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Impact of the Pre-Harvest Biocontrol Agent and Post-Harvest Massive Modified Atmosphere Packaging Application on Organic Table Grape (cv. '*Allison*') Quality during Storage

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Abstract: The marketing value of table grapes is contingent upon several quality requirements, mostly related to microbial decay, sugar/acidity ratio, and colour. This research explores the impact of combining organic-cultured compatible techniques to delay disorders along with organic grape distribution in post-harvest. *Aurebasidum pullulans* in-field application on grape bunches at three growing stages as a biocontrol agent against grey mould growth coupled with massive modified atmosphere packaging (MMAP; 20% CO_2, 10% O_2) equipped with a breathable valve was tested. The in-field treatment had a significant impact on the colour and sugar content of the grapes at harvest and the mould count evolution during storage, whilst the trend of the other parameters was mainly affected by the interaction of the variables tested. The untreated batch experienced the worst behaviour and the packaging was paramount in preserving the moisture content and appearance of the bunches. The findings of this study may contribute to developing novel practices for setting a smart distribution of organic table grapes and reducing food waste.

Keywords: *A. pullulans*; biocontrol; MAP; breathable film; post-harvest; packaging

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1. Introduction

Table and wine grapes (*Vitis vinifera* L.) are widely cultured in Italy, with an annual production of 8,437,970 tonnes, ranking third in the world [1]. With a constant annual output of about 865,839 tonnes in 2023 [2], Italy serves as Europe's primary table grape supplier. The majority of these grapes are sent to Northern Europe, particularly the organic ones.

Table grapes are susceptible to severe pathological and physiological decay that dramatically impairs the quality and reduces marketability after harvesting. Low storage temperatures (–1 ± 0 °C) and high relative humidity levels (>90%) are paramount to protecting table grapes against drying post-harvest [3]. Nonetheless, even during cold storage, some disorders could appear due to the high level of relative humidity and unfavourable oxygen (O_2) and carbon dioxide (CO_2) partial pressure inside the cold storage room. Grey mould, stem browning and desiccation, softening, and water loss are the main causes of losses occurring in post-harvest even at low temperatures [4–6].

In conventional production, table grape bunches are treated using sulphur dioxide (SO_2) to reduce the fungal decay, primarily caused by *Botrytis cinerea*, the aetiological agent of grey mould [7,8]. SO_2 treatment may cause unacceptable bleaching injuries on berries, such as colour changing, pitting, and compromising their flavour [9,10]. Furthermore, the

emerging attention to the exposure in sensitive consumers has prompted the US Food and Drug Administration to reduce SO_2 post-harvest use and have mandated the labelling of sulphites when residual levels exceed 10 μL L^{-1} [11] and the European Union to forbid post-harvest the use of SO_2 for organic crops [12]. Without SO_2 treatments, the life of the product does not exceed seven days at room temperature or roughly fourteen days at 0–2 °C [5,13].

Nowadays, many alternative techniques for SO_2 have been developed both at the pre- and post-harvest stages. Among in-field operations, biocontrol agents (BAs) are a promising alternative to reduce the incidence of grey mould decay caused by *B. cinerea*. BAs are antagonistic microorganisms such as fungi (*Aureobasidium pullulans*, *Trichoderma atroviride*) and bacteria (*Bacillus subtilis* QST 713, *Bacillus amyloliquefaciens* FZB24) that are used to counteract fungal diseases or plant pathogens, and their efficacy depends upon the temperature and humidity level of the environment [14,15]. *A. pullulans* can inhabit a wide variety of habitats, including saline, cold, water-stressed, and low-nutrient environments [16] and guarantee a high level of control of *Botrytis* spp. thanks to its high tolerance to various ecological stresses, antifungal activity, and biostimulant properties, chiefly due to pullulan production [17,18].

Different shock treatments in post-harvest to reduce organic table grape decay have been successfully explored, such as precooling [19], CO_2 [20], ozone (O_3) [21], ethanol (C_2H_6O) [22], followed by warehouse storage simulation in a controlled atmosphere (CA) environment of a massive bulk product (≥5 kg) in a box or modified atmosphere packaging (MAP) of small amount of product (<500 g) intended for household consumption. In those conditions, the shelf life of organic table grapes could be extended up to 90 days (Table 1).

Table 1. Post-harvest techniques to delay disorders in organic table grapes.

Grape Cultivar	Pre-Treatment	Tray/Bag/Tank Material	Mass	Atmosphere Composition	Storage Condition	Shelf Life	Reference
Redglobe	Precooling 0 °C	Sealed aluminium tank CF	NS	CA CO_2: 10%, O_2: 3/6/12%	0 °C	90	[19]
Flame Seedless	%CO_2 40 for 48 h	Sealed metal tank CF	NS	CA O_2: 12%, CO_2:12%	1 °C, 95% RH	66	[20]
Scarlotta Seedless	50–70% CO_2 for 24 h; 5/10/ 20 ppm O_3 for 30 min	PA/PE	NS	MAP O_2: 2%, CO_2: 5%	0 °C 95% RH	45	[21]
Palieri	Ethanol 50% for 5 min	OPP	100 g	MAP O_2: 5/10/15% CO_2: 3%	5 °C	70	[22]
Flame Seedless	-	Perforated OPP	140 g	Air	1 °C 90% RH	20	[23]
Superior Seedless	Pre-cooling 0 °C	Microperforated OPP/PP	500 g	Air	7 d at 0 °C, 80–90% RH, 4 d at 8 °C	12	[24]

CF: Continuous flow; NS: Not Specified; CA: Controlled Atmosphere; MAP: Modified Atmosphere Packaging; PA: Polyamide; PE: Polyethylene; OPP: Oriented Polypropylene; PP: Polypropylene; CO_2: Dioxide Carbon; O_3: Ozone.

The target of these techniques is to reduce water loss, mitigate the shrivelling of stems and berries and slow down the product's and endogenous microflora's metabolic activities. Storage of bulk table grapes, arranged in 5–8 kg per cardboard box, in CA environments

represents an effective technique to increase the shelf-life of organic table grapes [19,20], but it is eligible for the most innovative food industries. Currently, the major Italian table grape producers' factories are warehouses rather than food industries, and the exploitation of the above-mentioned techniques is still unusual or poorly spread.

On the other hand, MAP is a low-cost technology widely spread to preserve the quality of small amounts (<500 g) of packaged fruit and vegetables (F&V) during retail and household storage, as it allows to extend the shelf-life length of organic table grapes up to over 30 days [22–25]. The principle of MAP is to fill the package headspace with a specific gaseous mixture able to slow down the decay and chemical-physical disorder of the berries and rachis. The optimal gaseous mixture must contain a low O_2 percentage (%) to reduce the respiration rate of the product and avoid anoxia and a low-medium $\%CO_2$ as a hindrance against the growth of microorganisms, especially moulds.

Furthermore, through storage, because of the product respiration and microbiological activity, the initial gas ratio in the surrounding headspace in MAP is, unavoidably, altered. This process leads to the increase of CO_2 percentage and reduction of O_2 up to the steady state, with time characteristics dependent on the temperature, initial gaseous mixture, and packaging system involved. Impermeable packaging leads to high acetaldehyde and ethanol production even if packaged in a modified atmosphere [5].

The performance of the packaging materials to be permeable to water vapour or perm-selective to O_2 or CO_2 is crucial to avoid anoxia and high carbon dioxide-associated disorders, such as the onset of anomalous flavours and the browning of rasp and berries [5,13,24].

Researchers have developed a multitude of films made with different technologies, making their surface more or less permeable to one or more gases or volatile compounds. Gas-permeable packages made with micro- or macro-perforated films avoid the stack of humidity and, if properly designed (i.e., hole size and density adjusted for the items and storage conditions), they guarantee an approximate control of the O_2 and CO_2 diffusion throughout the holes. Concerning emerging food packaging solutions, a micro-machined adhesive with a micro-structure (BD) [26] allows the bidirectional gas flow to provide breathable properties and gas selectivity to every kind of film used for packaging.

The device's physical characteristics have been thoroughly documented, and it underwent testing to store small quantities (less than 0.5 kg) of fresh-cut products [27].

Given the potential of *A. pullulans* as BA and of the MAP, we tested the combination of these approaches to mitigate post-harvest decay and preserve the quality standards over the long-term storage of organic table grapes packaged with MMAP. The goal is to point out organic-compatible storage protocols to meet the large-scale retail logistical requests in terms of packaging and mass, that are up to market. Using an MMAP, after an initial bulk storage phase, the manufacturer could properly re-package the grapes based on the type and weight of packaging requested by the market, based on the commercial destination of the product.

2. Material and Methods

2.1. Plant Material and Biocontrol Treatment

Late-season organic table grape (*Vitis vinifera* L.) cv. 'Allison' was cultured in Gioia del Colle (40°48′07″ N, 16°51′44″ E, Bari province, Apulia region) using organic practices. The vineyard (2 ha) was planted in 2018, using 1103 P as a rootstock, and the trial was conducted in 2022. A formulation containing *Aureobasidium pullulans* (DSM 14940 and DSM 14941 strains, 2.5×10^{11} CFU of each; Manica, Italy) was applied in the field according to the producer's recommendations (BA treatment); water was used as a negative control. Treatments were carried out at the bunch pre-closure stage (BBCH 75–77), early veraison (BBCH 81), and preharvest (BBCH 89). Applications were arranged in a completely randomized block design with four replicates made of six plants each. Both microorganism-treated and water-treated blocks and replicates were separated by a buffer of untreated plants. Treatments were carried out using a commercial motor-driven back sprayer Fox Sprayers

40007 (Fox Motori snc, Poviglio, Italy) delivering an amount of solution corresponding to spraying 1000 L/ha (pressure 20 atm).

2.2. Packaging Equipment and Conditions

The grape was harvested in the morning (before 7 a.m.), on 6 October 2022. Randomly, from each block of the *A. pullulans* and water-treated grapes were collected the bunches up to arrange in-field 30 plastic cardboard boxes per treatment. The cardboard boxes were delivered to the laboratory within 2 h and then selected by trained personnel for size, colour, consistency uniformity, and absence of evident defects or diseases of the bunches.

Cardboard boxes were filled with unwashed bunches of about 350 ± 10 g each, up to reach the weight of 5 ± 0.1 kg per cardboard box (Figure 1). For each treatment (BA, Control), 20 out of 30 cardboard boxes were placed into LDPE bags (40 µm thickness, O_2 and CO_2 permeability, respectively, of 160 and 520 $cm^3/m^2/24$ h/bar at 23 °C; Gopack, Italy) and were filled with 10% O_2, 20% CO_2 and 70% N_2 and sealed using the MMAP equipment. A prototype machine for the MMAP of F&V (Figure 2) was designed and built by the Laboratory of Machine and Plant for Food Industry Processing (MAC-Lab) of the University of Basilicata.

Figure 1. *A. pullulans*-treated (BA) and untreated (Control) grapes upon their arrival in the laboratory.

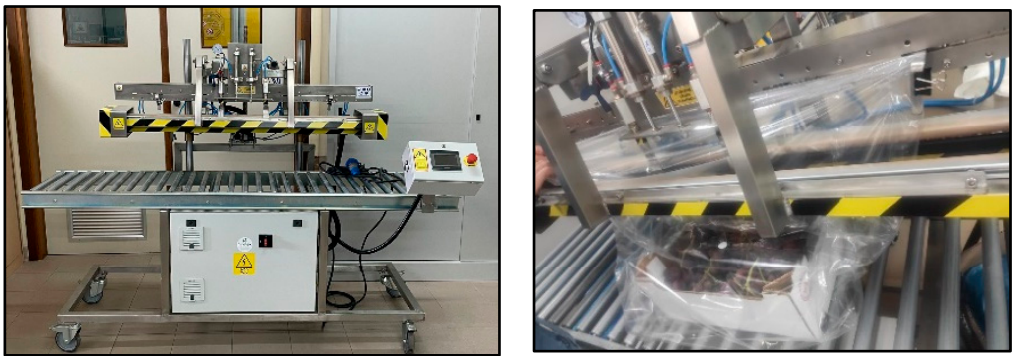

Figure 2. Pics of the MMAP machine at MAC-Lab (**left side**) and during the packaging cycle (**right side**).

Here, 10 out of 20 cardboard boxes were heat sealed with the device previously described [26] in correspondence of a 500 µm hole hand-made on film by a hot needle. Therefore, for every treatment, the following three packaging conditions were compared:

- Sealed packaging (SP) with 20% CO_2 and 10% O_2
- BlowDevice® (BlowDevice Ltd., Potenza, Italy) (International patent: PCT/IB2016/0506600) equipped packaging (BD) with 20% CO_2 and 10% O_2
- Open packaging (OP)

After the packaging, the samples were stored at 2 ± 1 °C, 80% RH. The quality standards were measured on day 0 in the fresh group and day 30 in the SP, BD, and OP groups. Therefore, 10 cardboard boxes from each test condition were arranged. From each replication (cardboard box), 15 berries were taken randomly and analysed in the following order: chemical-physical (colour), and mechanical analyses (detachment and firmness). Afterwards, the juice was squeezed from the berry and 1 mL was used for brix and acidity measurement.

2.3. Quality Standard Assessment

2.3.1. Mass Loss

The percentage variation of the net grape mass (% ML) in the cardboard box was determined by the weight difference between the initial net mass and the mass at each sampling time, using a precision digital electronic scale (±0.01 g) (Gibertini Europe, Italy) on 5 cardboard boxes.

2.3.2. Chemical and Physical-Chemical Attributes

The juice from 15 berries from each cardboard box was extracted to obtain a homogeneous sample to measure soluble solids content (SS) and titratable acidity (TA).

SS was measured by a digital refractometer (Atago, Japan). TA was determined by titration with 0.1 N NaOH up to pH 8.1, [28] and expressed in grams of tartaric acid per litre of grape juice. The colour of the skin was evaluated by a colorimeter (Minolta CR 400 ChromaMeter, Minolta Corp., Tokyo, Japan), on 15 berries for each cardboard box.

The parameters of the colour, L* (brightness) corresponding to a black-white scale (where 0 is black and 100 is white), a* (red trend), and b* (yellow trend), were recorded on 15 berries using the CIELAB colorimetric system. Using these values, the colour index of red grapes (CIRG) was measured [29].

2.3.3. Mechanical Properties of Berries

The hardness of 15 berries per cardboard box was measured with the compression test performed using a Universal Instron's 3340 Series Electromechanical Machine (Norwood, MA, USA) equipped with flat probe P/35 and load cell of 500 N. Operative conditions were selected on research results presented in the literature [30]. Berry detachment force was determined using the traction probe of the same machine at the speed of 1 mm/s. Data acquisition took place at 400 Hz using the dedicated Bluehill 2015 software version 3.66.41.60.

2.3.4. Total Counting Bacteria and Mould

Before the analysis, the grape suspensions were arranged. Briefly, for every treatment, the berries were collected randomly from each cardboard box and placed in a sterile bag up to 100 g; 3 bags per treatment were arranged. Each sample (100 g) in the bag was mixed with 900 mL of distilled sterilized H_2O (original suspension). The sample was crunched for 2 min and then the decimal dilutions were prepared by progressively pipetting 1 mL of the original suspension in 9 mL of water, and progressive decimal dilutions were prepared. For TBC analysis, 1 mL of previously prepared dilutions 10^{-3} and 10^{-5} were placed in a sterile Petri dish and about 15 mL of plate count agar media (Liofilchem, Roseto degli Abruzzi, Italy) was poured into the dish, and then incubated at 30 °C for 24 h [31]. Whereas, for TMC count one mL of previously prepared dilutions 10^{-2} was placed in a sterile Petri dish and about 15 mL of PDA media was added, and then incubated at 22 ± 2 °C for 96–120 h under aerobic conditions [32]. The total bacteria (TBC) and mould (TMC) colonies in grapefruit samples were expressed as colony form units per millilitre (cfu/mL). For every cardboard box, three biological replications were used, and each one was analysed in duplicate.

2.4. Statistical Analysis

Data were analysed using MatLab software v. R2016a. For every data set of the parameters investigated, the normality of the distribution was assessed. When consistent, a two-way analysis of variance was performed to investigate the effect of the pre-harvest treatment, the packaging system, and their interaction on each parameter. A general linear model was set using the packaging and the treatment as factors and the parameter investigated as a response. Tukey's honestly significant difference test was used to determine any significant difference ($\alpha = 0.05$) between the means. Therefore, in the table for each parameter investigated the values that do not share the same letters are significantly different.

3. Results and Discussion

3.1. Colour

The results of the colour CIELAB coordinates measured for the skin's berries are shown in Table 2. Statistical analysis (Table 3) suggests the treatment had a greater impact ($p = 0.000$) on the L* variability than packaging ($p = 0.01$). The L* average value at harvest of the control (33.61 ± 0.98) was significantly different from the BA-treated grapes (35.09 ± 1.01) and after the cold storage did not change and was quite similar in all the packaging conditions, apart from the BA-OP grapes that experienced after 30 days a significant L* value decreasing. Similar results were found by Burçak et al. [33] along with 30 days of passive MA storage of five organically cultured table grapes cultivars that experienced lowering or retaining of L*.

Table 2. Results of the grapes characterization expressed as the mean ± standard deviation at the harvest (Fresh) and after 30 days in the different packaging conditions (BD, SP, OP) of *A. pullulans*-treated (BA) and untreated (Control) grapes. For each parameter, values that do not share the same letters are significantly different. Different capital letters indicate significant differences between packaging conditions with the same pre-treatment. Different lowercase letters indicate a significant difference between pre-harvest treatment within the same packaging condition.

Parameter	Treatment	Fresh	CV%	Products BD	CV%	SP	CV%	OP	CV%
Mass Loss (%)	BA	-	-	$0.14 \pm 0.02^{\text{Ba}}$	17.55	$0.04 \pm 0.01^{\text{Aa}}$	25.00	$5.75 \pm 0.47^{\text{Ca}}$	8.20
	Control	-	-	$0.12 \pm 0.01^{\text{Ba}}$	12.06	$0.06 \pm 0.01^{\text{Aa}}$	22.91	$6.23 \pm 0.55^{\text{Ca}}$	8.83
Acidity (g/L)	BA	$7.18 \pm 0.49^{\text{Aa}}$	6.84	$6.61 \pm 0.14^{\text{Aa}}$	2.24	$6.75 \pm 0.54^{\text{Aa}}$	8.06	$6.68 \pm 0.44^{\text{Aa}}$	6.66
	Control	$6.72 \pm 0.37^{\text{Aa}}$	5.51	$6.51 \pm 0.19^{\text{Aa}}$	3.04	$6.78 \pm 0.11^{\text{Aa}}$	1.67	$6.45 \pm 0.44^{\text{Aa}}$	6.80
pH	BA	$3.78 \pm 0.04^{\text{Ba}}$	1.15	$3.73 \pm 0.07^{\text{Ba}}$	2.08	$3.63 \pm 0.01^{\text{Ba}}$	0.38	$3.39 \pm 0.01^{\text{Aa}}$	0.42
	Control	$3.75 \pm 0.01^{\text{Ba}}$	0.41	$3.78 \pm 0.04^{\text{Ba}}$	1.31	$3.56 \pm 0.07^{\text{Ba}}$	1.99	$3.52 \pm 0.01^{\text{Aa}}$	0.20
SSC (°Brix)	BA	$20.58 \pm 0.80^{\text{Ba}}$	3.93	$18.24 \pm 1.16^{\text{Aa}}$	6.41	$18.46 \pm 1.41^{\text{Aa}}$	7.64	$20.02 \pm 0.60^{\text{Ba}}$	3.03
	Control	$21.81 \pm 2.15^{\text{Cb}}$	9.89	$18.40 \pm 1.09^{\text{Aa}}$	5.93	$21.36 \pm 2.49^{\text{BCb}}$	11.68	$19.34 \pm 0.62^{\text{ABa}}$	3.23
L*	BA	$35.10 \pm 1.01^{\text{Bb}}$	2.88	$35.29 \pm 1.34^{\text{Bb}}$	3.79	$34.44 \pm 1.61^{\text{ABb}}$	4.68	$33.83 \pm 1.37^{\text{Aa}}$	4.05
	Control	$33.61 \pm 0.98^{\text{Aa}}$	2.91	$34.12 \pm 0.91^{\text{Aa}}$	2.66	$33.53 \pm 1.17^{\text{Aa}}$	3.49	$33.75 \pm 0.72^{\text{Aa}}$	2.12
a*	BA	$6.79 \pm 0.73^{\text{Aa}}$	10.76	$7.91 \pm 0.47^{\text{Ca}}$	6.05	$7.13 \pm 0.63^{\text{ABa}}$	8.85	$7.33 \pm 0.52^{\text{Ba}}$	7.21
	Control	$7.00 \pm 0.66^{\text{Aa}}$	9.50	$7.75 \pm 0.68^{\text{Ba}}$	8.74	$6.95 \pm 0.71^{\text{Aa}}$	10.32	$7.07 \pm 0.74^{\text{Aa}}$	10.56
b*	BA	$10.60 \pm 0.86^{\text{Ab}}$	8.14	$11.85 \pm 0.56^{\text{Ab}}$	4.76	$11.01 \pm 0.66^{\text{Ab}}$	6.00	$11.04 \pm 6.67^{\text{Ab}}$	6.04
	Control	$10.60 \pm 0.47^{\text{Aa}}$	4.46	$11.56 \pm 1.16^{\text{Aa}}$	10.08	$10.78 \pm 0.60^{\text{Aa}}$	5.54	$10.97 \pm 0.51^{\text{Aab}}$	4.64
CIRG	BA	$2.57 \pm 0.06^{\text{Ba}}$	2.72	$2.50 \pm 0.10^{\text{Aa}}$	4.21	$2.58 \pm 0.11^{\text{Ba}}$	4.59	$2.62 \pm 0.07^{\text{Ba}}$	2.78
	Control	$2.66 \pm 0.05^{\text{Bb}}$	1.83	$2.60 \pm 0.08^{\text{Ab}}$	3.17	$2.64 \pm 0.06^{\text{Bb}}$	2.28	$2.62 \pm 0.04^{\text{ABa}}$	1.60
Detachment	BA	$2.10 \pm 0.84^{\text{Aa}}$	40.11	$3.25 \pm 1.77^{\text{Aa}}$	54.60	$2.62 \pm 0.83^{\text{Ab}}$	32.01	$2.92 \pm 1.16^{\text{Ab}}$	39.77
	Control	$2.76 \pm 1.36^{\text{Ba}}$	49.40	$2.28 \pm 1.12^{\text{ABa}}$	49.02	$1.60 \pm 0.60^{\text{Aa}}$	37.58	$1.97 \pm 0.80^{\text{ABa}}$	40.60
Firmness	BA	$20.66 \pm 6.22^{\text{Aa}}$	30.13	$24.69 \pm 5.96^{\text{Aa}}$	24.13	$25.58 \pm 11.7^{\text{Aa}}$	45.99	$28.51 \pm 7.98^{\text{Aa}}$	28.02
	Control	$21.53 \pm 5.32^{\text{Aa}}$	24.72	$26.63 \pm 8.66^{\text{Aa}}$	32.51	$24.70 \pm 9.26^{\text{Aa}}$	37.49	$24.05 \pm 7.90^{\text{Aa}}$	32.08
LogTBC (cfu/mL)	BA	$2.80 \pm 0.15^{\text{Aa}}$	5.39	$5.75 \pm 0.18^{\text{Cb}}$	3.18	$5.27 \pm 0.33^{\text{Bb}}$	6.27	$5.45 \pm 0.14^{\text{Ba}}$	2.74
	Control	$2.68 \pm 0.18^{\text{Aa}}$	6.56	$3.77 \pm 0.25^{\text{Ba}}$	6.67	$4.66 \pm 0.20^{\text{Ca}}$	4.36	$6.38 \pm 0.10^{\text{Db}}$	1.65
LogTMC (cfu/mL)	BA	$2.41 \pm 0.17^{\text{Ab}}$	7.06	$2.44 \pm 0.31^{\text{Aa}}$	12.92	$2.97 \pm 0.15^{\text{Ba}}$	5.31	$2.91 \pm 0.26^{\text{Ba}}$	9.09
	Control	$1.75 \pm 0.17^{\text{Aa}}$	9.44	$3.40 \pm 0.44^{\text{BCb}}$	13.01	$3.20 \pm 0.07^{\text{Bb}}$	2.46	$2.86 \pm 0.12^{\text{Ca}}$	4.32

Table 3. The *p*-value calculated for each variability source by two-way ANOVA.

Parameter	*p*-Value		
	Packaging (P)	Treatment (T)	PxT
Mass Loss	0.000	0.256	0.297
Acidity	0.858	0.092	0.979
pH	0.000	0.691	0.037
SSC	0.025	0.000	0.012
L*	0.017	0.000	0.194
a*	0.000	0.994	0.966
b*	0.000	0.237	0.861
CIRG	0.000	0.000	0.048
Detachment	0.536	0.269	0.936
Firmness	0.927	0.907	0.558
TBC	0.000	0.014	0.000
TMC	0.083	0.116	0.019

Contrary to L*, the a* (redness) and b* (yellowness) values vary with characteristics depending mostly on the packaging (p = 0.000). At harvest, these values were similar both in BA and control grapes, and they increased significantly in both treatments when packaged using BD. Low variations were observed for b* in Control-OP and a* in BA-OP. The higher a* value found in BD may indicate a major proportion of anthocyanidins in this condition, which are the main polyphenols found in grapes [34] responsible for red colour intensity.

CIRG measured allowed the assessment of the external colour of the berry according to the criteria proposed by Carreño et al. [29], suggesting the grapes presented a pink to red skin colour, ranging between 2.57 (BA) and 2.66 (Control) at harvest. *A. pullans* in-field treatment was responsible for slight CIRG variation at harvest for the control grapes. After the storage, the evolution of the skin colour was influenced by the packaging system used and its interaction with the factor treatment. A shifting from red to pink in both treatments, that is a reduction of initial CIRG, was observed in those bunches packaged with BD, whilst SD and OP retained the initial values.

CIRG reduction along with cold storage was observed by Admane et al. [21] in organic cultured Sugranineteen Seedless after several CO_2- and O_3-based shock post-harvest treatments, followed by storage in MAP (2%O_2–5%CO_2). In that study, only the grapes treated with O_3 at 20 μL L^{-1} for 30 min retained CIRG value, confirming that the extent and type of pre-packaging treatment affect the colour. Although the impact of packaging on table grapes quality standards has been extensively studied in the literature, there is a lack of reporting in-field A. pullulans-based treatment influence on berry skin colour. Several authors have, however, evaluated its impact on winemaking. In our experiment, *A. pullulans*-treated grapes had different L* and b* values compared to the control, similar results were found by Merini and de Ambrosini [35,36], who reported higher total anthocyanins, total polyphenols and colour intensity (CI) of Malbec wines produced using *A. pullulans* GM-R-22, suggesting *A. pullulans* contribute to enhance the colour and the antioxidant capacity of red wine.

3.2. Weight Loss

Table 2 shows the results of mass loss, acidity, pH, and SSC of the product at harvest (Fresh) and after 30 days of storage in different packaging conditions. The mass loss had a very different trend due to the packaging system used (p = 0.000), whereas there is no effect of the *A. pullulans* treatment on its evolution. As expected, the unpackaged product (OP) met the greatest mass loss (5.75–6.23%), resulting in excessive drying of the peduncle and rachis (Figure 3). The lowest values were detected in sealed box (SP) ranging between 0.04 and 0.06%, whereas in BD, it was ten times higher (0.12–0.14%). In our experiment, the relative humidity in the cold room was set at around 80%; therefore, the higher weight loss in OP was expected, whilst in the other packaging tested it was negligible. This confirms

that LDPE film is an effective water vapour barrier [37] and that it can be used to store table grapes without causing significant weight loss. Broadly, weight loss occurs during cold storage as a natural process depending upon the temperature, packaging material, relative humidity of the storage environment, and grape variety. In a previous study [38], weight loss of *Red Globe* table grapes packaged in PP or PET film with a modified atmosphere ranged between 5 and 12% after 21 days of storage at 5 °C.

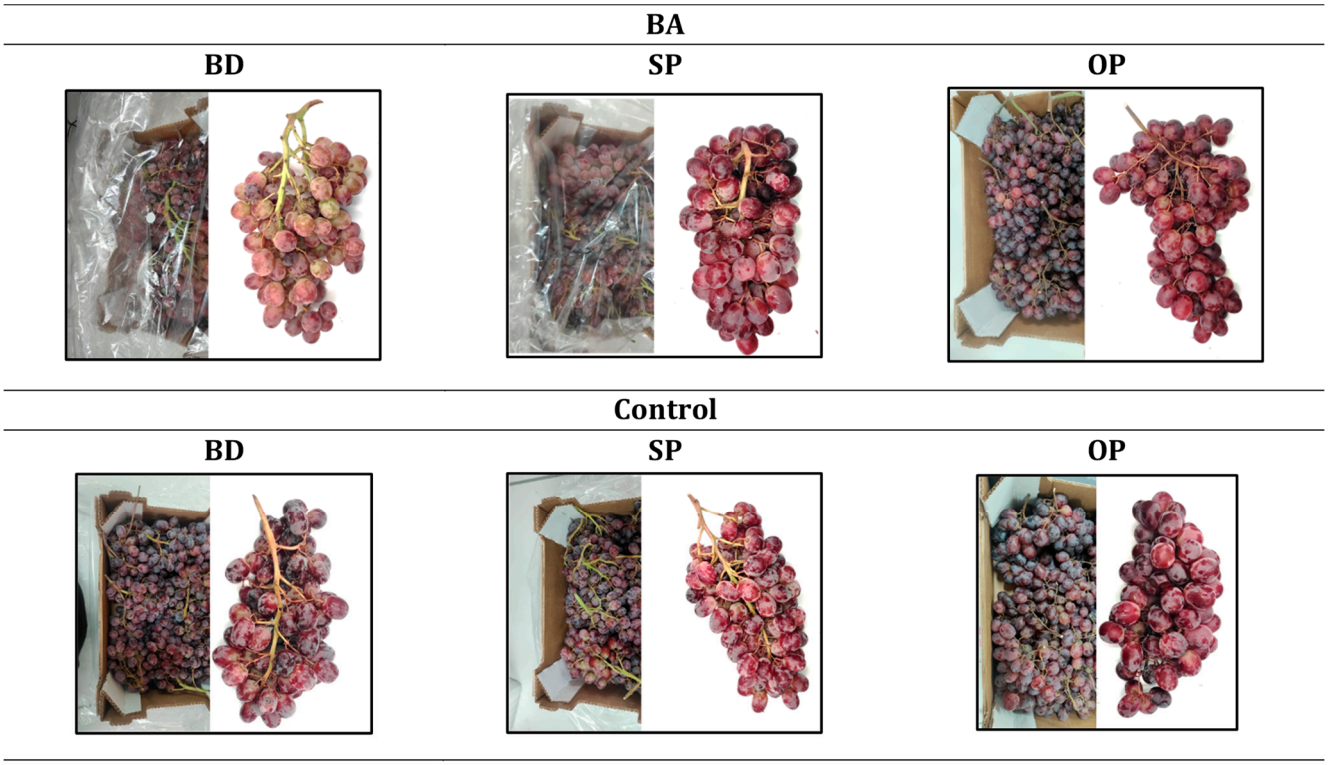

Figure 3. Bunches of the in-field *A. pullulans*-treated (BA) and untreated (Control) grapes after 30 days of storage in the tested packaging conditions (BD, SP, OP).

Mass loss lower than 1% was also reported by Costa et al. [22] in *Michele Palieri* table grapes packaged using various modified atmospheres and wrapped with oriented polypropylene (OPP) of 20 µm thickness. In contrast, breathable films ease mass loss as, if not properly designed, they do not represent a hindrance against water evaporation during storage. Martínez-Romero et al. [23] found mass loss of up to 1.5 and 4% after 18 days of storage at 1 °C in *Flame Seedless* packaged using, respectively, perforated and non-perforated OPP film. Five cultivars of organic table grapes packaged under passive MAP with sealed bags experienced up to 2% weight loss after 30 days of storage at 0.5 °C, where the lowest values (0.5%) were found in red grape *Vitis vinifera* L. cv. 'Çeşme Pembesi' [33].

3.3. Acidity, pH, and SSC

Over the course of storage, berries' SSC, TA, SSC/TA, and pH changed (Table 2) because of the breakdown of insoluble polysaccharides into soluble sugars and organic acids during respiration and of the water loss [39]. The average titratable acidity at harvest, expressed as tartaric acid (g/L), ranged between 6.72 and 7.18 for BA and Control samples, respectively. The values were not significantly different at harvest, and during storage, they did not change deeply. Slight variations were observed but without any influence of the factors tested. This is in general agreement with the results of several studies conducted on different grape cultivars, such as *Flame Seedless* [23], which reported the MAP did not affect acidity, even with high $\%CO_2$ (30–40) in the headspace [38]. On the other hand, the titratable acidity of *Thompson Seedless* decreased after 30 and 60 days of storage in air at 1 °C [40]. As

for acidity, the pH of the grapes at harvest was unaffected by the in-field treatment. pH variability is mostly due to the packaging system (p = 0.000), as it decreased significantly in OP, probably it may due to the water evaporation. The bunches treated with *A. pullulans* if packaged with BD or sealed (SP) did not experience any significant pH variation, in agreement with other studies that previously reported MAP did not influence pH [41,42]. The SSC average values of the *A. pullulans*-treated and untreated grapes at harvest were significantly different, respectively, 20.58 and 21.81 °Brix. The SSC at end storage decreased in all conditions, with different trends. Statistical analysis suggests the treatment and storage period the most influenced SSC (p = 0.000), while the packaging system used had a weak influence (p = 0.026). The lowest value reached was observed in those samples packaged with BD, in both BA and Control samples. Concerning the interactions of the variables, treatment*packaging was quite relevant (p = 0.014) and the highest SCC values were observed in the BA-OP and Control-SP. The increase of the SSC expected in OP, due to water loss, was observed in BA but did not occur in the Control batch, suggesting that physiological or microbial degradation of sugars may have contributed to the decrease in SSC in that condition. Cold storage coupled with 2%O_2–5%CO_2 did not affect the SSC in tale grapes [21], but MAP influenced the sugar content upon the headspace mixture used, in particular, high %CO_2 (20–40) were correlated with the lower SSC [5,38]. Sugar and organic acid content and composition have a major impact on table grape organoleptic quality [43], the sugar to acidity ratio is paramount for the taste of the grape and should not drop by 20 based on the standard table marketing requirements of large-scale retail trade. At harvest, the ratio was higher in Control rather than in BA-treated grapes, and after the cold storage the value dropped in all conditions without falling below 20.

3.4. Mechanical Properties of the Berries

Table 2 shows the results of the firmness and berry detachment force analysis at the harvest (Fresh) and after 30 days in the different packaging conditions (BD, SP, OP) of *A. pullulans*-treated (BA) and untreated (Control) grapes. The average values of firmness at harvest were different amongst the BA and Control batches reaching, respectively, 20.66 ± 6.22 and 21.52 ± 5.32 N and were retained after 30 days. The storage period, even in MAP, affected negatively grape berry firmness [21,23], but statistical analysis suggests in our experiment there is no effect of the factor investigated on firmness. Berry detachment force measured at harvest was not affected by the in-field treatment, the average values were 2.1 (BA) and 2.7 N (Control). The trend of this parameter indicates that the average values of the BA batch increased, whilst decreasing in Control samples. The SP batch experienced the highest slope, resulting in the lowest berry detachment force in Control. The variability of the recorded data did not allow for the discrimination of the BA samples. Our results agree with those found by Burçak et al. [33], which reported any significant variation of berry detachment force of five *Vitis vinifera* cv. over 60 days of storage in passive MAP.

3.5. Microbiological Analyses

The results of microbiological analysis are also shown in Table 2. The aerobic bacterial mesophilic population (TBC) in the fresh sample was quite low, ranging between 2.68 and 2.88 Log(cfu/g) for the Control and BA samples, respectively. Statistical analysis suggests the pre-harvest treatment did not affect the TBC of the grapes. At end storage, the TBC in BA rose in all packaging conditions, ranging between 5 and 6 Log(cfu/g). On the other hand, the experimental packaging solutions tested were effective in controlling the contamination rate in the Control batch. The Control-BD samples had the lowest contamination, whilst in the Control-OP TBC was considerable, reaching over 6 Log(cfu/g). As expected, TMC results confirm the higher contamination of the BA batch with respect to the Control, which may be due to the in-field treatment. Over the cold storage, the mould growth was unchanged only in the BA-BD batch, whereas it rose to 3 Log(cfu/g) in the BA-SP and BA-OP samples, and experienced the highest value in the Control-

BD batch, reaching over 3.5 Log(cfu/g). Analysis of variance carried out showed the highest weight of the packaging ($p = 0.082$) rather than the treatment ($p = 0.110$) on TMC evolution in the experiment, but the interaction between factors was prominent. The number of aerobic mesophilic bacteria is widely used to evaluate the microbiological quality and safety of ready-to-eat produce, making them one of the most significant food quality indicators. It indicates possible sources of contamination during manufacture and reflects the suitability of temperature and hygienic management during processing, transport, and storage. Although EU Regulation does not limit the TBC in fresh food, several studies and worldwide public guidelines claim the limits are not applicable to fresh F&V [44–47], due to the wide variety of F&V products and production techniques. Hence, a thorough comprehension of the product type is required in order to completely interpret the TBC (i.e., it is truly ready-to-eat or an ingredient that requires a further heating process before consumption). The stage of shelf-life should also be considered, if sampled at the point of production TBC is likely to categorise foods as "satisfactory", whereas if sampled at the end of shelf-life TBC can normally be expected to approach the upper "borderline" limit. TBC of less than 6 Log cfu/g is usually associated with a mixed flora. Above this point, a predominant organism often is present and the kind of organism that predominates will determine the acceptability and organoleptic quality of the food. For raw, ready-to-eat food commodities such as salad vegetables, TBC may already be high at harvest, between 6 and 8 Log cfu/g. That microbiological profile shortens their shelf life because spoiling can happen quickly and is usually noticeable [45]. Storage in MAP may hinder microbial growth, upon the CO_2 and O_2 partial pressure in the headspace. Liguori et al. [38] stored *Red Globe* cv. in different MAP conditions, achieving between 30 and 40% CO_2 and anaerobic condition after 21 days of storage at 5 °C, and they did not find any variation of TBC (averaging 4.5 Log cfu/g), whilst the higher the CO_2 the lower TMC (ranging between 3 and 5 Log cfu/g). In our study, we used 20% CO_2 in order to mitigate the bacterial and mould growth, but the hindrance effect was detected upon the interaction between the packaging solution used and in-field treatment, as only the combination of *A.pullulans* and 20% CO_2 was effective to slow the fungal growth. This may be due to the high antagonistic activity against *B. cinerea*, mainly associated with the antimicrobial organic volatile compound (ethanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-phenylethanol) produced by *A. pullulans* [48] that enhance the oxidative stress and compromise the membrane wall permeability in *B. cinerea* [49].

4. Conclusions

The in-field application of *A. pullulans* affected the SSC, TMC, and berries' colour at harvests. Along with the storage, the major effect of the BA treatment was observed on microbiological decay, with characteristics depending on the packaging. The biocontrol in-field treatment did not prevent aerobic bacteria development in any of the packaging conditions tested, whilst the untreated grapes were contaminated with characteristics depending on the packaging used. The unpackaged and untreated grapes experienced the highest bacterial growth rate. On the contrary, mould growth was much higher in untreated grapes sealed with LDPE, which may be due to the high relative humidity inside the bag. Acidity, pH, and mechanical properties were affected neither by the in-field treatment nor the packaging used. The combination of the variability factors revealed a fair influence on microbiological counts and SSC, which are paramount to the marketing classification. The practical implications of these findings are crucial to developing food waste-reducing practices. The differences in quality characteristics between the tested conditions underline the importance of setting targeted approaches for the smart distribution of F&V.

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