# Active packaging for table grapes: Evaluation of antimicrobial performances of packaging for shelf life of the grapes under thermal stress

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## ARTICLE INFO

## ABSTRACT

The paper reports the formulation of an active packaging based on PET coated with a Layered Double Hydroxide (LDH) hosting 2-acetoxybenzoic anion (salicylate) as antimicrobial molecule. The release of the molecule anchored to the LDH, compared to the molecule free dispersed into the coating, appeared much slower. Permeability of carbon dioxide and oxygen through the packaging at different temperatures was evaluated, as well as the capability of the active material to inhibit *Pseudomonas*, *Listeria* and *Lactobacillus*. Table grape was stored in thermal stress conditions (*i.e.* 10 °C) into the active packaging and the total mesophilic aerobic count and yeasts and moulds population was evaluated up to 14 days of storage. The experimental results were used for a theoretical prediction of shelf life of the packed grapes and compared with the same fruit packed into untreated material. Global and specific migration of salicylic acid from the active packaging demonstrated, in compliance with the migration limits of the EU regulation, the suitability of the considered material for food contact.

## 1. Introduction

In recent years, the food packaging materials based on polymers are in continuous evolution, in response to the increasingly complex needs of the markets, of the distribution times and conditions and of consumers' safety. Therefore, packaging systems are becoming increasingly sophisticated thanks to the rapid evolution of science and technology in producing new materials. The fast development of nanotechnologies is accelerating the innovation in food packaging field and several applications of nanomaterials in packaging and food safety have been studied and developed. They include, for instance, composites based on polymer and active nano-fillers as high barrier packaging materials, reservoirs of antimicrobial agents, nanosensors for the detection of food contaminants or monitoring the packaging conditions integrity (Bugatti, Vertuccio, Zuppardi, Vittoria, & Gorrasi, 2019; Duncan, 2011; Gan & Chow, 2018; Pucciariello et al., 2004; Sorrentino, Gorrasi, & Vittoria, 2007; Souza & Fernando, 2016). Active packaging systems are developed with the goal of extending shelf life for foods and increasing the period of time in which the food is of high quality. The controlled release of antimicrobials from packaging materials represents an important innovation in active packaging. Antimicrobials incorporated in packaging materials could extend shelf life by preventing bacterial growth and spoilage. This system could potentially increase the stability and specificity of preservation and reduce the amount of chemicals needed in foods. It has been demonstrated that the use of nanostructures can achieve levels of functionalities in some cases very difficult to reach using bulk materials. The use of hybrid inorganic-organic materials is increasing considerably, in fact manipulating on nanometer scale the matter it is possible to design advanced materials with specific structure and functionalities. Among the inorganic fillers that can modify the polymers' properties and functionalities, Layered Double Hydroxides (LDH) are very attractive and versatile (Costantino, Marmottini, Nocchetti, & Vivani, 1998; Costantino, Nocchetti, Gorrasi, & Tammaro, 2011). They are composed of positively charged brucite-like layers of divalent and trivalent metal hydroxides in which the excess of positive charge is compensated by anions and water molecules between the interstitial position. They can be represented by the general formula:

$$[M(II)^{1-x}M(III)_x(OH)_2]^{x+}[A_{x/n}^{n-}YH_2O]^{x-}$$

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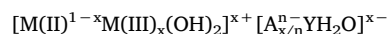
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where M(II) = Mg, Ni, Co, Cu, Zn, Mn; M(III) = Al, Fe, Cr, V; $A^{n-}$ = $CO_3^{2-}$, $Cl^-$, $SO_4^{2-}$, *etc.*, and x = 0.1 − 0.35. High performance thermoplastic polymers generally need high temperatures and shear forces during the processing of incorporation of fillers and nano-fillers, such conditions can cause the degradation of the active molecules that are thermolabile. It has been demonstrated that LDH layers can protect the intercalated molecules which maintain their activity even after drastic processing conditions (Sorrentino et al., 2007). They are biocompatible and can be produced with simple procedures and high level of purity, and have been widely proposed in drug delivery and as carrier of antimicrobials as hybrid nanofillers into polymers (Gorrasi & Sorrentino, 2015; Velasco, Ardanuy, & Antunes, 2012). Table grape (*Vitis Vinifera* L.) is a nonclimateric fruit with serious problems during postharvest handling, storage and marketing. It loses quality accompanied to weight loss, color changes and softening (Carvajal-Millàn et al., 2001; Crisosto, Garner, & Crisosto, 2002). All these detrimental effects lead to quality losses and high occurrence of decay during prolonged storage (Palou, Crisosto, Smilanick, Adaskaveg, & Zoffoli, 2002; Valverde et al., 2005). Disease in table grape is caused by different microorganisms: acetic acid bacteria (*Gluconobacter* spp., *Acetobacter* spp.), yeasts (*Zygosaccharomyces* spp.*)* and moulds (*Botrytis cinerea*); these latter induce uncontrolled infections that allow the development of aerial mycelium spreading rapidly to adjacent berries with severe economic repercussions. Several methods have been used to preserve table grape, the most common is $SO_2$, but sulfite residue is a problem associated with $SO_2$ fumigations. Food and Drug Administration (FDA) established the maximum tolerance to sulfite residues at 10 μL/L, while the European Union forbidden the use of $SO_2$ (EU Directive 95/2/CE). In this optic, the pesticide usage associated to the development of fungicide-resistant strains and the public's concern for the environmental pollution and the human health are stimulating the research toward new strategies as alternative means for controlling postharvest decay. It is, then, fundamental to find alternative and safe active systems able to preserve fresh fruits, like table grapes, and to extend their shelf life with the same performances of $SO_2$. In this paper, we report the preparation of an active packaging based on a PET tray with an active coating composed of a food grade resin filled with LDH hosting salicylate anion as antimicrobial agent (listed in EC-Directive 10/2011/EC of 14 January 2011). Structure and thermal properties of the filler was evaluated. Barrier properties against $O_2$ and $CO_2$ at different temperatures and *in vitro* inhibition of bacteria spoilage were analyzed. Table grapes were packaged at 10 °C and it was evaluated the total mesophilic aerobic count, yeasts and moulds population, and compared to the same table grapes packaged in not treated packaging. The shelf life of the packaged grapes was estimated on the basis of the evolution of yeasts and moulds during the 14 days of storage, using the Gompertz's model.

## 2. Experimental

### 2.1. Materials

Poly(ethylene terephthalate) (PET) (B.PET TRA S2 F740 S350, 2157400350) used for the trays, was supplied by Aristea spa, Salerno (Italy) in laminate form 350 μm thick. These laminates were then processed in trays. The active filler, having the trade name of A6B6® and based on a LDH intercalated with antimicrobial salicylate anion (listed in EC-Directive 10/ 2011), was produced by Nicefiller Ltd, a startup of the University of Salerno (Italy). The synthesis was conducted accordingly to a previously reported procedure (Frunza, Lisa, Popa, Miron, & Nistor, 2008). The resin used for coating was a water-based paint normally used for food packaging (Inx 1-7801-7000, solid content 42 ± 2 %, viscosity 20 s at 20 °C) purchased from Inx srl (Lodi, Italy). Its constituents are in accordance with the EC-Directive 2002/72 including amendments. The resin and the active filler (A6B6®) at 5 wt% were mixed using high energy ball milling at room temperature, for 30 min at 450 rpm and coated on PET (sample named PET/Active) by using an automatic coater. The composite filler weight was 12 ± 0.5 g/$m^2$ on dry resin. The laminates after the coating phase were thermoformed to obtain the active trays. A sample of PET with the active molecule simply mixed into the coating was prepared in the same described experimental conditions.

### 2.2. Methods

*X-ray diffraction (XRD)* patterns were taken in reflection mode with an automatic Bruker diffractometer D8, using nickel-filtered Cu Kα radiation (Kα = 1.54050 Å) and operating at 40 kV and 40 mA, with a step scan of 0.05° of 2ϑ and 3 s of counting time.

*Gas permeation rates* were measured on circular samples with an effective area of 34.2 $cm^2$ that were cut from the top of the trays. Their thickness was obtained as the average of multiple measurements taken with a digital micrometer (Series 293, Mitutoyo, Milano, Italy). The fixed volume/pressure increase instrument (Elektro & Elektronik Service Reuter, Geesthacht, Germany) and the testing procedure are described elsewhere (Clarizia, Bernardo, Gorrasi, Zampino, & Carroccio, 2018). The tests were conducted at a feed pressure of 1 bar in the temperature range 15 − 55 °C with single gases ($O_2$ and $CO_2$ from SAPIO, Milano, Italy). Before each test, the samples were evacuated with a series of membrane and turbo molecular pumps to remove humidity or other dissolved species. In particular, owing to the combination of high sample thickness and low permeability, the membrane evacuation time was about two weeks at the lowest temperature. According to the 'solution-diffusion' mechanism, that describes the gas transport through dense polymeric films (Wijmans & Baker, 1995), the gas permeability (P) can be expressed as product of diffusion (D) and solubility (S) coefficients, $P = DS$. The gas permeability (P) was obtained from the slope of the pressure *vs*. time curve at steady-state conditions. According to the time-lag method, the diffusion coefficient (D) was calculated combining the membrane thickness (l) and the gas time-lag (θ) that is the intercept on the time axis of the linear portion of the pressure curve. The solubility coefficient was indirectly obtained from permeability and diffusion ($S = P/D$). The ideal selectivity for the two gases was obtained as the ratio of their individual permeability values. This parameter can be decoupled in solubility- and diffusion-selectivity terms.

*The release kinetics* of salicylate were followed using a Shimadzu UV-2401 PC spectrometer. The tests were performed using 4 $cm^2$ rectangular specimens placed into 25 mL physiological solution and stirred at 100 rpm in an orbital shaker (VDRL MOD. 711 + , Asal). The release medium was withdrawn at fixed time intervals and replenished with fresh medium. The considered band was 230 nm.

The *in-vitro* effect of inhibition against *Pseudomonas, Listeria* and *Lactobacillus* by the treated PET trays was analyzed following the directive ISO 22196:2011: such method evaluates the antibacterial activity of treated plastics, surfaces and other non-porous materials.

*Overall migration tests* were performed on the PET trays treated with A6B6®filler samples according to the following procedure: lamina specimens having 1 $dm^2$ of surface area (10 cm × 10 cm, 0.10 mm thickness) were put into contact with 100 mL of simulant (preconditioned at 40 °C) in a borosilicate glass tube closed with a screw cap internally layered with Teflon®. The obtained surface/volume ratio was 10 $dm^2$/L. Migration tests after contact for 10 days at 40 °C were performed using C (ethanol at 20 %) and D1 (ethanol at 50 %) as simulants. The overall migration test was performed on different aliquots from the same contact sample. The overall migration results were calculated by using 6 $dm^2$/kg food (6 $dm^2$/L simulant) as conventional EU surface/volume ratio. A known aliquot of the simulant from the contact solution was transferred into a weighted quartz capsule and evaporated to dryness until constant weight. From the differences between the weights, the overall migration was derived in accordance to EN 1186 Migration Testing for Food Contact Materials. The data were averaged on five samples.

*Microbiological tests* were carried out on table grapes (*Vitis vinifera* L., cv Egnathia) provided by an experimental farm conducted by Research Centre for Viticolture and Enology (CREA, Turi, Italy). Bunches were harvested at the maturity stage (total soluble solid content of about 20° Brix) and transported within two hours from harvest to the Postharvest laboratory. Samples were selected on the basis of the absence of defects, divided in small pieces (berries with rachis of about 450 g) and randomly closed in PET trays (165 × 135 × 45 mm) with active filler (AF) or without (Control, C). A total of 24 trays (3 replicates x 2 packages x 4 storage times after 4, 7, 11 and 14 days) were stored at 10 °C. Just after harvest (fresh sample) and at each storage time, samples were analysed for the incidence of visible mould on berries and microbiological analysis measuring the lactic acid bacteria, total mesophilic aerobic bacteria, and moulds and yeasts.

The mould incidence on berries was evaluated counting berries with visible mould on each bunch for each replicate in C or AF packages. It was expressed as percentage of infected berries respect to total one.

For microbiology analysis, fifty grams of berries from each packaging (C or AF) were rinsed, in triplicate, in 450 mL of 0.1 % peptone water with 0.02 % Tween 20 by orbital shaking (CERTOMAT® HK, Sartorius AG, Göttingen, Germany) in a flask at 170 strokes/min for 30 min (Garofalo, Tristezza, Grieco, Spano, & Capozzi, 2016). Then, each sample was decimally diluted in 0.1 % (w/v) sterile saline solution (0.9 % NaCl) before plating on the selective media. In particular, yeasts and moulds were detected on Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Biolife Italiana srl, Milan, Italy) and incubated at 25 °C for 3–5 days (ISO 21527-1, 2008); lactic acid bacteria population (LABs) were counted on De Man, Rogosa and Sharpe agar (MRS, Biolife Italiana srl, Milan, Italy) supplemented with 100 mg/L of cycloheximide after incubation at 30 °C for 48 h (ISO-15214, 1998); total mesophilic aerobic counts (TBC) were enumerated on Plate Count Agar (PCA, Biolife Italiana srl, Milan, Italy) supplemented with 100 mg/L of cycloheximide after incubation at 30 °C for 24 h (ISO 4833, 2003).

A two-way ANOVA test was performed using SPSS 20.0 (IBM, Armonk, NY, USA) to examine the effects of storage and packaging (C or AF) on the incidence of visible moulds and on the microbial load of different microbial groups after assessing variance homogeneity with Levene's test ($P < 0.05$). Multiple comparisons within the same microbial group were made by Fisher's least significant difference (LSD, C.I. $> = 95$ %); wherever required, a two-tail indipendent *t*-test ($\alpha = 0.05$) was also performed.

## 3. Results and discussion

Fig. 1 reports the XRD of the pristine LDH in nitrate form (Fig. 1(A)) with the basal spacing at 10.2° of 2ϑ (d = 8.6 Å). The basal spacing increased from 8.6 Å to 16.3 Å, corresponding to 2ϑ ≅ 6.02° (Fig. 1(B)),
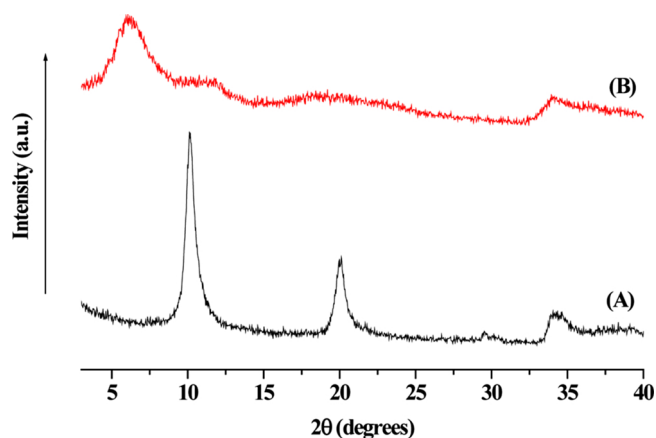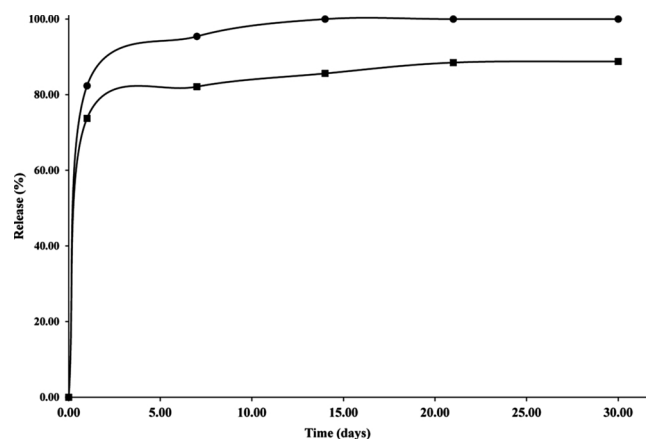


**Fig. 2.** Release kinetics of the active molecule anchored to the active filler in PET (■) and of the active molecule simply dispersed into PET (•).

and this is a clear probe of the successful intercalation of the salicylate anion into LDH layers.

The possibility to obtain a polymer/active filler system with slow and controlled release is crucial in the active packaging, in order to increase the shelf life of the packed food. When an active molecule is simply dispersed into a polymer matrix its release is driven by a fast diffusion. On the other hand, if it is fixed with a chemical bond, the release can be dependent on several factors, such as medium of interaction of the whole system that influences the ionic exchange reaction between the intercalated molecular anions and the counter-ions present into the release medium. Fig. 2 reports the release kinetics from the PET trays of the active molecule anchored to the active filler and the salicylate simply dispersed into the polymer matrix. A first stage, corresponding to the release from the surface, is followed by a second stage that corresponds to the de-intercalation of the salicylate bonded to the LDH lamellae. It is evident that, at any release time, the molecule from the active packaging shows a slower release. It is worth to note that, in the considered contact time (30 days), the active molecule anchored to the filler was not completely released.

The data obtained from the gas permeation tests on the trays are summarized in Table 1.

$CO_2$ was the most permeable gas in both tray types. A detailed analysis of the two contributions to permeability (namely, diffusion and solubility) allows to recognize that the $CO_2$ molecules has a higher solubility than $O_2$ and this contribute is predominant in determining its higher permeability.

The PET/Active trays displayed a partial change in the transport properties with respect to those not treated. In particular, they presented a faster $CO_2$ transport, with a 65 % increase in permeability. On the contrary, the $O_2$ permeability was almost unchanged. Consequently, the $CO_2/O_2$ ideal selectivity increased in the PET/Active trays (+68 %).

The apparent diffusion coefficient for $CO_2$ was unaffected in the PET/Active trays, while it was reduced for the $O_2$. On the other hand, the presence of the inorganic fillers in the PET/Active trays lead to an



**Fig. 1.** XRD of pristine LDH in nitrate form (A) and LDH-salicylate anion (B).

**Table 1**
Gas transport properties evaluated in the trays at 25 °C.

| Material | | $O_2$ | $CO_2$ | $CO_2/O_2$ |
|---|---|---|---|---|
| PET | *Permeability* (Barrer) | 0.099 | 0.26 | 2.6 |
| | $D$ ($10^{-8}$ cm²/s) | 0.43 | 0.11 | 0.26 |
| | $S$ (cm³(STP) cm⁻³ bar⁻¹) | 0.17 | 1.7 | 10 |
| PET/Active | *Permeability* (Barrer) | 0.097 | 0.43 | 4.4 |
| | $D$ ($10^{-8}$ cm²/s) | 0.36 | 0.11 | 0.31 |
| | $S$ (cm³(STP) cm⁻³ bar⁻¹) | 0.20 | 2.9 | 15 |

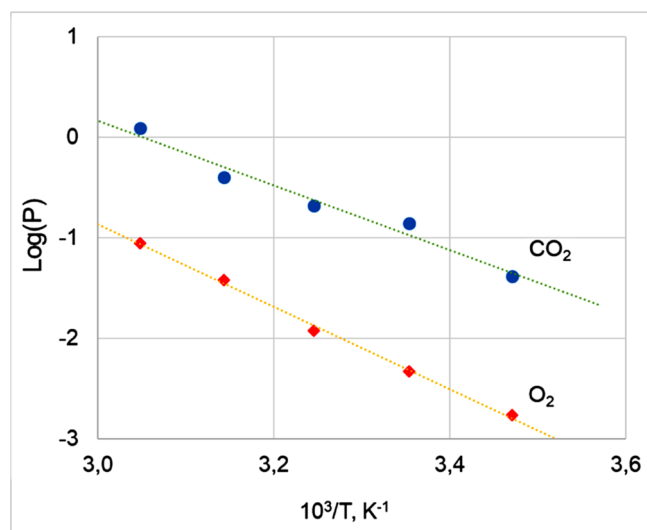1 Barrer = $10^{-10}$ cm³ (STP) cm cm⁻²cmHg⁻¹ s⁻¹.

**Fig. 3.** Permeability logarithm versus reciprocal of absolute temperature plot, for $CO_2$ and $O_2$ measured in the PET/Active trays.

increased solubility for both gases, particularly for $CO_2$. Accordingly, it was observed increment in the $CO_2/O_2$ ideal selectivity, and this contribution was due mainly to the solubility parameter. The gas permeation tests demonstrated that the active coating combined to the thermoforming process was able to keep unchanged the barrier properties for the $O_2$ transport, but at the same time, the $CO_2$ transport through the trays was enhanced. These features favor the $CO_2$ outflow from the packaged product. The calculation of activation energy for the permeation process of both gas species ($CO_2$ and $O_2$) allows to analyze the response of material to the gas transport in dependence of temperature change. An Arrhenius trend was observed (Fig. 3), that is, there is a linear relationship between the gas permeability logarithm and the reciprocal of absolute temperature ($T$). Thus, $P$ can be expressed as:

$$P = P_0 \exp(-E_P/RT) \tag{1}$$

where $P_0$ is the pre-exponential factor of permeation, $E_P$ is the activation energy for permeation, and $R$ is the gas constant.

As expected from the order of gas permeability and endothermicity of the sorption process, $E$p for $CO_2$ was lower than for $O_2$ (25.2 and 35.5 kJ mol$^{-1}$), respectively. Additionally, as the temperature increased, $CO_2/O_2$ selectivity decreased. It results since the diffusion coefficient tends to increase with temperature as consequence of the enhanced mobility of gas molecules as well as the segmental chain mobility in polymer, whereas the solubility coefficient decreases. Therefore, the permeability of $CO_2$, depending on solubility term more

markedly than $O_2$, was less influenced on temperature with respect to $O_2$.

The evaluation of the bacterial inhibition from the treated PET trays was carried out using *Pseudomonas, Listeria* and *Lactobacillus* bacterial strains. The test microorganisms were prepared by growth in a liquid culture medium. The suspension of the test microorganisms was standardized by dilution in a nutritive broth, then control and test surfaces were inoculated with microorganisms, in triplicate, and the microbial inoculum was covered with a sterile film. In order to provide a comparison, all microbiological assay runs were performed with the necessary parallel controls for the whole time of the experiments. Microbial concentrations were determined at "time zero" by elution followed by dilution and plating. A control was run to verify that the neutralization/elution method effectively neutralized the antimicrobial agents in the antimicrobial surface being tested. Inoculated, covered control and antimicrobial test surfaces were allowed to incubate in a humid environment for 24 h and microbial concentrations were determined. The reduction of microorganisms relative to initial concentrations and the control surface was calculated. By including the proper controls and being able to make these reduction calculations, such assay allowed to evaluate whether the treated PET tray is bacteriostatic, having the ability to inhibit the growth of microorganisms. Table 2 reports the experimental conditions and results. For all considered strains, a significant antibacterial activity from the studied active packaging is evident.

The incidence of visible mould on berries was significantly affected only by packaging (p < 0.001); in detail, a mean incidence of 2.8 % was recorded on C, while 0.9 % was the value calculated for AF packages on the whole storage. Thus, based on this visual evaluation, the presence of the AF resulted able to limit the mould development on berries. As for microbial evaluation, there was a statistically significant interaction between factors level on load within each microbial group, ($p < 0.00001$). As shown in Fig. 4(A), table grapes registered a low initial value of LABs load that reached 4.59 ( ± 0.13) log CFU/g for fruits packed in C trays at the 7th day of storage. These results were in accordance with Barata, Malfeito-Ferreira, and Loureiro (2012) which found low populations of LAB on both healthy and sour rotten grapes. Starting from the 4th day of storage, a significantly lower value (3.5 log CFU/g, on average; $P < 0.05$) was instead registered in PET/Active trays in comparison to control sample (4.15 log CFU/g, on average) (Fig. 4(A)). As regard total mesophilic aerobic count (TBC), simple main effects analysis showed that time of incubation did not affect microbial load for both samples ($P > 0.05$); however, TBC values on samples from AF packaging were significantly lower ($P < 0.05$; Student's $t$-test) than those from C trays throughout the entire period of incubation (Fig. 4(B). The significant antimicrobial effect of salicylate anion released from PET/Active tray was also registered for yeasts and moulds population (Fig. 4(C); $P < 0.001$); this latter, primarily responsible for microbial spoilage activity on grapes (Pinto, Caputo, Quintieri, de

**Table 2**
Inhibition of *Pseudomonas*, *Listeria* and *Lactobacillus* from the PET/Active trays.

| Bacterial strain | *Pseudomonas aeruginosa* ATCC 15442 | *Listeria monocytogenes* ATCC13932 | *Lactobacillus sakei*- ATCC 15521 |
|---|---|---|---|
| Sample size | 50 × 50 (mm × mm) | 50 × 50 (mm × mm) | 50 × 50 (mm × mm) |
| Sample thickness | 1.0 mm | 2.0 mm | 0.1 mm |
| Inoculum volume | 0.4 mL | 0.4 mL | 0.4 mL |
| Uo: Number of bacteria available in the inoculum | 410.000 | 380.000 | 35.000 |
| Ut: Count of bacteria recovered from non-treated samples after 24 h from inoculation | 5.3 (log) | 4.8 (log) | 2.5 (log) |
| At: Count of bacteria recovered from treated samples after 24 h from inoculation | n.d.[a] | 3.0 (log) | 0.8 (log) |
| Antibacterial activity R = (Ut-Uo)-(At-Uo) (ISO 22196:2011) | > 5.3 | 1.7 | 1.6 |

[a] Not detectable.
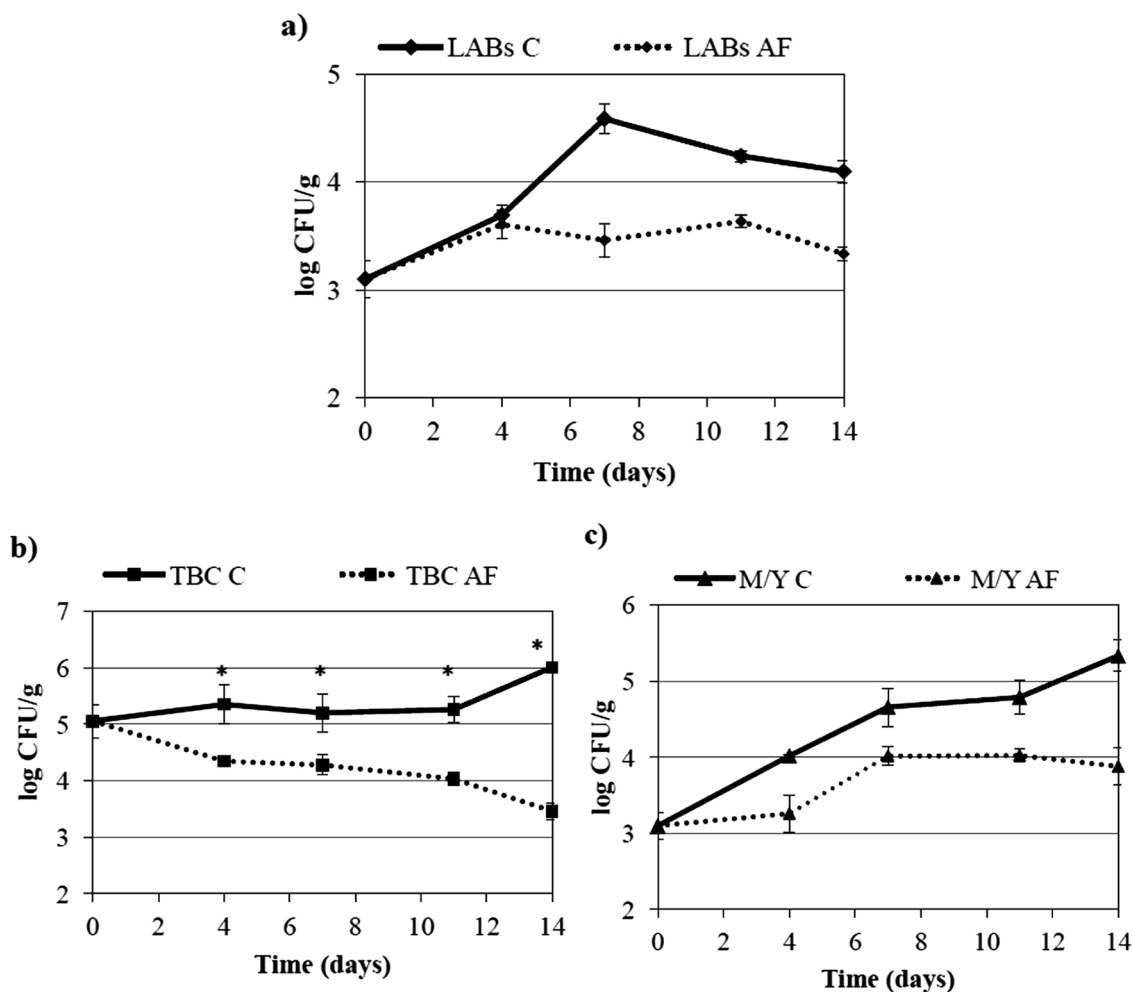
**Fig. 4.** Bacterial counts (expressed as log CFU/g) of *A)* lactic acid bacteria, LABs, *B)* total mesophilic aerobic bacteria, TBC, and *C)* mould and yeast, M/Y in table grapes packed in PET (C) or PET/Active (AF) trays during incubation at 10 °C for 14 days. Values represent the average ± the standard deviation (n = 3). Differences among ΔlogCFU/g values higher than 0.41 (A) and 0.64 (C) (Fisher's LSD) separate significantly different means. B) Asterisks indicate statistically significance differences (Student's test, α < 0.05 assuming equal variances).
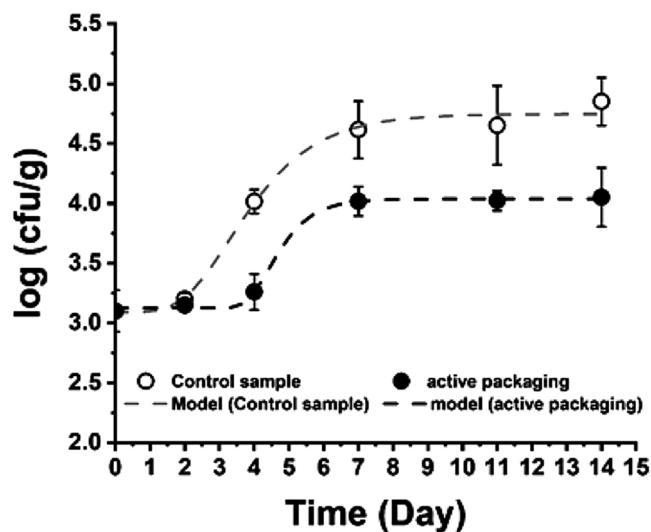


**Fig. 5.** Time course during storage of grapes at 10 °C in PET (control) and PET active film. The curves are the best fit using Eq. (2).

**Table 3**

Model parameters referred to Eq. (2): the increase in log CFU/g between time = 0 and the maximum population density achieved at the stationary phase (A), the maximal specific growth rate ($\mu_{max}$) and the lag time ($\lambda$).

| Sample | A [log (CFU/g)] | | $\mu_{max}$ [log (CFU/g)/d] | | lag time ($\lambda$) [d] | |
|---|---|---|---|---|---|---|
| Control | 1.658 [1.640 | 1.676] | 0.451 [0.439 | 0.462] | 1.921 [1.837 | 2.006] |
| Active film | 0.913 [0.902 | 0.923] | 0.486 [0.464 | 0.507] | 3.770 [3.730 | 3.809] |

Candia, & Baruzzi, 2017; Sanzani, Schena, De Cicco, & Ippolito, 2012), showed a reduction of *ca.* 1.5 log CFU/g after 14 days of storage in PET/Active trays in comparison to C ones. Decay of table grape during storage is mainly due to moulds development, which limits the product marketability favoring waste (Romanazzi, Lichter, Gabler, & Smilanick, 2012). The antimicrobial effect of PET/Active trays is due to the salicylate anion release responsible for changes in cell membrane permeability of several Gram-positive and Gram-negative bacteria, as reported by other authors (Kaduskar, et al., 2017; Bandara, Sankaridurg, Zhu, Hume & Willcox, 2016). To prevent table grape decay in

**Table 4**
Global migration and specific migration of salicylic acid from the active PET trays.

| Simulant | C - ethanol at 20 % (v/v) | D1 - ethanol at 50 % (v/v) | Limits |
|---|---|---|---|
| | Global migration into aqueous food simulant by filling a container UNI EN 1186-1: 2003 + UNI EN 1186-9: 2003 | Global migration into aqueous food simulant by filling a container UNI EN 1186-1: 2003 + UNI EN 1186-9: 2003 | |
| Temperature of the test | 40 °C | 40 °C | |
| Contact time | 10 days | 10 days | |
| Global migration average in the simulant | 5.2 (mg/dm$^2$) | 9.0 (mg/dm$^2$) | 10 (mg/dm$^2$) |

postharvest caused by mould development, different antimicrobial agents and physical treatments were found effective; mould contamination is also responsible for mycotoxin production impacting on human health (Romanazzi et al., 2012; Ubeda, Hornedo-Ortega, Cerezo, Garcia-Parrilla, & Troncoso, 2020). However, most of these treatments should be applied to bunches immediately after harvesting and in specific equipments; thus, their antimicrobial effect decline during the next first days of storage. By contrast, the use of the antimicrobial packaging allow to control deterioration of table grapes throughout storage period by preserving shelf life until to the domestic use. In addition to the functionalization of packaging materials with an antimicrobial agent, the use of modified atmosphere (such as 20 % $O_2$ plus 10 % $CO_2$) is a further strategy to preserve grape shelf life during its transport and cold storage (Cefola & Pace, 2016). Indeed, table grapes packed in active PET bags and closed in modified atmosphere resulted in an extension of marketability and antioxidant activity over the storage period, also in comparison to the use of $SO_2$ pads (Cefola, Pace, Bugatti & Vittoria, 2015). In addition, the use of this activated PET bags (Cefola et al., 2015) allowed a release in antioxidants able to control the rachis browning, associated with water loss (Lichter et al., 2011) and oxidation processes (Carvajal-Millàn et al., 2001).

The results of microbial growth for yeasts and moulds, and the best fitting of the experimental data are reported in Fig. 5. The fitting of the experimental data has been obtained with a coefficient of determination $R^2 = 0.99$, whereas the model parameters, obtained according to the proposed approach in reference (Corbo, Del Nobile, & Sinigaglia, 2006) (95 % confidence intervals), are reported in Table 4.

The generally adopted method (Zwietering, Jongenburger, Roumbouts, & van't Riet, 1990) consists in estimating the Gompertz's parameters by fitting the following equation to the experimental data:

$$log\left(\frac{CFU}{g}\right) = K + A*exp\left\{-exp\left[\left(\mu_{max}*2.7182\frac{\lambda - t}{A}\right) + 1\right]\right\} \qquad (2)$$

where: $K$ is the initial level of bacterial count (log CFU/g), $A$ is the increase in log CFU/g between start time and the maximum population density achieved at the stationary phase, $\mu_{max}$ is the maximal growth rate ($\Delta$log (CFU/g)/day), $\lambda$ is the lag time (days) and $t$ is the time (days).

As shown in Table 3, the active system reduces the growth of yeasts and moulds, leading to a decrease of the plateau value of around one order magnitude with respect to the control system.

In the present study, the maximal specific growth rate value ($\mu_{max}$) is essentially the same for both tray types, while the salicylate anion based film inhibits the growth of yeasts and moulds as shown by the increase of the lag time from 1.9 days to 3.8 days. Therefore, the shelf life is extended in the active packaging.

In order to demonstrate that the prepared active packaging is suitable for food contact, we performed overall migration tests on the base of the trays (part in contact with the fruits). Table 4 reports the global migration and specific migration of salicylic acid, evaluated on the active packaging, in different food simulants, accordingly to UNI EN 1186-1:2003 and UNI EN 1186-9: 2003 (ethanol (20 % v/v) and ethanol (50 % v/v). The experimental results, in compliance with the

migration limits, demonstrate the suitability of the considered material for food contact.

## 4. Concluding remarks

PET trays with an active coating were prepared and tested for the application in the packaging of table grapes. The Active coating, is based on a food grade acrylic resin filled with Layered Double Hydroxide (LDH) nanofiller hosting antimicrobial 2-acetoxybenzoic anion (salicylate) (listed in EC-Directive 10/2011/EC of 14 January 2011). XRD analysis, revealing an increase of the basal spacing of the pristine clay, proves the successful intercalation of the active molecule inside the LDH galleries. Accordingly, the salicylate has a slower and not complete release from the trays when it is anchored to the LDH fillers compared to a sample in which the molecule is directly dispersed inside the polymer matrix. The active coating, combined to the thermoforming process, maintains the barrier properties for the $O_2$ transport, enhancing the $CO_2$ transport rates through the trays. The *in-vitro* bacterial inhibition on the active packaging against *Pseudomonas, Listeria* and *Lactobacillus* was analyzed prior to storage of the table grapes, and a significant antibacterial activity was evidenced. Table grape berries were stored in thermal stress conditions (*i.e.* 10 °C) into the active packaging and a significant reduction in total mesophilic aerobic count (TBC) and mould and yeast population, the main responsibles for grape spoilage, was observed with respect to control samples. The prediction of shelf life of the fruits stored in the active packaging was elaborated using the Gompertz's equation. Finally, the global migration and specific migration of salicylic acid from the active PET trays in different food simulants, accordingly to UNI EN 1186-1: 2003 and UNI EN 1186-9: 2003 (ethanol (20 % v/v) and ethanol (50 % v/v)), complies with the migration limits. Therefore, the prepared active material is suitable for food contact.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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