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Combination of edible coatings containing oregano essential oil nanoemulsion and pulsed light treatments for improving the shelf life of tomatoes

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Edible coatings (ECs) have attracted increasing attention in the last years as a simple yet effective approach to increase the storability of perishable foods, such as fresh or fresh-cut fruits and vegetables, contributing to maintaining their quality by reducing respiration rate and water loss. The incorporation of antimicrobial agents, such as essential oils, was reported to add also antimicrobial properties to the coatings, through the controlled release of the antimicrobial compounds on the food surface, contributing to further reduce microbial growth over extended periods of storage. Pulsed light (PL) treatments have been widely investigated as non-thermal processes for superficial decontamination of food and food-contact surfaces, because of their ability to cause, through a short exposition, a significant reduction in the microbial population. Therefore, the combination of ECs and PL treatments represents a promising hurdle approach in food preservation, for extending the shelf life of fresh products. ECs in combination with optimum PL treatment condition (4 J/cm^2) improved the quality of tomato fruits in terms of reducing the growth of the endogenous flora, as well as of preserving the quality attributes (pH, total soluble solids, and color) over a 15-d storage at room temperature.

1. Introduction

With the increased globalization of the agri-food sector, leading to longer distances traveled by fresh produce, agricultural waste significantly increased. Therefore, new and improved measures to mitigate food waste are required. A promising nonthermal food preservation technology, capable to efficiently inactivate microorganisms on food surfaces is pulsed light (PL) technology. It is based on short-time pulses of an intense broad spectrum of light, ranging from UV to near-infrared (200–1,100 nm) for the elimination of bacteria, yeasts, molds, and viruses. Its use has been approved for food surface decontamination, with a maximum cumulative dosage of 12 J/cm^2 (FDA, 1996). The PL treatment has been reported to be very effective in the inactivation of microbial surface load on fresh fruits and vegetables, greatly contributing to quality preservation and shelf life extension (Mahendran et al., 2019). Edible coatings (ECs) are a consolidated technology used, in the replacement of chemical additives, in the preservation of the postharvest quality of fruits and vegetables, whose action is mainly based on slowing down respiration rate, water loss, and oxidation processes. ECs, consist of thin layers of proteins, polysaccharides, or lipids, acting as a physical barrier against moisture, gas (O_2, CO_2), and solute movement by creating a semi-permeable membrane on the food surface. Numerous studies have shown how the application of ECs can effectively preserve the quality and extend the shelf life of fresh and fresh-cut products (Maringgal et al., 2020). Recently, antimicrobial ECs have emerged as a new innovative approach to reduce microbial growth and consequently, to improve the safety and delay the spoilage of different food products (Das et al., 2020). Antimicrobial ECs are based on the incorporation of natural antimicrobial agents, such as organic acids and their salts, parabens and other food additives, chitosan, essential oils, or natural plant extracts, to couple the physical barrier effect with the sustained release of the antimicrobial agent over time (Pirozzi et al., 2020b). Therefore, antimicrobial ECs have gained a growing interest in extending the shelf life of highly

perishable and/or high value-added fruits and vegetables. Among the antimicrobial agents used in ECs, essential oils represent an extremely promising option for their non-specific action on the cell membranes, as well as easy use as EC additives in emulsified form (Donsì and Ferrari, 2016; Sessa et al., 2015). In particular, oregano essential oil (OEO) is rich in the GRAS monoterpene carvacrol, which exhibits excellent antimicrobial properties by inhibiting both gram-positive and gram-negative bacteria, and fungi (Mauriello et al., 2021).
A hurdle approach, based on the combination of PL and ECs, offers the possibility to reduce the intensity of the individual technologies by exploiting their eventual synergistic effect for food preservation. The combination of rapid surface microbial reduction by PL with the inhibition of microbial growth over an extended time by antimicrobial ECs was previously tested for fresh-cut cucumbers (Ta_tan et al., 2017). In this work, for the first time, the effect of the combined methods was tested on fresh tomatoes, whose microbial and physicochemical quality were monitored over a 15-d storage at 25 °C, using PL treatments, applied either before or after the deposition of ECs made of calcium chloride cross-linked alginate, incorporating an OEO nanoemulsion.

2. Materials and Methods

2.1 Materials

Fresh cherry tomatoes (*Solanum lycopersicum* cerasiforme) were purchased from a local market (Salerno, Italy), and stored at 4 °C until used. Before each experiment, tomatoes of uniform shape and without damages or visible signs of disease were washed with distillate water and manually dried with absorbent paper. Sodium alginate (purity 99.98%, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used as the coating material, while calcium chloride (purity 99% min, Thermo Fisher GmbH, Kandel, Germany,) as the crosslinking agent. Oregano essential oil (OEO, Frey & Lau GmbH, Henstedt, Ulzburg, Germany) was used as an antimicrobial agent in nanoemulsions, stabilized by soy lecithin (Solec IP, Milan, Italy). Buffered peptone water, plate count agar, and agar dichloran rose Bengal (VWR International, Milan, Italy) were used in microbiological viability tests. All chemicals and solvents were purchased from Sigma Aldrich (Milan, Italy) unless otherwise specified.

2.2 Edible Coating preparation and application

The coating formulation was based on a previously optimized coating carried out for shelf life extension of tomatoes (Pirozzi et al., 2020a). Details on the preparation of the EC and OEO nanoemulsions are provided elsewhere (Pirozzi et al., 2020a). Briefly, two film-forming solutions were prepared and sterilized separately for sodium alginate (0.5% w/w) and calcium chloride (2% w/w) in distilled water. Nanoemulsions were prepared by finely dispersing OEO (0.5% w/w) in distilled water containing lecithin (0.5% w/w), using the high-pressure homogenization method (200 MPa, 5 passes, and 5 °C). The nanoemulsion replaced part of the water in the alginate solution to reach a final OEO concentration of 0.17% (w/w).
Tomatoes were submerged for 2 min, using a sterile pin pricked in the pit, first in the coating solutions of sodium alginate, with or without OEO nanoemulsion, and then in calcium chloride solution to allow the cross-linkage of the polysaccharide chains, and finally, the excess coating was drained off. Tomatoes dipped in sterilized Milli-Q water for the same time were used as control samples. The coated tomatoes were drained and dried in a laminar-flow hood (24 ± 1 °C) in aseptic conditions for 3 h before further analysis.

2.3 Pulsed light treatment

PL treatments were carried out in a bench-top PL unit (RS-3000C SteriPulse-XL system, Xenon Corp., Wilmington, Mass., USA), which includes a power/control module and a treatment chamber. Tomatoes were placed at the center of the tray inside the treatment chamber (1 cm of distance between tomatoes) and the distance between the upper surface of the fruit and the lamp source was 14.1 cm. At this distance, the fluence per each pulse was 0.60 J/cm^2. During the treatments, the tomatoes were exposed to the desired energy dose on one side and then rotated 180° horizontally and exposed again to the same dose. The total fluences applied were 2, 4, and 8 J/cm^2/side and were changed using exposure times of 1.1, 2.2, and 4.4 s/side, respectively. The temperature increase on the surface of the samples upon irradiation treatments was monitored utilizing a K-type thermocouple and never exceeded 2 °C (Pataro et al., 2015). All the experiments were carried out at least in duplicate. PL treatment was combined with EC in two different sequences, as previously proposed (Pirozzi et al., 2020b): EC deposition followed by PL treatment, and PL treatment applied before EC deposition.

2.4 Microbial viability analysis

Mesophilic aerobic and yeast and mold counts of tomatoes subjected to the different treatments were evaluated throughout a 15-d storage period under a laminar-flow hood at 24 ± 1 °C. Tests were carried out after 1 or 2 d after treatment, to leave sufficient time to the PL or EC treatment to express its potential. For each microbial determination, two tomatoes were placed into a filter stomacher bag containing buffered peptone water (1:5 mass ratio) and aseptically homogenized for 1 min in a Stomacher 400 Circulator (Seward, FermionX, provided

by VWR International PBI s.r.l., Milan, Italy). Serial dilutions were made with the diluent and 1 mL were included in appropriate culture media, PCA for the total bacterial count, and DRBC for yeasts and molds. The total bacterial count was enumerated after incubation at 30 °C for 72 h, according to ISO 4833-1/2013, while yeasts and molds were determined after incubation at 25 °C for 120 h, according to ISO 21527-1/2008. All tests were performed in triplicate, and for each sample, the evaluation was carried out in duplicate for each dilution and analyzed according to ISO TS 19036:2006. The sensitivity of the analysis was e 1 log CFU/g.

2.5 Quality attributes: pH, total soluble solids, and color

At each storage time, two tomatoes for each formulation were homogenized in the Stomacher for 1 min to obtain homogenized samples for chemical analyses. The pH was determined with a pH meter (pH-Meter BASIC 20+ Crison Instruments) at 25 °C, according to the AOAC method 981.12. The soluble solid content was determined at 25 °C as °Brix, by measuring the refraction index with an Abbe digital refractometer (DR-A1 Atago).
The surface color was determined using a colorimeter CR400 Chroma Meter (Konika Minolta Inc., Japan). Color changes were quantified in the CIE L*a*b* color space. L* (lightness), a* (green-red chromaticity), and b* (blue-yellow chromaticity) were recorded and used to calculate the whiteness index (WI), chromaticity (C*), and hue angle (h°) (Koh et al., 2018). Before each analysis, the colorimeter was calibrated with a standard white plate. Ten readings were taken at random positions from each fruit, for two independently treated fruits.

2.6 Statistical analysis

Treatments and analyses were performed in triplicate unless differently specified and the results were reported as means ± standard deviations. Differences among mean values were analyzed by one-way variance (ANOVA), performed with SPSS 20 (SPSS IBM., Chicago, USA) statistical package, and Tukey test was performed to determine statistically significant differences ($p < 0.05$).

3. Results and Discussion

3.1 Pulsed light treatment effect on microbiological analysis

The effect of PL treatments on total microbial count and yeast and mold over 15-d storage was investigated at different fluence intensity (2, 4, and 8 J/cm^2) on the tomato surface decontamination (Figure 1). The microbial population decreased rapidly when PL treatment was applied, with significant inactivation effect observed already at low doses. At 2 J/cm^2, about 1 log CFU/g reduction of the mesophilic aerobic bacteria was recorded, while at 4 J/cm^2, the microbial populations decreased to 1.92 log CFU/g from 3.08 log CFU/g, providing 1.16 log CFU/g reduction. Remarkably, exposure beyond 4 J/cm^2 failed to deliver any additional reduction in the total microbial population. This behavior might be explained by several causes, such as non-uniform exposure of fruit surface to light flashes, the existence of a distribution of sensitivities to the PL treatment within the microbial population as well as shadow effect among microorganisms or surface irregularities (Pataro et al., 2016).
Yeast and mold inactivation (Figure 1b) was aligned to total microbial decontamination, highlighting the efficiency of PL treatment in promoting microbial stabilization and, hence, prolonging the tomato shelf life.
Based on the data of Figure 1, the dose selected for subsequent PL experiments was 4 J/cm^2, which ensured a high inactivation level of both total microbial count and yeasts and molds, without any visible surface damage.

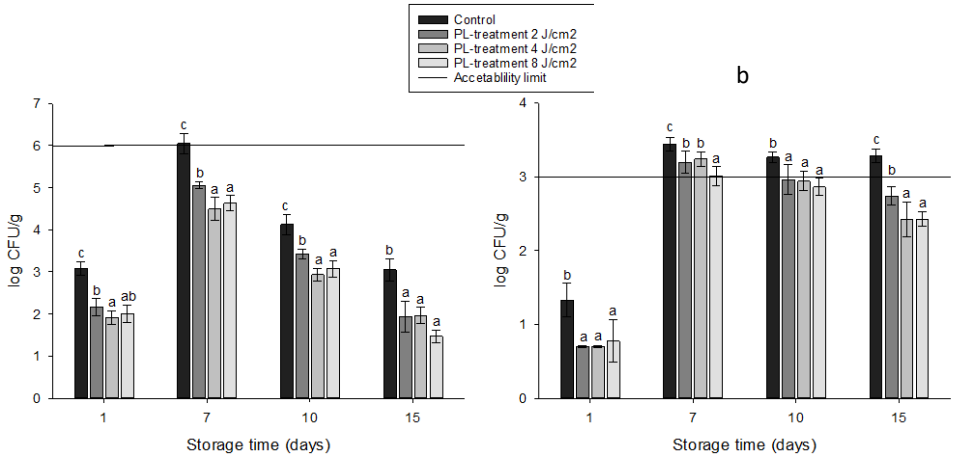

Figure 1: Total microbial (a) and yeast and mold (b) count over a 15-d storage period at 24 ± 1 °C of untreated tomatoes (control) and PL-treated tomatoes at 2, 4, and 8 J/cm^2. Different letters denote significant differences ($p < 0.05$) among the different samples for each day of storage.

3.2 Effect of combination processes on microbial behavior and shelf life of tomatoes

The surface decontamination of tomatoes was investigated by means of the deposition of ECs based on calcium chloride cross-linked alginate-based coating without and with the addition of OEO nanoemulsion, and of PL treatments, alone and in combination with ECs in different orders (Figure 2) over 15 d. As shown in Figure 2a, the aerobic plate count increased throughout the storage, reaching a maximum value between 8 and 12 d, regardless of treatments. The three preservation technologies (SA, SA_OEO, and PL) applied individually led to a substantial reduction of the initial counts. Overall, regardless of the storage day, PL treatment was more effective than SA_OEO and SA, in this order, leading to 3.06, 2.20, and 1.07 log reductions, respectively, after 8 d. Beyond the already discussed decontamination effect of PL-treatment, the ECs (without OEO) contributed to limiting the microbial growth on the product surface. The addition of OEO nanoemulsion caused an initial microbial reduction and contributed to extending the product shelf life, maintaining the total microbial count below the acceptability limit for fresh tomatoes of 6 log CFU/g (EC Regulation n. 1441/2007), and to values significantly lower ($p < 0.05$) than control and ECs without OEO, as previously observed (Pirozzi et al., 2020a).

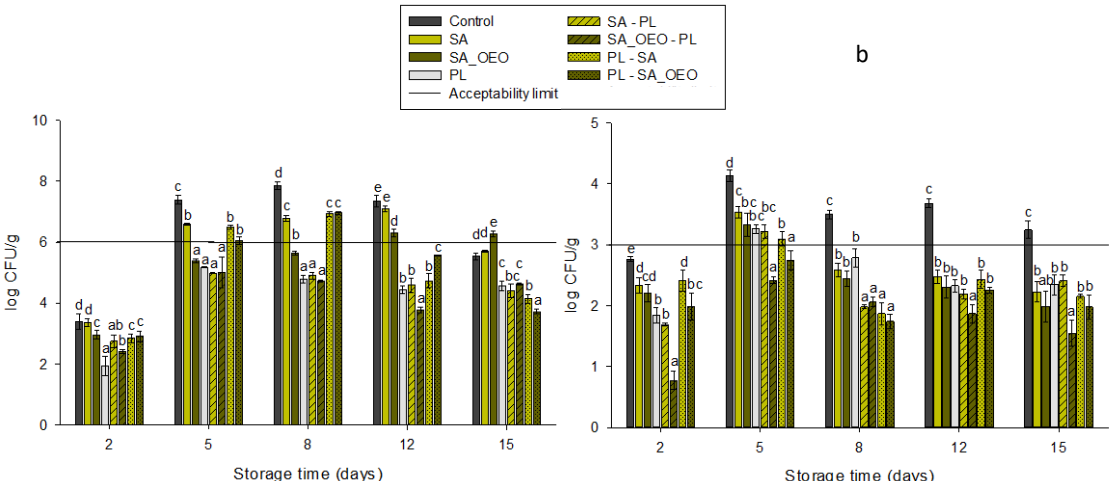

Figure 2: Total microbial (a) and yeast and mold (b) count over a 15-d storage period at 24 ± 1 °C for ECs application (SA), ECs with OEO nanoemulsion (SA_OEO), PL treatment (PL), ECs followed by PL (SA - PL), ECs with OEO nanoemulsion followed by PL (SA_OEO - PL), PL followed by ECs (PL - SA) and PL followed by ECs with OEO nanoemulsion (PL - SA_OEO). Different letters denote significant differences.

Remarkably, the combined treatments did not induce any synergistic effects, but the inactivation level and microbial growth depended on the order of treatment application. The deposition of ECs without and with OEO nanoemulsion applied before PL treatment led to a maximum reduction of the total microbial populations of 2.77 and 3.58 log cycles, respectively, after 12 d of storage. Instead, the PL treatment before coating application without and with OEO led to a significantly milder reduction of 2.61 and 1.78 log, respectively (Figure 2). This fact could be attributed to a potential antagonistic action of the PL treatment, which may have changed the microstructure of tomato skin, limiting the effectiveness of adhesion, cohesion, and durability of ECs, and hence their capability to act as barriers. This aspect needs further investigation to be fully elucidated. In the case of yeast and mold, during storage the population of all samples increased until reaching a maximum value at 5 d, followed by a slight decay towards an asymptotic value after 8 d. Remarkably, the combined treatments based on PL and ECs showed no significant ($p < 0.05$) difference in inactivation level in comparison with the individual treatments. When tomatoes were treated by PL after the deposition of EC with OEO, a statistically significant lower value of yeast and mold population than for all other samples was recorded during the entire storage period. Remarkably, it never exceeded the yeast and mold acceptability limit of 3 CFU/g (Stannard, 1997). Only limited information is available in the literature on the combination of edible coatings or active edible coating with PL treatments for surface decontamination. However, the present results suggest that PL treatments should be applied after alginate coating for reducing the aerobic mesophilic populations growing on tomatoes.

3.3 Physicochemical properties

The pH of foods, which typically increases with maturation, plays an important role in affecting the growth and type of microbial flora spoiling the product as well as its sensory and organoleptic properties (Pataro et al., 2015). The initial value of the pH of untreated tomatoes was 4.51 ± 0.02 and was not significantly ($p > 0.05$) influenced by the different treatments. Throughout the entire storage, no statistical differences ($p > 0.05$) in pH

values were observed among the treatments, except for PL-treated samples followed by EC deposition, which reached the values in the range of 4.0-5.1 (data not reported). In addition to pH, also the content of soluble solid can be used as an indicator of tomato quality and maturity. Total soluble solids (data not reported) increased during the storage period for all the applied treatments. However, no significant ($p > 0.05$) changes were detected between control and all treated samples for each storage day, which remained in the range 5.5 - 5.9. Color is one of the most important sensory characteristics and a major factor in the consumer's purchase. Color of tomatoes is related to two simultaneous processes: the chlorophyll degradation from green to colorless compounds and the synthesis of carotenoids from a colorless precursor (phytoene) to lycopene (red), ²-carotene (orange), xanthophylls, and hydroxylated carotenoids (yellow) (Radzevi•ius et al., 2009). Table 1 shows the changes in the color of tomatoes during storage according to the instrumental (L*, a* and b*) and derived color parameters (WI, C* and h°), as a consequence ECs application and PL treatments.

Table 1: Color parameters of tomatoes coated with edible coatings, treated with PL, and a combination of these two treatments stored under room temperature (sample legend as in Figure 2).

Sample	Storage (days)	L*	a*	b*	WI	C*	h°
Control	0	39.5±1.4^{b,AB}	29.8±1.7^{a,A}	22.8±2.2^{b,A}	28.8±0.7^{a,E}	49.5±1.7^{b,A}	0.65±0.03^{a,ABC}
	5	38.2±1.0^{ab,A}	28.4±2.2^{a,A}	20.6±1.6^{ab,A}	29.0±0.8^{a,C}	48.6±1.9^{ab,A}	0.63±0.03^{a,AB}
	15	37.2±1.4^{a,A}	27.4±2.5^{a,A}	19.7±2.5^{a,A}	28.7±0.7^{a,BC}	47.8±2.5^{a,A}	0.61±0.03^{a,AB}
SA	0	40.0±2.3^{a,AB}	34.5±1.9^{b,B}	24.3±3.9^{b,AB}	26.5±0.5^{a,BC}	49.8±2.8^{b,B}	0.61±0.03^{b,A}
	5	38.9±1.8^{a,AB}	33.5±1.7^{ab,B}	22.4±2.9^{ab,AB}	26.7±0.6^{a,A}	49.1±2.4^{ab,BC}	0.59±0.04^{ab,A}
	15	37.9±1.6^{a,A}	31.4±2.4^{a,BC}	19.7±2.8^{a,A}	27.6±2.0^{a,ABC}	48.8±1.9^{a,BCD}	0.56±0.02^{a,A}
SA_OEO	0	40.4±2.0^{b,ABC}	36.4±1.8^{b,B}	27.1±3.4b^{b,ABC}	25.0±1.5^{a,A}	50.2±1.2^{b,B}	0.64±0.04^{a,AB}
	5	39.1±1.4^{ab,AB}	33.8±1.4^{a,B}	23.7±2.2^{a,ABC}	26.4±0.9^{b,A}	49.2±1.5^{a,BC}	0.61±0.04^{a,A}
	15	38.4±1.2^{a,AB}	32.3±1.6^{a,CD}	22.2±2.1^{a,AB}	26.9±0.9^{b,AB}	48.7±1.7^{a,CDE}	0.60±0.04^{a,A}
PL	0	42.8±2.6^{a,C}	34.4±2.3^{b,B}	28.7±4.75^{a,CD}	27.2±1.2^{a,CD}	52.2±1.6^{b,B}	0.71±0.05^{a,CD}
	5	42.3±2.4^{a,C}	32.2±1.8^{ab,B}	27.8±4.11^{a,C}	28.2±1.0^{a,BC}	51.1±1.9^{b,C}	0.69±0.06^{a,BC}
	15	40.50±1.9^{a,BC}	30.7±2.3^{a,BC}	26.4±3.00^{a,C}	27.9±1.2^{a,BC}	49.6±2.0^{a,DE}	0.66±0.05^{a,C}
SA–PL	0	41.91±1.6^{a,BC}	34.0±1.7^{a,B}	27.5±2.91^{a,ABC}	27.2±0.8^{a,CD}	51.4±1.7^{a,B}	0.69±0.03^{a,BC}
	5	41.04±3.3^{a,BC}	32.2±2.7^{a,B}	26.2±6.13^{a,BC}	27.6±1.3^{a,AB}	50.3±4.1^{a,BC}	0.68±0.04^{a,BC}
	15	41.34±1.4^{a,C}	32.1±1.3^{a,CD}	26.3±2.36^{a,C}	28.1±0.9^{a,BC}	50.1±1.5^{a,E}	0.67±0.03^{a,BC}
SA_OEO–	0	42.6±1.7^{b,C}	30.9±1.9^{b,A}	29.5±2.9^{b,C}	28.4±1.3^{a,DE}	51.1±2.2^{b,B}	0.76±0.03^{b,D}
PL	5	40.4±1.9^{a,ABC}	28.3±2.5^{a,A}	25.0±3.5^{a,ABC}	29.3±0.9^{a,C}	49.9±2.8^{a,AB}	0.73±0.04^{ab,C}
	15	38.8±1.7^{a,AB}	27.1±2.2^{a,A}	22.9±3.1^{a,ABC}	29.2±1.2^{a,C}	48.9±2.3^{a,AB}	0.70±0.05^{a,C}
PL–SA	0	40.5±1.6^{b,ABC}	33.9±1.6^{c,B}	24.7±3.4^{b,AB}	27.1±0.8^{a,CD}	50.3±2.1^{c,B}	0.63±0.04^{a,AB}
	5	38.8±1.6^{a,AB}	31.7±1.9^{b,B}	22.3±2.9^{ab,AB}	27.5±1.1^{a,AB}	48.8±2.2^{b,ABC}	0.61±0.04^{a,A}
	15	37.6±1.4^{a,A}	29.2±1.5^{a,AB}	19.5±2.3^{a,A}	28.3±1.8^{a,BC}	47.9±1.2^{a,ABC}	0.59±0.04^{a,A}
PL–	0	39.0±1.5^{a,A}	35.6±1.2^{b,B}	24.6±2.7^{b,AB}	25.1±0.5^{a,AB}	49.0±1.8^{b,B}	0.60±0.02^{b,A}
SA_OEO	5	38.0±1.4^{a,A}	33.3±1.9^{a,B}	21.9±1.9^{a,AB}	26.3±0.7^{b,A}	48.3±2.1^{a,ABC}	0.60±0.03^{a,A}
	15	38.9±1.8^{a,AB}	34.4±1.1^{ab,D}	23.4±2.1^{ab,BC}	26.0±1.3^{ab,A}	48.1±1.7^{ab,E}	0.58±0.02^{a,A}

The results are expressed as mean•±•standard deviation (n•=•3). Different lowercase letters indicate significant differences within the same sample for different storage times ($p < 0.05$). Different uppercase letters indicate significant differences between samples within the same storage time ($p < 0.05$).

Both L* and b* values for tomatoes treated by PL alone or by ECs followed by PL remained unchanged for 15 d during storage, whereas the other samples exhibited significant changes. The a* values tended to decrease for all the samples during the storage period, except for the control. Moreover, the a* values of treated samples were significantly ($p <0.05$) higher than control. This aspect needs further investigation to be fully understood. WI, C*, and h° are key parameters affecting consumer acceptance and food quality. Low WI will harm product marketability and desirability (Das et al., 2020). In this study, WI did not significantly change during storage time for all samples. It reached a maximum value of 29.1±0.3 for ECs with OEO followed by PL treatment, and a minimum value of 25.7±0.4 for PL-treated sample followed by ECs with OEO. The incorporation of the nanoemulsion in ECs likely promoted a higher level of light scattering, enhancing surface brightness, opacity, and whiteness. The C* parameter, which quantifies color intensity, slightly decreased during the storage period for the untreated and treated samples. However, no significant ($p < 0.05$) variations were detected among samples, with the intensity of red coloration maintained during storage, regardless of the applied treatment. The color hue (or redness) of tomatoes can be estimated through h°, which was constant during storage, without any significant ($p < 0.05$) difference detected over time for both untreated and treated tomatoes. In comparison

with control, h° was not significantly affected ($p < 0.05$), except for EC application, and PL treatment followed by EC. Overall, it can be summarized that the color of tomatoes was substantially unaffected during storage time for all treatments, and no signs of fruit damage were observed for PL, ECs, and their combination.

4. Conclusion

The combination of pulsed light (PL) treatments with antimicrobial edible coatings (ECs) was investigated for tomato preservation. Remarkably, the developed alginate EC formulation containing an oregano essential oil nanoemulsion, followed by PL treatment, significantly improved the quality of tomato fruits in terms of physicochemical properties as well as microbiological shelf life, in comparison with the untreated sample and with the individual (EC alone or PL alone) treatments. Indeed, the proposed hurdle approach led to a higher reduction of both mesophilic aerobic bacteria, as well as of yeast and molds throughout the entire storage period at room temperature, while minimizing the changes in physicochemical properties. Apparently, ECs primarily contributed to maintaining the physical quality of tomatoes, while PL treatment controlling microbial growth.

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