Article

A Food-Grade Resin with LDH–Salicylate to Extend Mozzarella Cheese Shelf Life

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Abstract: Mozzarella cheese can be considered by far the world's most popular Italian dairy product. Extending the shelf life of mozzarella cheese is an important issue in the dairy industry due to the high risk of contamination by several bacteria species, including spoilage pseudomonads. In this work, active packaging was prepared by coating traditional polyethylene terephthalate (PET) containers of "ovoline" mozzarella cheese with a food-grade resin mixed with a layered double hydroxide (LDH) in which salicylate anion was intercalatedby ionic exchange.. This antimicrobial molecule is listed in EC-Directive 10/2011/EC of 14 January 2011. Morphological arrangement of the molecule into the LDH layers was evaluated by X-ray diffraction (XRD) and controlled release followed by UV spectroscopy. Then, active trays were used to pack the mozzarella cheeses stored for 20 days at 4 °C and under thermal abuse (15 °C). Samples from both conditions showed coliform reduction (by ca. 2 log CFU/g) throughout the storage period. Depending on temperature, total mesophilic aerobic bacteria, *Pseudomonas* spp., yeasts, and mold loads were reduced in the first 3 days; at 4 °C. Slower acidification and lower proteolysis were also found in treated samples in comparison to control ones. The fitting of the Gompertz function to coliforms and spoilage pseudomonads highlighted an increase in the shelf life of mozzarella cheese of ca. 2 days at 4 °C. These results suggest that salicylate–LDH-coated PET may be applied to extend the shelf-life of mozzarella cheese and also counteract its spoilage if accidental interruptions to refrigeration occur.

Keywords: layered double hydroxides; salicylate; PET; active coating; *Pseudomonas* spp.; coliforms

1. Introduction

Mozzarella is a typical Mediterranean pasta filata cheese with a moisture content of around 50–60%. It is mild, soft, and can be cut and manufactured in different shapes [1]. It is packed in a liquid, called brine conditioning, based on tap water or a water dilute solution of sodium chloride and/or calcium chloride and whey. Fresh mozzarella cheese has a short shelf life, from approximately 5 to 7 days, because of its high moisture content, which makes it highly perishable. Although it is submitted to heat treatment during curd stretching, post-processing contamination by microorganisms may occur, causing spoilage and severe health risks for the consumers [2]. The main spoilage microorganisms of mozzarella cheese are *Pseudomonas* spp. and coliforms, which can cause proteolysis, pigmentation, discolorations, and the development of off-flavors [3,4]. In addition, the presence of antibiotic-resistant genes (ARGs), which can be acquired and transmitted by horizontal genetic transfer, further increases their risk and the need to be controlled [5,6].

Extending the shelf life of mozzarella cheese is an important issue in the dairy industry due to the high interest in widening the distribution of this traditional product beyond the market borders. Several approaches have been attempted to extend the shelf life of the

food product, based on the quality improvement of the raw materials, on making process innovations, and on the use of different active packaging and storage conditions [7–11].

Nanoscience and nanotechnology are providing a fundamental contribution to innovation in the food packaging field, and several applications of nanomaterials in active packaging have been studied and developed. Among these, composites based on polymers and active nano-fillers as high-barrier packaging materials and reservoirs of antimicrobial agents are attracting particular interest [12–17]. In fact, the potential for the controlled release of antimicrobials from packaging materials could extend food products' shelf life by preventing bacterial growth and spoilage. Among nano-fillers that can modify the properties and functionalities of polymers, layered double hydroxides (LDHs) are very attractive and versatile [18–21]. They are 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 are biocompatible and can be produced with simple procedures and high levels of purity, and they have been widely proposed for use in drug delivery and as carriers of antimicrobials as hybrid nano-fillers into polymers [22–24]. The intercalation of antimicrobials into the interlayer lamellae of LDHs, indeed, results in greater chemical stability, cell targeting function, and high surface area; it reduces concentration fluctuations and maintains concentrations at the desired level for longer periods of time due to the controlled release of the active molecule [25]. Besides their application in drug delivery systems, these properties make an LDH intercalation system a promising strategy in drug delivery systems as well as in food applications to counteract microbial growth by favoring shelf life elongation.

This paper reports the preparation of active packaging based on a coating composed of a food-grade resin filled with LDHs hosting salicylate anion as an antimicrobial molecule [26] on traditional PET trays usually used to pack and store mozzarella pieces (ovoline). The intercalation degree of salicylate into the filler layers was evaluated and the controlled release of the active molecule analyzed. Both barrier properties against O_2 and CO_2 and in vitro inhibition against pathogenic bacteria were also analyzed. Then, the active packaging was evaluated for its antimicrobial efficacy and quality preservation of mozzarella cheese stored under both conventional refrigerated conditions (4 °C) and thermal abuse (15 °C). This latter condition was considered in order to simulate the effects of eventual temperature fluctuations during mozzarella cheese storage. Then, microbiological results were used in Gompertz modeling to predict mozzarella cheese shelf life during its storage in active packaging at 4 °C.

2. Materials and Methods

2.1. Materials

Poly(ethylene terephthalate) (PET) (R.PET TRA S2 F740 S350, 2157400350), used for the trays, was supplied by Selepack spa, Salerno (Italy) in laminae form with a thickness of 350 μm. These laminae were then processed in trays. The active filler, having the trade name of A3B9® and based on an LDH intercalated with antimicrobial salicylate anion [26], was produced by Nicefiller Ltd., a startup at the University of Salerno (Italy). The synthesis was conducted according as previously reported [27]. The resin (from Inx srl Lodi, Italy) 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) and in accordance with the EC-Directive 2002/72 including amendments [28]. The resin and the active filler at 7 wt% (4.2% of active molecule) were mixed using high-energy ball milling at ambient temperature, for 30 min at 450 rpm and coated on PET (sample named Active Packaging) by using an automatic coater. The composite filler weight was 12 ± 0.5 g/m^2 on dry resin. The laminae after the coating phase were thermoformed to obtain the active trays.

2.2. Characterization of Active Packaging Film

X-ray diffraction (XRD) patterns were acquired in reflection 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.

The release kinetics of salicylate were evaluated at room temperature using a Shimadzu UV-2401 PC spectrometer. The tests were performed using 4 cm^2 specimens placed into 25 mL of physiological solution and stirred at 100 rpm in an orbital shaker (VDRL MOD. 711+, Asal S.r.l., Cernusco, Milan, Italy). 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 aeruginosa, Escherichia coli,* and *Staphylococcus aureus* by the active PET trays was analyzed following the ISO 22196:2011 directive [29].

The migration tests were performed on the PET trays treated with A3B9®filler samples as follows: lamina specimens with 1 dm^2 of surface area (10 cm × 10 cm, 0.10 mm thickness) were placed into contact with 100 mL 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 as simulants B (Acetic acid at 3%) and D1 (Ethanol at 50%). The overall migration test was performed on different aliquots from the same contact sample and 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 a constant weight was achieved. From the differences between the weights, the overall migration was derived in accordance with EN 1186 Migration Testing for Food Contact Materials. The data were averaged for five samples.

2.3. Active Packaging of Mozzarella Cheese and Shelf Life Evaluation during Storage

Ball-shaped, high-moisture, cow's milk mozzarella cheese ("ovoline", weighing ca. 30 g/sample) was industrially manufactured and purchased from an Apulian dairy farm on the same day. Cheese samples were cold-stored for 3 h before draining their governing liquid and transferred in triplicate into coated and uncoated plastic trays (three samples/tray). Each tray was filled with 190 mL of cold tap water previously autoclaved (following the same ratio product/governing liquid of commercial mozzarella cheese). Then, the trays were incubated at 4 and 15 °C for 20 days. At different times of cold storage (0, 3, 7, 10, 14, and 20 days), cheese samples were withdrawn and subjected to pH determination with the Φ 340 pH/Temp Meter system Beckman Coulter (Fullerton, CA, USA), followed by microbiological analyses and proteolysis. Color determination and texture profile analyses were also carried out on samples stored for 10 days at 4 and 15 °C.

2.3.1. Microbiological Analyses

Ten grams of mozzarella cheese sample were, in triplicate, aseptically homogenized in 90 mL of sterile 0.9% NaCl solution in a stomacher (Lab-Blender 400, PBI International, Milano, Italy), and decimally diluted in 0.1% (*w/v*) sterile peptone saline solution (0.9% NaCl) before plating (100 μL) on selective media. In particular, yeasts and molds were detected on potato dextrose agar (PDA) supplemented with 100 mg/L of chloramphenicol and incubated at 25 °C for 3–5 days [30]; presumptive coliforms were enumerated on violet red bile agar [31] and incubated at 37 °C for 24 h; pseudomonads were grown on a *Pseudomonas* agar base (PSA, amended with *Pseudomonas* CFC selective supplement, Oxoid, Italy) at 30 °C for 24 h [32]; lactic acid bacteria population (LABs) was counted on De Man, Rogosa, and Sharpe agar (MRS) supplemented with 100 mg/L of cycloheximide after incubation at 30 °C under anaerobic conditions for 48 h [33]; total mesophilic aerobic counts (TBC) were enumerated on Plate Count Agar (PCA) supplemented with 100 mg/L of cycloheximide after incubation at 30 °C for 24 h [34].

2.3.2. Proteolysis

Small peptides and amino acids were extracted by adding 2 mL of methanol to 1 mL of tap water and withdrawn from the trays with mozzarella cheese samples at defined times (0, 3, 7, 10, 14, and 20 days). Then, samples in triplicate were dipped in ice for 5 min before centrifuging at 4 °C at 13,000 rpm for 5 min; the *o*-phthaldialdehyde method was performed to quantify small peptide and amino acid concentrations [35]. These values were calibrated against glycine solutions at different concentrations (0.8–0.01 mg/mL) and expressed as glycine (Gly)-equivalent concentration.

2.3.3. Texture Profile Analysis

Three replicates of uncut mozzarella cheese (ca. 30 g) with an aspect ratio (L/D = 0.9) close to the value required for mozzarella cheese specimens were drained and allowed to equilibrate to room temperature for half an hour before being submitted to texture profile analysis (TPA) according to Baruzzi et al. [3]. The analysis of data was performed by the software TestXpert v10.11 master (Zwick/Roell GmbH e Co, Ulm, Germany), calculating the TPA parameters—hardness, cohesiveness, springiness, and chewiness (Gunasekaran and Ak, 2003)—of cheese samples stored at 4 and 15 °C for 0 and 10 days.

2.3.4. Color Determination

Color appearance of HM mozzarella cheese samples stored at 4 and 15 °C for 0 and 10 days was determined by measuring colorimetric CIE (Commission Internationale de l'Eclairage) coordinates L* (lightness), a* (redness), and b* (yellowness) on 3 random points of the cheese surface with ChromaMeter CR-400 (Konica Minolta, Osaka, Japan; illuminant: D65; observer: 2°) and Color Data Software SpectraMagic NX (Konica Minolta), as previously reported [4]. Hue (h°) and chroma (C*) of cheese samples (corresponding to the basic tint and the saturation of color, respectively) as well as color differences (ΔE* ab) were calculated as described for a soft cheese [4]. CIELab coordinates were converted to HEX values using a Nix color sensor (https://www.nixsensor.com/free-color-converter/; accessed 17 January 2021) regardless of chroma values.

2.3.5. Gompertz Analysis

The modified Gompertz's Equation (1) was used to determine the shelf life of packaged mozzarella cheese [36]:

$$
\begin{aligned}
\mathrm{Log}\left(\tfrac{\mathrm{CFU}}{\mathrm{g}}\right) &= \left|\mathrm{Log}\left(\tfrac{\mathrm{CFU}}{\mathrm{g}}\right)\right|_{\max} - \mathrm{A} * \exp\left\{-\exp\left\{\left[\mu_{\max} * 2.71\tfrac{\lambda-\mathrm{S.L.}}{\mathrm{A}}\right]+1\right\}\right\} + \mathrm{A} \\
&\quad * \exp\left\{-\exp\left\{\left[\mu_{\max} * 2.71\tfrac{\lambda-\mathrm{t}}{\mathrm{A}}\right]+1\right\}\right\}
\end{aligned} \quad (1)
$$

in which the shelf life (S.L.) appears as a parameter of the equation Log (CFU/g) vs. time, and where A (expressed in Log (CFU/g)) is the maximum bacteria growth attained at the stationary phase, μ_{max} is the maximal growth rate (ΔLog (CFU/g)/day), λ is the lag time (days), and $\left|\log\left(\tfrac{\mathrm{cfu}}{\mathrm{g}}\right)\right|_{\max}$ is the decimal logarithm of the microbial acceptability limit; this value of $\left|\log\left(\tfrac{\mathrm{cfu}}{\mathrm{g}}\right)\right|_{\max}$ max was set to 4 and 6 for coliforms and pseudomonads, respectively [7,9,11].

The average values and related standard deviations were calculated for four repeated measurements. The confidence intervals of the model's parameters were evaluated as reported elsewhere [11,36].

2.4. Statistical Analyses

Before statistical analysis, raw data of microbiological counts were log-transformed, whereas an arcsine-root transformation was applied to texture cohesiveness. All texture data were also standardized according to a z-value distribution with mean value zero and

standard deviation of 1. Analysis of variance was performed after assessing homogeneity of variance by using Levene's test ($p < 0.05$) and SPSS 20.0 (IBM SPSS, Armonk, NY, USA). A two-way ANOVA was carried out to evaluate the effect of coating and storage time counts of the main microbiological groups found in mozzarella cheeses stored at 4 and 15 °C, respectively. A one-way ANOVA was applied to evaluate the effects between the coating treatment and the incubation period for each storage condition on the texture characteristics and CIELab coordinates. Multi-comparison analyses of means were performed with Fisher's least significant difference test (LSD, 95% confidence interval) and Tukey's HSD post hoc test ($p < 0.05$). Acidification and proteolysis pairwise comparisons between cheese samples stored in active packaging and control at the same incubation time and temperature were analyzed by 2-tailed independent Student's t test ($p < 0.05$).

3. Results and Discussion

Figure 1A reports the XRD of the pristine LDH (a) having NO^{3-} as an intercalated anion showing basal spacing of 0.86 nm ($2\vartheta = 10.2°$). The active nano-filler, with the intercalated salicylate anion between the LDH layers (b), shows increased spacing from 0.86 nm to 1.63 nm ($2\vartheta = 6.02°$). Such an increase in the interlayer distance is a demonstration of the successful intercalation of the salicylate anion into LDH galleries.

(A)

(B)
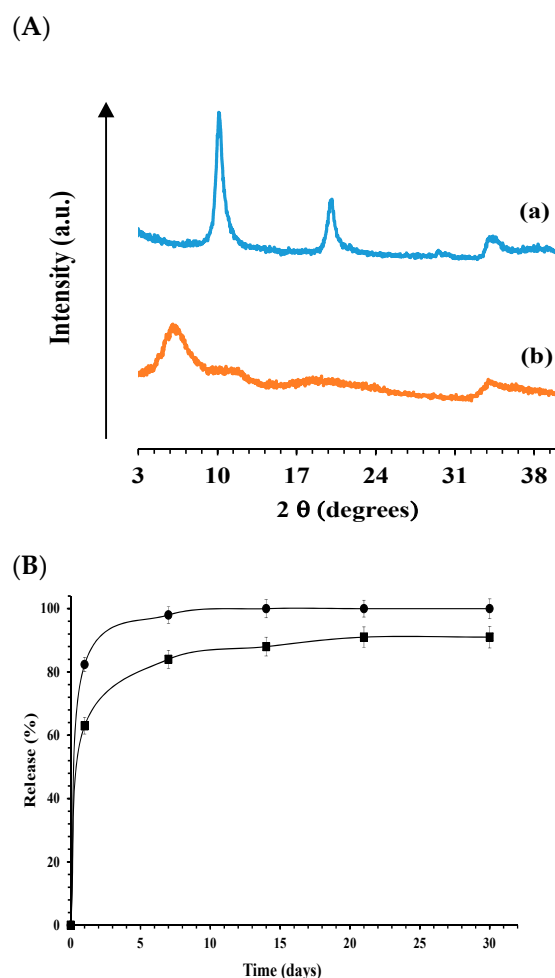
Figure 1. (**A**): Pristine LDH–nitrate (a) and LDH–salicylate (b); (**B**) Release kinetics of salicylate inside the active filler (■), and the active molecule simply dispersed into PET (●).

Figure 1B reports the release of the salicylate from the nano-carrier inside the active packaging, as a function of contact time (days). A sample of PET coated with food-grade resin and salicylate, with the same percentage of the active molecule in the nano-hybrid (i.e.,

4.2%) simply mixed into the coating, was prepared under the same experimental conditions reported in Section 2.1. The first stage, corresponding to the release from the surface, is followed by the second stage that corresponds to the de-intercalation of the salicylate ionically bonded to the LDH lamellae. It is evident that, at any release time, the molecule inside the active packaging shows a slower release, in terms of released percentage. It is worth noting that, in the investigated contact time (30 days), the molecule anchored to the nano-carrier was not completely released. Such results are in agreement with the XRD results, where we observed an intercalation of salicylate between the LDH galleries.

The evaluation of the bacterial inhibition from the active packaging was carried out using *P. aeruginosa*, *E. coli*, and *S. aureus* strains. As shown in Supplementary Table S1, the studied active packaging showed significant antibacterial activity against all target strains. These results were in accordance with those reported by other authors demonstrating the antimicrobial activity of salicylate against several Gram-positive and Gram-negative bacteria through a modification of the cell membrane permeability [37,38].

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. Supplementary Table S2 reports the global migration and specific migration of salicylic acid, evaluated on the active packaging, in different food simulants, according to UNI EN 1186-1:2003 and UNI EN 1186-9: 2003 (acetic acid, 3% *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.

3.1. Shelf Life Evaluation of Mozzarella Cheese

3.1.1. Microbiological Results

The efficacy of active packaging to counteract the growth of naturally contaminating microorganisms of mozzarella cheese was evaluated under optimal storage conditions (4 °C) and thermal abuse (15 °C); the latter was selected to simulate accidental interruptions of the conventional storage conditions (exposure of the product to ambient temperatures, thermal fluctuations during transport, etc.) that could accelerate spoilage phenomena. As shown in Figure 2, temperature- and time-dependent increases in all microbial populations were observed.

Overall, mild interactive effects between coating and time were registered against the enumerated populations only in the first 3 days of incubation both at 4 and 15 °C.

In particular, in cheese samples stored at 4 °C in coated trays, a significant decrease ($p < 0.05$) in the microbial load was registered for TBC (ca. 1.4 log CFU/g of reduction in comparison to the untreated sample), pseudomonads (ca. 0.8 log CFU/g of reduction in comparison to the untreated sample), and presumptive coliforms (0.7 log CFU/g of reduction in comparison to the untreated sample; Figure 2C). For this latter population, the antimicrobial effect was registered throughout the entire period of storage, by reaching a significant ($p < 0.05$) decrease by an average of ca. 1 log CFU/g in the treated sample in comparison to the control one (Figure 2C). The determination of presumptive coliforms (including *E. coli*) was generally chosen as a microbial target among the process hygiene criteria in the milk and dairy products [39]. Coliform recontamination can occur in the processing or aging facility through the contact of the cheese with contaminated water (especially for high-moisture mozzarella cheese), humans, air, and biofilms on dairy equipment, implying that any coliforms present in the finished product result from post-processing contamination [39]. In addition, some coliform genera are psychrotolerant and thus able to grow to high levels at refrigeration temperatures [40]. Thus, although coliform testing is no longer recommended for dairy products, we have chosen to consider them in relation to the shelf life of traditional Italian mozzarella due to the utilization of governing liquid.
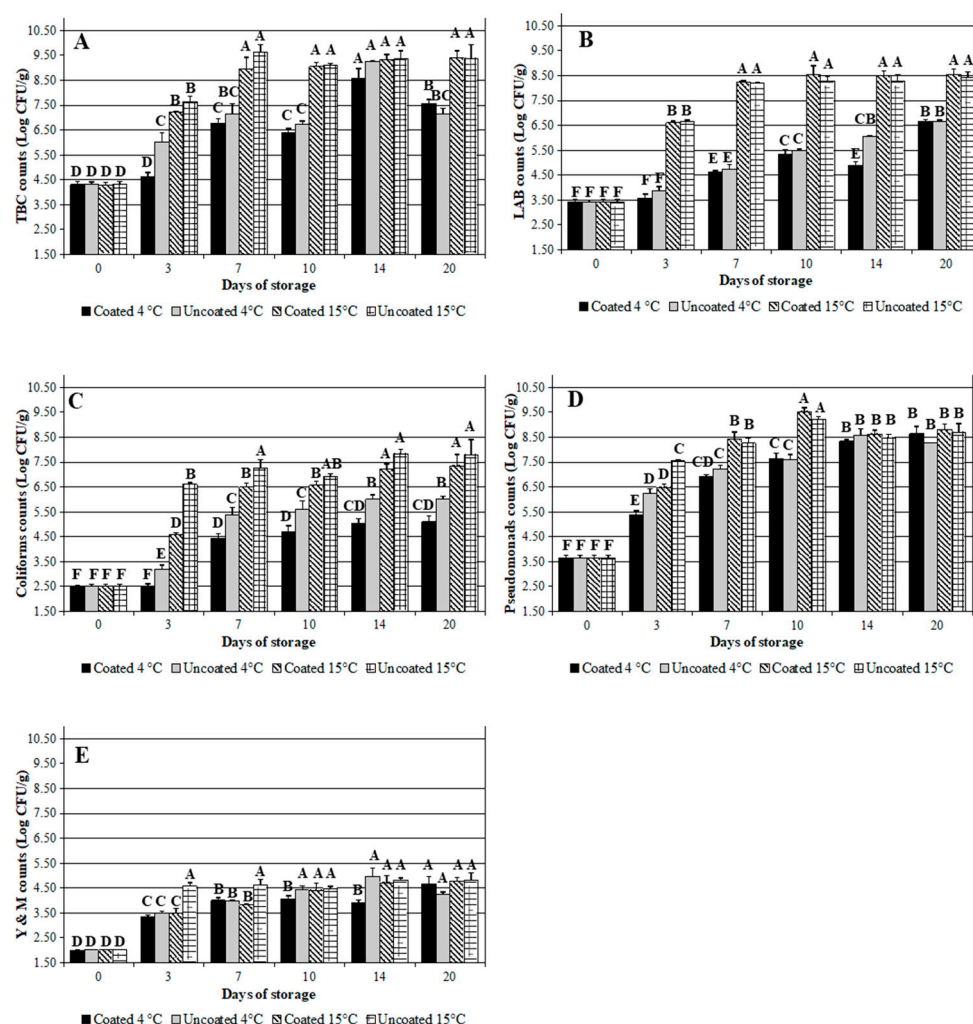
Figure 2. Counts of different microbial groups ((**A**): total mesophilic aerobic counts, TBC; (**B**): lactic acid bacteria, LAB; (**C**): coliforms; (**D**): *Pseudomonas* spp.; (**E**): yeasts and molds, Y & M) from mozzarella cheese samples dipped in autoclaved governing liquid and packed in coated and uncoated PET packages during incubation at 4 and 15 °C for 20 days. Bars represent mean $\pm$ standard deviation (N = 6). Different uppercase letters represent significant differences between the results using the Fisher's least significant difference (LSD) test with a 95% confidence interval. Y&M: yeast and mold counts.

Thus, the application of an active coating could allow the herein assayed product to maintain an acceptable presumptive coliform limit up to the third day of storage at 4 °C. By contrast, the effect of treatment on the remaining microorganisms (lactic acid bacteria, yeasts, and molds) was not different from those of control cheese samples (Figure 2B). Similar results were also registered on cheese samples stored under thermal abuse (15 °C; Figure 2). Indeed, at 3 days of incubation, significant reductions ($p < 0.05$) in microbial load in cheeses stored in the coated trays in comparison with control samples were found for the microbial groups of *Pseudomonas* spp., coliforms, yeasts, and molds (reduction of ca. 1.1, 2.0, and 1.1 log CFU/g, respectively). Although the inhibitory effect persisted for less time under thermal abuse in comparison to a lower temperature, an average reduction of 0.8 log CFU/g was observed for coliforms, yeasts, and molds up to the seventh day of incubation compared to control samples. These results agree with those obtained in a previous paper [11]. Indeed, no activity was previously found against LAB populations in mozzarella cheese packed in PET active pouches and incubated under thermal abuse (18 °C) for 22 days; by contrast, the PET active film registered the highest inhibitory effect against coliforms, molds, and yeast within the first three days of storage; then, on the

following days, their growth in the treated sample was slowed down in comparison to the untreated one [11]. The effective control of microbial growth (especially pseudomonads and presumptive coliforms) during the first three days of storage was supported by the concomitant increase in the salicylate concentration released by the active packaging.

3.1.2. Physical and Chemical Characteristics

Mozzarella cheese samples showed a different progressive decrease in pH over time, consistent with the increase in lactic acid bacteria concentration; however, no significant pH difference was found between cheese samples packed in coated and uncoated trays (Supplementary Figure S1).

During storage at 4 and 15 °C, mozzarella cheese samples showed different behavior to the acidification rate, as shown in Supplementary Figure S1. Indeed, at 4 °C, pH values remained similar to the initial value (6.45) for up to 10 days of storage in both samples of cheese stored in active packaging together with the control samples. Despite a minor subsequent decrease, the pH of the treated cheese sample was significantly higher than that registered in the control ones (6.15 vs. 6.02). As expected under thermal abuse, the pH of cheese samples dramatically decreased during up to 7 days of storage by an average of 0.59 pH units, regardless of the packaging used. Subsequently, no significant variation was registered for samples in active trays, whereas control samples showed a further significant decrease, reaching pH values of 5.60. This pH profile is quite consistent with the time-dependent increases in LAB counts (Figure 2B), suggesting their direct involvement in mozzarella cheese acidification, as also previously reported [41]. Nevertheless, the control of the acidification rate of mozzarella cheese during storage could become a useful method to extend its shelf life. Indeed, during storage, mozzarella cheese might spoil because of excessive microbial growth and a time-dependent mass transfer between the product and the governing liquid [42]. The latter, mostly based on the migration of salts and water, is generally triggered by a pH decrease that, in turn, promotes the release of colloidal calcium phosphate bound to caseins, resulting in the swelling of the casein network, loss of taste and organic matter, and disruption of the cheese's surface [43–45]. Based on this evidence, an effective control strategy for pH changes in mozzarella cheese was recently developed to counteract the rise in the pH of the governing liquid but also caused minor cheese acidification [46]. By contrast, our results showed that the active tray tested here may counteract the pH decrease in mozzarella cheese and the consequent loss of organic matter in the governing liquid. Interestingly, at 4 °C, the concentration of free amino acids and small peptides, released in tap water (used as governing liquid) by the hydrolytic activity of mozzarella cheese autochthonous microbes, was lower in the treated samples than in control ones during the storage period (Supplementary Figure S2).

This result was also consistent with an apparently lower governing liquid turbidity in the treated trays compared to that observed in control ones (Figure 3).
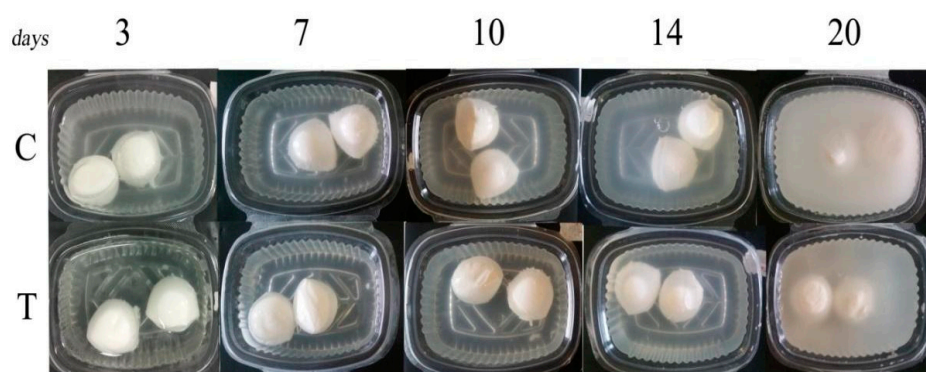
Figure 3. HM mozzarella cheeses packed in treated (T) or untreated (C) trays and incubated at 4 °C for 20 days.

In addition to higher pH values, the lower proteolysis registered in samples packed in active trays in comparison to control ones could be also attributed to the reduction in coliform counts; indeed, in addition to pseudomonads, lipolytic and proteolytic activities of psychrotolerant coliforms isolated from milk were previously reported [40].

By contrast, at 15 °C, during 14 days of storage, the treated sample showed an FAA concentration higher than those registered in control ones. Since no differences in microbial load were found for most of the analyzed microbial groups, these results could be explained by salicylate's mechanisms of action. Indeed, it has been reported to negatively interfere with the energy metabolism of bacterial cells, in turn correlated with amino acid catabolism [47]; therefore, we can hypothesize a down-regulation or repression of related enzymes under the treatment.

After 10 days of storage under the herein tested experimental conditions, a slight but significant softening was registered for all assayed mozzarella cheese samples regardless of the thermal conditions (F(4,10) = 20.082, $p = 9.057 \times 10^{-5}$; Supplementary Table S3).

Mozzarella cheeses stored for 10 days under thermal abuse in active coated packages showed values of hardness and chewiness similar ($p > 0.05$) to those stored at 4 °C. Cohesiveness slightly decreased, whereas springiness showed no significant changes ($p > 0.05$). Therefore, storage in active packaging preserved the consistency of the mozzarella cheese at 15 °C, while it had no effect on those at 4 °C. These data seem to be consistent with the results of sensory analysis performed on mozzarella cheese after 22 days of storage [11]. Likewise, hue values of cheese samples showed mild but significant reductions only in comparison to those of fresh products, maintaining the initial light greyish yellow appearance regardless of the treatment of the packaging (Supplementary Table S4).

3.1.3. Gompertz Fitting

The fitting of the modified Gompertz function from Equation (1) to coliforms and pseudomonads data at 4 °C is reported in Figure 4. Data related to control samples were also included. The results highlighted that, under optimal storage conditions, coliforms were the microbial group mainly inhibited by active packaging during the first days of incubation. In fact, the maximum cell load relating to the active packaging is reduced by around one order of magnitude with respect to the control sample throughout cold storage. The experimental data indicate both a reduction in microbial load and a slowing of the coliforms' growth kinetics. The main model parameters obtained as well as the confidence intervals are summarized in Table 1. As expected, the slowing of coliform growth kinetics is confirmed by the average values of the main parameters of the modified Gompertz model. The use of active packaging allows a reduction in maximal specific growth of around 18% with an increase in the lag time from 2.0 to 3.4 days, corresponding to an increase of 67%.

As concerns pseudomonads, their growth started in the early stage of cheese storage regardless of the treatment, as also shown by the lag time values (Table 1). Although the maximum cell load of pseudomonads related to the active packaging was comparable to that of the control system; the active packaging caused a reduction in pseudomonads' growth rate (μ_{max}) in the first 7 days that was markedly higher than that registered for coliforms (Table 1). These results are consistent with the greater adaptation of pseudomonads to low temperatures compared to coliforms, as also reported by other authors in mozzarella [7,9] and stracciatella cheese [48].

Except for *P. aeruginosa*, non-pathogenic *Pseudomonas* spp. have not yet been taken into consideration in European and national legislation and therefore no acceptability limit in food has been established for this microbial group. The only Italian regulatory reference concerning pseudomonads is contained in Legislative Decree no. 31 of 2001, which requires the absence of *P. aeruginosa* in drinking water. Recent evidence [5,6,49] highlights that the risk assessment associated with spoilage of *Pseudomonas* spp. needs to be improved, as well as the strategies counteracting their spread. Several authors report the appearance of spoilage at around 6 log CFU/g of pseudomonad load [9,50]. Thus, based on this limit of acceptability, the results of our work allowed an increase in mozzarella

cheese's shelf life from 3 days to almost 5 days, with an increment of ca. 54% in comparison to the untreated samples (Figure 4). Furthermore, since psychrotrophic pseudomonads are mostly associated with spoilage of refrigerated fresh cheeses [3,51], this technology could be exploited for other dairy products.

(**A**)

(**B**)
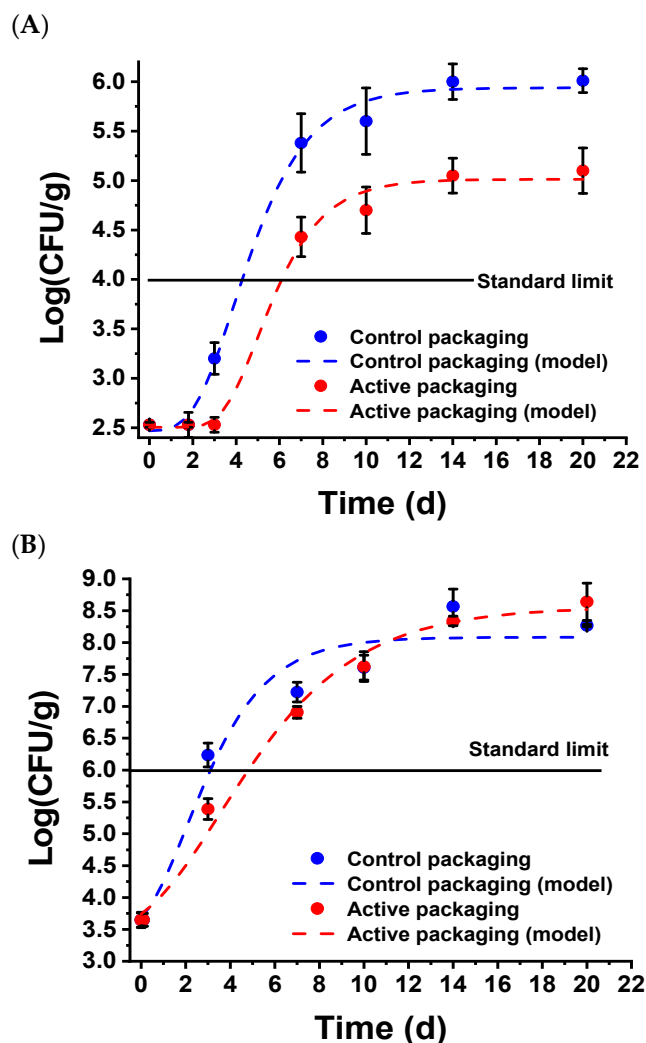
Figure 4. Behavior of microbial load of coliforms (**A**) and *Pseudomonas* spp. (**B**) at 4 °C; log (CFU/g) in the packaged mozzarella cheese vs. time (days), using control packaging and active packaging: experimental data and best fitting of Equation (1).

Table 1. Parameters from fitting the experimental data relating to microbial load of coliforms and pseudomonads (incubation temperature T = 4 °C) of Figure 4, using Equation (1): shelf life (S.L.), maximal growth rate (μ_{max}), lag time (λ), and coefficient of determination (R^2).

	S.L. [Days]	μ_{max} [μ_{max} Log(CFU/g)/Day]	Lag Time [Days]	R^2
Coliforms				
Control	4.308 [4.115–4.500]	0.680 [0.638–0.722]	2.021 [2.008–2.034]	0.98
Active film	6.122 [5.955–6.288]	0.560 [0.543–0.577]	3.372 [3.330–3.415]	0.98
Pseudomonads				
Control	3.119 [2.950–3.288]	0.871 [0.843–0.898]	≈ 0	0.97
Active film	4.812 [4.686–4.937]	0.549 [0.547–0.550]	≈ 0	0.99

This result may be considered most relevant in the evaluation of the shelf life of treated mozzarella cheese, since psychrotrophicpseudomonads are considered to be the bacterial group involved in the most important spoilage phenomena of fresh cheeses under cold storage [3,51]. Thus, even though coliforms were more sensitive than pseudomonads to the treatment, the results from this latter group allowed us to establish that the microbial acceptability of mozzarella cheese in active packaging was increased by 1.70 days in comparison to the control.

4. Conclusions

This paper reported a type of active packaging based on a food-grade resin and layered double hydroxide as the host of a salicylate antimicrobial anion, as an active coating on traditional poly(ethylene terephthalate) (PET) trays to package "ovoline" mozzarella cheese. The experimental results confirmed the potential of the active coating to counteract the microbial growth of TBC, pseudomonads, during the first 3 days of incubation at 4 °C, whilst coliforms were inhibited throughout the incubation period. Although lower, the antimicrobial activity of the coating against coliforms was also observed under thermal abuse conditions. The fitting of pseudomonad and coliform growth at 4 °C, using a modified Gompertz equation, demonstrated an increase in shelf life of ca. 2 days for mozzarella cheese stored in the active tray. Thus, these results suggest that salicylate–LDH-coated PET may be applied to extend the shelf life of mozzarella cheese and also counteract its spoilage if accidental interruptions to refrigeration occur.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/pr9050884/s1, Table S1: Inhibition growth of three pathogenic strains on the prepared active packaging after 24 h of incubation (ISO 22196:2011). U0: Counts of bacteria recovered from non-treated specimens after the inoculation; Ut: Count of bacteria recovered from non-treated samples 24 h after inoculation; At: Count of bacteria recovered from treated samples 24 h after inoculation. R: Antibacterial activity, Table S2: Global migration and specific migration of salicylic acid from the active packaging, Table S3: Texture profile analysis of mozzarella cheese samples stored at 4 and 15 °C in coated and uncoated packaging for 10 days, Table S4: CIELab coordinates of mozzarella cheese samples stored at 4 and 15 °C in coated and uncoated packaging for 10 days, Figure S1: pH values of mozzarella cheese samples packed with governing liquid in active and control packaging and stored at 4 °C and 15 °C. Values represent average ± standard deviation (N = 3). *,**: significant pairwise differences ($p < 0.05$; $p < 0.001$, respectively) between control and active samples at the same incubation temperature following the 2-tailed independent Student's t test ($p < 0.05$), Figure S2: Free amino acids and small peptide contents (FAA, μg/mL, as glycine equivalent) registered in governing liquid of mozzarella cheese samples packed in active and control trays during incubation at 15 and 4 °C for 20 days. Bars represent average ± standard deviation (N = 3). *,**: significant pairwise differences ($p < 0.05$; $p < 0.001$, respectively) between control and active samples at the same incubation temperature according to 2-tailed independent Student's t test ($p < 0.05$); n.s.: not significant.

Author Contributions: Conceptualization, L.Q., G.G.; methodology, L.Q., V.B., L.C., L.V.; validation, L.V.; formal analysis, L.Q., V.B., L.C., L.V.; investigation, L.Q., V.B., L.C.; resources, G.G.; data curation, L.C., L.V.; writing—original draft preparation, L.Q.; writing—review and editing, V.B., L.C., G.G.; visualization, L.C., L.Q.; supervision, L.Q., G.G.; project administration, G.G.; funding acquisition, G.G. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

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