and colonise fruit surfaces even at cold temperatures in high-CO_2 atmospheres.[11,24]

SL trials showed an increase in TAM counts throughout the sampling points, reaching maximum values of 6.63 ± 0.19 $\log_{10}$ CFU g^{-1}, below the acceptability limit of 8 $\log_{10}$ CFU g^{-1} for fresh products stored in MAP.[42] Interestingly, fludioxonil and inoculations with antagonistic yeast reduced the increase in TAM at sampling points during SL ($P < 0.001$). The global mean increase in TAM counts for untreated cherries was 2.03 ± 1.47 $\log_{10}$ CFU g^{-1} according to Serradilla *et al.*,[9] whereas 0.56 ± 0.59 $\log_{10}$ CFU g^{-1}, 0.43 ± 0.43 $\log_{10}$ CFU g^{-1}, and 0.66 ± 0.51 $\log_{10}$ CFU g^{-1} were the global mean increases for fludioxonil, PK18, and MPL672 batches respectively. However, these differences were not detected among batches when comparing yeast counts. The global mean increase in yeast counts between CS and SL was from 0.37 ± 1.20 $\log_{10}$ CFU g^{-1} of untreated cherries to 1.08 ± 1.09 $\log_{10}$ CFU g^{-1} of fludioxonil-treated cherries.

High-CO_2 atmospheres confer an effective control of browning and fungal decay in 'Lapins' and '0900 Ziraat' sweet cherries, as reported by Tian *et al.*[43] and Aglar *et al.*[30] respectively. Our results showed a lower effectiveness of MAPs in controlling rot than the aforementioned studies, with percentages higher than 10% at 12 days of CS. The different susceptibility of cultivars to rot could explain the reason for these high percentages.[31] Combined applications of MAPs with antifungal substances or antagonistic microbes have shown better control of rots from the first sampling point at 5 days of CS, although significant reduction appeared only on the final sampling day at 40 days of CS. The antagonistic mechanisms reported in *M. pulcherrima*, competition for nutrients such as iron depletion,[32,44] parasitism,[32] and induction of host defence

response,[45] and in *P. kudriavzevii* the production of antifungal volatile organic compounds[33] may be involved in the control of common cherry postharvest pathogens, such as *Monilinia* spp., *Cladosporium cladosporioides*, *B. cinerea*, *P. expansum*, and *Penicillium glabrum*. The application of these three antifungal treatments was also effective in controlling decay during SL, mainly at the final sampling point, where significant reductions were observed ($P < 0.050$).

Previous studies indicated that MAP slows down the increase in soluble solids concentration (SSC) and pH and delays reductions in TA and firmness during CS, and the subsequent influence of SL simulation on these parameters.[22,30] The results shown in Tables 1 and 2 are consistent with previous literature. Nevertheless, combined treatments with MAP can interfere in these parameters; thus, Parka + MAP and natural antifungal compounds + MAP delayed the effects of senescence on SSC, pH, TA, and firmness with respect to MAP during CS.[30] The application of antagonistic microbes does not usually modify fruit quality traits.[46,47] However, treatment of cherries with PK18 and MPL672 conferred slight but significant changes on quality traits during CS. These results agree with those obtained by Qin *et al.*[48] and Tian *et al.*,[49] who showed changes in grapes after *Hanseniaspora uvarum* inoculation and in mango inoculated with *M. pulcherrima* respectively. The observed effects on cherry quality parameters associated with inoculated batches did not indicate a loss of quality compared with control cherries; in fact, some parameters showed better values in CS, such as SSC and firmness evolution. In contrast, skin colour exhibited some relevant differences among batches, mainly at the beginning of the sampling period. However, there is limited literature analysing the impact of antagonistic microbes on colour parameters. In this regard, application of *Debaryomyces nepalensis* on mango slightly modified L^*, a^*, and b^* coordinates of mango after 30 days of storage.[50] Interference in cherry skin colour by antagonistic yeast such as *M. pulcherrima* could be related to the ability to modify the synthesis of some endogenous enzymes, such as polyphenol oxidase, peroxidase, and particularly phenylalanine ammonia lyase.[51] These enzymes are involved in the synthesis and stability of anthocyanins, which are the main pigments that characterise cherry skin colour.[4] Guo *et al.*[52] also observed the stimulation of anthocyanin accumulation in blood oranges of the Tarocco cultivar (*Citrus sinensis* L.) after application of *Bacillus amyloliquefaciens*.

To determine the effect of treatments on volatile compounds in cherries, three marker compounds were selected: hexanal, [*E*]-2-hexenal, and 1-hexanol, related to fresh fruit flavours and positively correlated with desirable flavours in cherry,[53,54] and benzaldehyde, associated with bitter almond flavour[55] and negatively associated with panellists' preferences.[54] Treatments with antagonistic yeasts showed a similar evolution of marker volatiles to that of the fludioxonil batch during CS and SL. Compared with the control batch, a decrease in volatile compounds associated with fresh cherry aroma was observed at 40 days; nevertheless, there was a lower presence of benzaldehyde associated with lower cherry assessments by panellists.[54]

CONCLUSIONS

The storage of 'Burlat' cherries in MAP (~1.5 kPa O_2, ~9 kPa CO_2) at 2 °C provides adequate quality maintenance during 40 days of storage. The combination of MAP with the antagonistic yeasts *M. pulcherrima* L672 and *P. kudriavzevii* PK18 increases the control of microbiological spoilage with results comparable to the application of fludioxonil. No significant differences in cherry quality traits were observed with these yeast antagonist treatments. The same effect of control of spoilage was observed during SL at 25 °C for 2 days, so the control effect of yeasts on spoilage moulds is prolonged in conditions of higher temperature and aerobic atmosphere.

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DATA AVAILABILITY STATEMENT

Research data are not shared.

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