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Essential Oils and Bacteriocin-Based Active Edible Coating: An Innovative, Natural and Sustainable Approach for the Control of *Listeria monocytogenes* in Seafoods

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Abstract: The anti-*Listeria monocytogenes* activity of four essential oils (EOs) (*Salvia officinalis, Citrus limon, Mentha piperita and Thymus vulgaris*) and bacteriocin bacLP17, added alone or in mixture in active edible coatings, was determined in artificially contaminated shrimps. The minimal inhibitory concentration (MIC) values of the EOs were determined against the NCTC 10888 strain of *L. monocytogenes* by using the broth microdilution method. The checkerboard method was carried out in tryptic soy broth (TSB), using microdilution to obtain the Fractional Inhibitory Concentration Index (FIC-Index) for six associations of EOs, chosen based on the best MIC results. All the EOs confirmed their anti-*Listeria* activity, both "in vitro" and inside the coatings. The coating matrix was suitable for use in the food field, allowing a gradual release of the EOs in packaged food. When the EOs were used in association (EO/EO) they were demonstrated to act synergistically, leading to a significant reduction in the amount (10–20 times) of EOs needing to be used, and consequently a decrease in the strong smell on the food. This effect was also confirmed when the compounds were incorporated into the coatings. The inclusion of the EOs within the coating not only ensured the anti-*Listeria* activity by increasing the shelf-life of food products, but also further mitigated the strong smell of the EOs, improving the organoleptic impact on the food and its sensory properties.

Keywords: *Listeria monocytogenes*; shrimp; essential oils; bacteriocin; edible coating

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1. Introduction

The employment of chemical additives in food preservation is increasingly less accepted by consumers and limited by restrictive laws [1], and new methodologies based on natural additives are necessary to improve the prospects of sustainable packaging for future applications in the food industry. Foodborne infections due to bacterial pathogens such as *Listeria monocytogenes* remain a serious health concern, as this foodborne pathogen, responsible for over 90% of all cases of food poisoning, is also associated with high mortality in humans, notably for vulnerable sub-populations: young, old, pregnant, and immunocompromised [2]. *L. monocytogenes* causes septicemia, meningitis, and other infections of the central nervous system in immunocompromised patients. If a woman is infected during pregnancy, it may lead to spontaneous abortion, neonatal sepsis or fetal death. In 2021, 2268 confirmed cases of listeriosis were reported by 30 EU/EEA Member States. Germany, France and Italy were the countries with the highest number of reported cases (560, 435 and 241 respectively), corresponding to 54.5% of all cases reported in the EU/EEA [3]. Listeriosis has the second-highest fatality rate (21%) and the highest hospitalization rate (90.50%), with 2536 European cases of listeriosis reported in 2016 [4,5]. *L. monocytogenes* is a psychrotrophic bacteria that can grow at refrigeration temperatures and can persist in food processing plants and on equipment [6]. This pathogen can contaminate food

during all stages of production, and it is most frequently found in perishable foods that can also be eaten raw, such as: fish and fishery products (6%), ready to eat salads (4.2%), meat and meat products (1.8%), soft and semi-soft cheeses (0.9%), fruit and vegetables (0.6%) and hard cheeses (0.1%) [5]. Fish and seafood products, notably those consumed without cooking and preserved at refrigeration temperatures for a long time (such as smoked fish), support the growth of the pathogen, providing sufficient nutrients for its growth. *L. monocytogenes* can both directly contaminate the raw seafood from the aquatic environment or be reintroduced at the end of the production process [7]. Smoked mussels, smoked trout, raw oysters, gravid and cold-smoked fish are all fishery foods responsible for outbreaks of listeriosis [8–11]. The persistence of *L. monocytogenes* in food processing plants is an important factor both in the transmission of this foodborne pathogen and in the contamination of food and food-associated environments. In this context, a primary role is played by the biofilm [12,13], a microbial community which allows the persistence and therefore the environmental spread of the pathogen. *L. monocytogenes* is capable of surviving, adapting and persisting in various environments due to the presence of a biofilm. Inside this structure it can find both nutrients for growth and protection from different adverse conditions, including preservation treatments [14,15]. Within the biofilm, microorganisms are more resistant to sanitation treatments than their planktonic form, and this complex microbial structure is more difficult to eradicate, becoming a continuous source of contamination in food processing plants [16,17]. Cleaning and sanitizing procedures are currently applied to food processing plants by employing chemicals, but recently the need for chemical-free foodstuffs and concern for the environment have increased the demand for treatments that do not involve their use in the food field. Among the most widely used procedures is refrigeration, a treatment that uses low temperatures to slow down the growth of pathogenic microorganisms (and the possible production of toxins), which is certainly the most-used means of preservation for all types of food. This preservation method is particularly used for those foods defined as "fresh" which have undergone low levels of heat treatments or transformations and are free from additives. However, while refrigeration is effective against mesophilic pathogens, it is unable to slow down the development of a psychrophilic microorganism such as listeria which survives and grows at 2–4 °C. For this reason, most current research is aimed at identifying natural additives that can contribute to overcoming this problem [18,19]. Following the ever-increasing demand from consumers for foods free from chemical preservatives, while also wanting to guarantee quality and safe products, many recent studies have been oriented towards a bio-preservation approach. Bio-preservation is a technique of food preservation in which the antimicrobial potential of selected microorganisms, their metabolites or other natural substances against spoilage and pathogenic bacteria are exploited. Notably, the antimicrobial properties of biomolecules (bacteriocins) produced by Lactic Acid Bacteria (LAB), and of plant-derived compounds such as essential oils [20], have been widely investigated. LAB possess a major potential for use in bio-preservation. They are commonly used in food fermentation, may produce several metabolites with beneficial health effects, and thus are generally recognized as safe (GRAS). LAB exert strong antagonist activity against many related and unrelated microorganisms, including food spoilage and pathogenic bacteria such as *Listeria* and *Staphylococcus* spp. The antagonistic effect of LAB is due to lowering the pH of the food, the competition for nutrients, and the production of inhibitory metabolites such as organic acid (lactic acid or acetic acid), hydrogen peroxide and antimicrobial peptides such as bacteriocins active against bacterial pathogens [21]. The beneficial characteristics of Essential Oils (EOs) have long been known and have attracted the attention of researchers for their biological properties, such as anti-inflammatory, antioxidant, fungicidal, anticancer and antimicrobial activities. Their wide range of antimicrobial activity, in particular, is extensively studied in the food field, as they are natural products endowed with many characteristics that make them suitable for food preservation [22]. EOs are volatile compounds of vegetable origin produced by plants as defensive agents against herbivores, insects and phytopathogenic microorganisms. The discovery of the inhibitory activity of

EOs against human pathogens has also led to the development of numerous studies on their mode of action. Interaction with the microbial cell membrane seems to be the main target of the EOs [23–25]. The damage of this microbial structure is the main cause of the growth inhibition of some Gram-positive and Gram-negative bacteria, which, however, show a differential sensitivity to these compounds, being more marked in Gram-positive pathogens, including *L. monocytogenes* [26].

A further step forward was taken when different categories of these natural antimicrobial compounds were added to edible films and coatings, leading to significantly improved antimicrobial properties of packaging [27–29]. Both companies and research in the food industry are increasingly aiming at developing packaging materials of natural origin, which are made with renewable polymers, derived from by-products, biodegradable and/or produced in total respect for the environment [30]. Moreover, the study of edible packaging for use in fresh foods aligns with this trend, where such materials can represent an edible barrier capable of prolonging the preservation of fresh foods [31,32]. Edible coatings or films may be a new green preservation method, notably if they are based on the addition of natural substances which make it possible to reduce the use of synthetic polymers. Therefore, bacteriocins, EOs and edible coatings represent a natural promise in food preservation and the food industry to reduce the addition of chemical preservatives and antibiotics. All these materials could therefore be increasingly used to reduce or eliminate the growth of pathogenic and spoilage bacteria, particularly in perishable foods such as fish products. The consumption of fish products is increasing in many places in the world, mainly due to the presence of high quality proteins with low fat content [33]. However, due to its abundance of nutrients, this type of food represents a suitable medium for the development of microorganisms, both spoilage and food-borne pathogens [34]. Foodborne outbreaks due to the consumption of seafood account for about 10–20% of all episodes caused by the consumption of contaminated food, but this percentage may differ between countries depending on the level of consumption, local traditions (use of raw fish or bivalve molluscs) and the monitoring system for the microbial quality of the product [33]. *L. monocytogenes* is frequently isolated in raw fresh fish, and the level of contamination ranges between 0% and about 30% of the products, depending on geographical area [35]. It is frequently isolated from smoked salmon, fermented fish and fish salads, all products subjected to light preservation processes; treatments which, while bringing pleasant organoleptic properties, are not able to sanitize the product [36,37]. High isolation rates of listeria are also reported for shrimp and shrimp products. In a study carried out in Chile [38], *L. monocytogenes* was detected in 28% of tested fresh shrimp, whereas in Iceland, it was observed in 20.9% of tested fresh products [39]. Lastly, even if *L. monocytogenes* has been isolated from a fish's surface, stomach, gills and intestines, seafood products can also be contaminated from different sources through cross-contamination, during production processes, transportation and in the market environment [36,40].

The per capita consumption of seafood has greatly increased over the past two decades, and the consumption of raw seafood such as fish, crustaceans and molluscs pose a threat to consumer health. Hence, considering the public health implications of listeriosis and the persistence of the pathogen in seafood processing plants and on equipment [41], the aim of the present study was to create a natural and ecological antimicrobial barrier capable of counteracting the development of *L. monocytogenes* on foods that come to our tables. Therefore, the present investigation was carried out in order to (i) evaluate the anti-*Listeria* activity of two types of natural compounds used by themselves or in combination, viz., bacteriocin LP17 (bacLP17) produced by *Enterococcus mundtii* LP17 (isolated from red mullet) and EOs derived from *Salvia officinalis, Citrus limon, Mentha piperita and Thymus vulgaris*, spices widely used in the kitchen as natural flavourings; and (ii) determine in artificially contaminated shrimps the anti-*Listeria* activity of the same, added alone or in combination, to an edible coating.

2. Materials and Methods

2.1. Natural Compounds and Strains

The EOs *Citrus limon* (*C.l.*), *Mentha piperita* (*M.p.*), *Salvia officinalis* (*S.o.*) and *Thymus vulgaris* (*T.v.*), obtained by hydrodistillation, and purchased from a local herbalist's shop in Modena, Italy, have been studied and characterized in previous investigations by GC-MS and GC-FID for quali- and semi-quantitative analysis [42]. The bacteriocin bacLP17 is produced by *Enterococcus mundtii* LP17, a strain previously isolated from red mullet and endowed with strong activity toward the pathogen [43]. The edible coating, provided by the SSICA research foundation (Parma, Italy), was obtained from pea proteins coming from processing residues [44]. The frozen shrimps, purchased from a supermarket in Modena, were brought to the laboratory using dry ice and the defrosting process was performed in a refrigerator (4 °C). *Listeria monocytogenes* NCTC 10888 was used as a microorganism test.

2.2. Minimal Inhibitory Concentration (MIC) and Fractional Inhibitory Concentration Index (FIC-Index) Determination

The MIC values of *C. lemon*, *M. piperita*, *S. officinalis* and *T. vulgaris* EOs were determined against *L. monocytogenes* NCTC 10888 by using the broth microdilution method in 96-well microplates, according to the Clinical Laboratory Standards Institute (CLSI) guidelines 2012 [45]. The test was performed in sterile 96-well microplates, with a well working volume of up to 200 mL of solution, by dispensing into each well 95 mL of Tryptic Soy Broth (Oxoid S.p.A, Milan, Italy) and 5 mL of bacterial suspensions, to a final inoculum concentration of 10^4 CFU/mL. Then, 100 µL of EOs serial dilutions were added to obtain concentrations ranging from 512 to 0.125 µL/mL. Negative control wells consisted of bacteria in TSB without EOs. The plates were incubated at 37 °C for 24 h, mixed on a plate shaker at 300 rpm for 20 s, and the MIC was defined by the growth of the bacteria on the TSA plate. The checkerboard method was carried out in TSB by using the microdilution method to obtain the FIC-Index for the following six EO/EO associations, chosen based on the best MICs results obtained by the single compounds: *C. lemon*/*M. piperita*, *C. lemon*/*S. officinalis*, *C. lemon*/*T. vulgaris*, *M. piperita*/*S. officinalis*, *M. piperita*/*T. vulgaris* and *S. officinalis*/*T. vulgaris* [46]. The same associations were also used in the experiments carried out with the addition of the bacteriocin bacLP17 (Tables 1–4).

Table 1. MICs of EOs and bacLP17 used alone against *Listeria monocytogenes* NCTC 10888.

C. lemon	*M. piperita*	*S. officinalis*	*T. vulgaris*	**bacLP17**
32 µL/mL	32 µL/mL	128 µL/mL	8 µL/mL	16 µL/mL

Table 2. MICs of the most advantageous EO/EO associations (chosen based on the FIC-Index) added to the coatings.

C. lemon *M. piperita*	*C. lemon* *S. officinalis*	*C. lemon* *T. vulgaris*	*M. piperita* *S. officinalis*	*M. piperita* *T. vulgaris*	*S. officinalis* *T. vulgaris*
3 µL/mL +3 µL/mL	1.5 µL/mL +6 µL/mL	3 µL/mL +0.8 µL/mL	1.5 µL/mL +6 µL/mL	3 µL/mL +0.8 µL/mL	6 µL/mL +0.8 µL/mL

Table 3. MICs of the most advantageous EO/bacLP17 combinations (chosen based on the FIC-Index) added to the coatings.

C. lemon **bacLP17**	*M. piperita* **bacLP17**	*S. officinalis* **bacLP17**	*T. vulgaris* **bacLP17**
3 µL/mL +0.8 µL/mL	1.5 µL/mL +1.5 µL/mL	12.5 µL/mL +0.4 µL/mL	1.5 µL/mL +0.8 µL/mL

Table 4. MICs of the most advantageous EO/EO/bacLP17 combinations (chosen based on the FIC-Index) added to the coatings.

C. lemon *M. piperita* bacLP17	*C. lemon* *S. officinalis* bacLP17	*C. lemon* *T. vulgaris* bacLP17	*M. piperita* *S. officinalis* bacLP17	*M.piperita* *T. vulgaris* bacLP17	*S. officinalis* *T. vulgaris* bacLP17
3 µL/mL	3 µL/mL	3 µL/mL	3 µL/mL	3 µL/mL	6 µL/mL
+1.5 µL/mL	+3 µL/mL	+0.8 µL/mL	+3 µL/mL	+1.5 µL/mL	+0.4 µL/mL
+0.8 µL/mL	+1.5 µL/mL	+0.8 µL/mL	+0.8 µL/mL	+0.4 µL/mL	+1.5 µL/mL

2.3. Shrimp Contamination and Coating

The defrosted shrimps (44 samples) were contaminated in sterile conditions by inoculating each shrimp with a Hamilton syringe with approximately 10^6 CFU/mL [47]. Suspension of an overnight *L. monocytogenes* NCTC 10888 culture diluted in sterile saline solution (NaCl 0.85%) resulted in a final absorption of about 10^4 CFU/g of *L. monocytogenes* in the food samples. Edible coating added with different concentrations of EOs used by themselves, in association with each other and in combination with bacLP17 (see Tables 1–4), was applied to all samples by dipping, including the controls inoculated with *L. monocytogenes* NCTC 10888 but coated without essential oils. Samples of doped and undoped coating stored at 4 °C were analyzed at regular intervals: 0 h, 24 h, 72 h, 4 days and 7 days. Experiments were carried out in triplicate.

2.4. Anti-Listeria Activity Determination

Portions of samples (25 g) were placed in sterile plastic bags, with 225 mL of buffered peptone water (Oxoid, Milan, Italy) added, and homogenized for 1 min in a Stomacher (Lab Blender, Seward, London, UK) [48]. Serial tenfold dilutions of the obtained homogenates were spread in triplicate on Palcam agar with added selective supplement (Oxoid, Milan, Italy) and plates were incubated aerobically at 37 °C for 24 h. All bacterial counts were recorded as CFU/g.

2.5. Statistical Analysis

Each experiment was carried out in triplicate. The statistical significance was determined by the *t*-test and ANOVA test using the statistical program GraphPad Prism 9.2.0. (San Diego, CA, USA). The *p*-values were declared significant at ≤ 0.05.

3. Results

3.1. Minimal Inhibitory Concentration (MIC) of the Single Natural Compounds and of the Most Advantageous Synergistic Mixtures

Table 1 shows the MICs of EOs and bacLP17 used alone against the indicator strain *L. monocytogenes* NCTC 10888. Among the plant-derived compounds, *T. vulgaris* was the most effective, and good anti-*Listeria* activity also emerged from the bacteriocin, bacLP17.

Tables 2–4 show the antibacterial activity against *L. monocytogenes* NCTC 10888 of the most advantageous synergistic mixtures (expressed as MIC values), chosen based on the FIC-Index. In all tests, carried out using EO/EO associations and EO/bacLP17 combinations, a synergistic effect emerged. EO/EO and EO/bacLP17 were effective at very low concentrations that decreased up to 20 and 40 times for EO/EO and EO/bacLP17, respectively, compared to the MIC of the single compounds.

3.2. Edible Antimicrobial Coating Added with EOs by Themselves or in Association against L. monocytogenes NCTC10888 on Artificially Contaminated Shrimp Samples

The anti-*Listeria* properties of all the chosen EOs were confirmed even when singularly added to the coatings. In this study, the synergistic effects of EOs mixtures on *L. monocytogenes* were also investigated. When the EOs were used in association, the FIC-Index showed good synergy between the natural compounds inside the coating, with an anti-*Listeria* activity obtained at values much lower than when used alone (Tables 1–3).

Figures 1 and 2 report the mean values of the *L. monocytogenes* NCTC 10888 viable counts (log CFU/g) detected in the contaminated samples packaged either with doped (EOs by themselves or in association) or undoped (control) coatings, after storage at 4 °C.

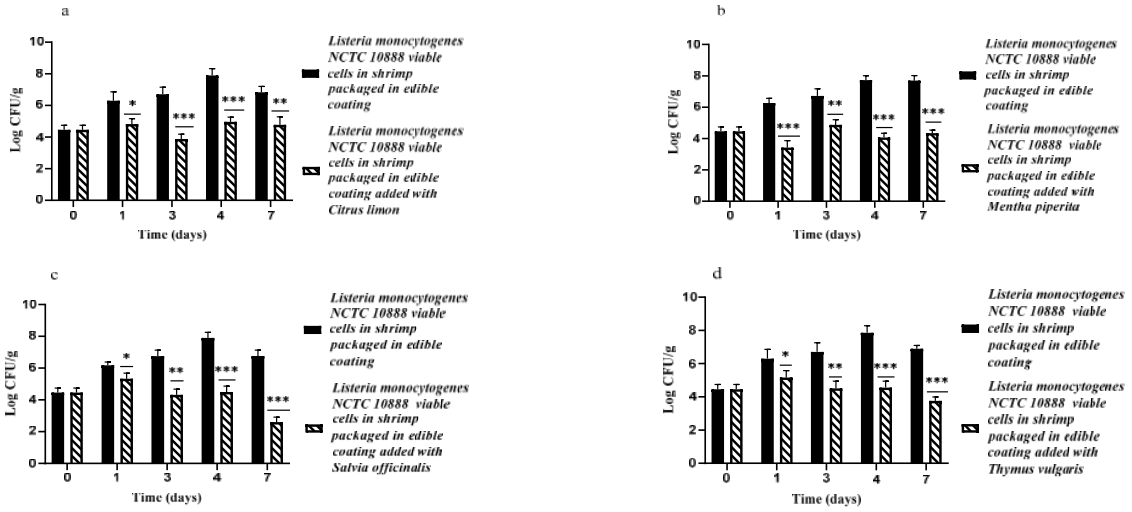

Figure 1. Coating added with EOs by themselves and at the respective MIC values, applied on artificially contaminated shrimp samples: *Citrus lemon* (**a**), *Menta piperita* (**b**), *Salvia officinalis* (**c**), *Thymus vulgaris* (**d**). Experiments were performed in triplicate. *p*-values of <0.05 (*), $p < 0.01$ (**) and $p < 0.001$ (***) were considered significant by *t*-test and ANOVA.

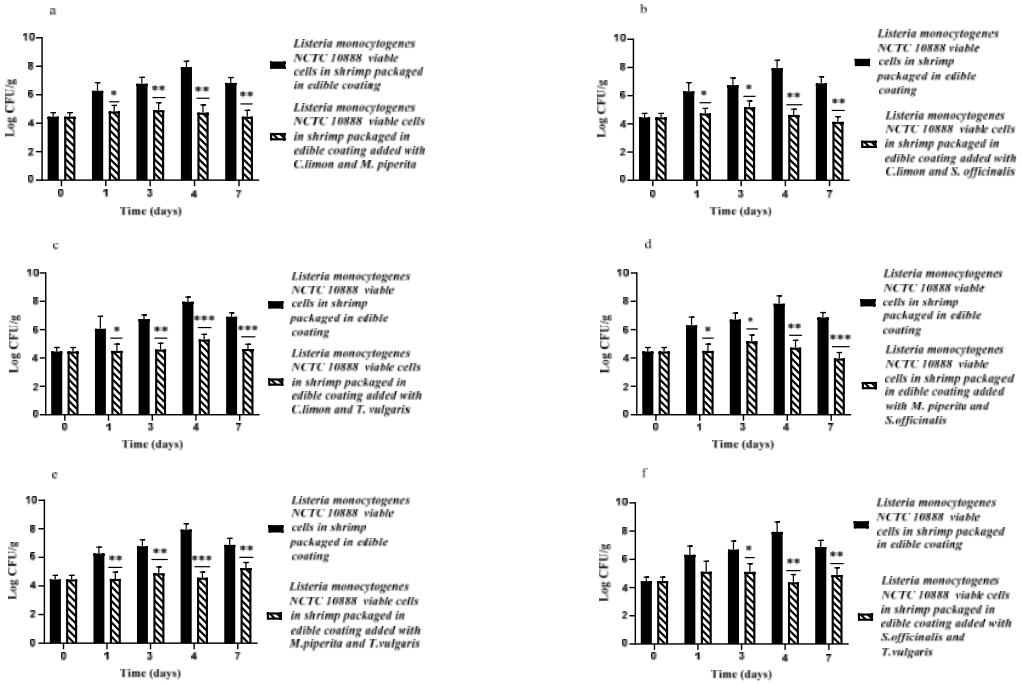

Figure 2. Coating added with different EOs association, applied on artificially contaminated shrimp samples: *C. limon*/*M. piperita* (**a**), *C. limon*/*S. officinalis* (**b**), *C. limon*/*T. vulgaris* (**c**), *M. piperita*/*S. officinalis* (**d**), *M. piperita*/*T. vulgaris* (**e**) and *S. officinalis*/*T. vulgaris* (**f**). Experiments were performed in triplicate. *p*-values of < 0.05 (*), $p < 0.01$ (**) and $p < 0.001$ (***) were considered significant by *t*-test and ANOVA.

The results showed good anti-*Listeria* activity for the coating with EOs added by themselves, compared to the control, but with significant differences in the action times (Figure 1). *M. piperita* was effective in controlling the growth of *L. monocytogenes* NCTC10888 as early as 24 h after the study, showing a 3 log difference compared to the control ($p < 0.001$) (Figure 1b). The same result was obtained using *C. limon* ($p < 0.001$), but from the third day (Figure 1a). In both cases the activity was maintained until the end of the experiments. *S. officinalis* and *T. vulgaris* showed the best activity against viable *Listeria* cells on the fourth day ($p = 0.0003$ and $p = 0.0005$, respectively), with a 4 log ($p = 0.0001$) and 3 log ($p < 0.0001$) reduction in microbial load, respectively, observed at the end of the study (Figure 1c,d).

Good anti-*Listeria* activity also emerged when the mixtures of EOs were used, confirming that the synergy between the selected associated compounds is still maintained when added to the coating (Figure 2).

After only 24 h the *M. piperita*/*T. vulgaris* mixture showed a significant difference compared to the control ($p = 0.009$), displaying a 2 log reduction in the microbial load of the pathogen, and reaching a 4 log reduction on the fourth day (Figure 2e). This EO/EO association allows the reduction of the concentration of use of both compounds (see Table 2), combining the efficacy of *M. piperita*, in the first hours of experimentation, with the marked anti-*Listeria* activity of *T. vulgaris*, a synergy that was detectable until the end of the experiment. An excellent efficacy was also observed using the associations *C. limon*/*T. vulgaris* ($p = 0.002$), followed by C. *limon*/*M. piperita* ($p = 0.009$), on the third day of testing (Figure 2a,c). The association *C. limon*/*S. officinalis* and *M. piperita*/*S. officinalis* yielded the best activity against viable *Listeria* cells on the 4th and 7th day, respectively, and this good synergistic effect was maintained by the last mixture until the end of the study ($p = 0.0009$). The association *S. officinalis*/*T. vulgaris* showed the worst result than the other mixtures.

3.3. Edible Antimicrobial Coating Added with a Mixture of EOs and bacLP17 against L. monocytogenes NCTC10888 on Artificially Contaminated Shrimp Samples

The results showed good anti-*Listeria* activity for the coatings added with the four EOs and bacLP17 in combination, with significant differences in *L. monocytogenes* NCTC10888 viable counts compared to the control. The combination of the two types of natural compounds again showed good synergy inside the coatings as well, with consequent anti-*Listeria* activity detectable at much lower values than their singular use.

Figure 3 shows the mean of the *L. monocytogenes* NCTC 10888 bacterial load (log CFU/g) found in the contaminated samples packaged either with doped (bacLP17 alone and in combination with the EOs) or undoped (control) coatings, after storage at 4 °C.

When added to the coatings, bacLP17 showed an early activity against *L. monocytogenes* NCTC10888 at 24 h, with clear efficacy maintained and improved until the end of the experiments (Figure 3a). Once again, the presence of *C. limon* in the bacLP17/*C. limon* mixture ensured its efficacy from the first hours of contact (24 h), as well as a significant reduction in bacterial counts on days 4 and 7 ($p = 0.0001$ and $p < 0.0001$, respectively) (Figure 3b). The best result in terms of reduction of *L. monocytogenes* bacterial load was obtained using the bacLP17/*M. piperita* combination, with a marked reduction of viable cells already after 24h ($p = 0.002$), and a subsequent increase until the end of the experiment ($p < 0.0001$) (Figure 3c). Lastly, regarding the combinations between bacLP17 and two EOs, all the latter compounds exerted a synergistic effect with bacLP17 against *L. monocytogenes* NCTC10888. Here, we report that the best result obtained was by adding to the coating the bacLP17/*M. piperita*/*T. vulgaris* combination. As shown in Figure 3d, there was a significant antibacterial effect on viable *Listeria*, with an increasing trend from the first hours to the end of the experiment (7 days) ($p < 0.0001$).

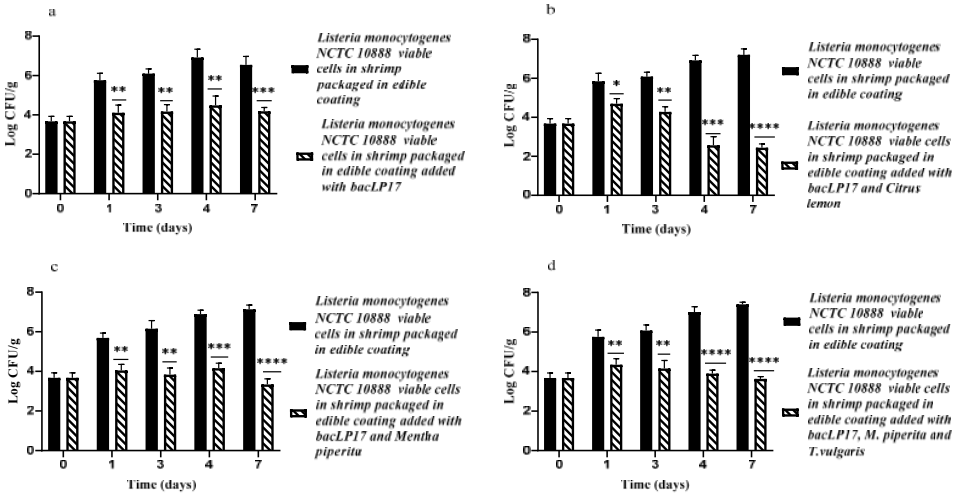

Figure 3. Coating together with bacLP17 (**a**) and with the best combination bacLP17/EOs: bacLP17/*Citrus lemon* (**b**), bacLP17/*M. piperita* (**c**), bacLP17/*M. piperita*/*T. vulgaris* (**d**), applied on artificially contaminated shrimp samples. Experiments were performed in triplicate p-values of < 0.05 (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****) were considered significant by t-test and ANOVA.

4. Discussion

The primary function of food packaging is to protect food from external contamination, with the aim of both improving its shelf life and ensuring its safety for the consumer. During the last few years, the changing demands of the market have induced food industries to direct their research towards innovative and sustainable technologies of food preservation. Active food packaging is the most innovative system of interaction between packaging and food, capable of extending its shelf-life and increasing the quality, safety and organoleptic characteristics during the storage period. The development of technological innovations in the agri-food field to expand the "life" of perishable foods, however, have not prevented the increased incidence of bacteria with high adaptability to survive in adverse environmental conditions. The most relevant pathogens are those endowed with psychrotrophic characteristics, able to survive and even multiply at temperatures close to zero. Among the psychrotrophic bacteria, *L. monocytogenes* appears to be the main human food-borne pathogen abundantly widespread in nature and isolated from a wide variety of foods.

The data obtained in the present investigation can represent an important step forward in the control of *L. monocytogenes* in foods that require storage at refrigeration temperature, notably perishable products such as seafood.

All the EOs employed in the study confirmed their anti-*L. monocytogenes* activity in the "in vitro" studies, even when incorporated into the coatings. The coating matrix has been found to be suitable for its use in the food field, allowing a gradual release of the EOs on packaged food, thereby sustaining a marked antibacterial activity during preservation at refrigeration temperature. At the same time, the EOs have shown to act synergistically even when incorporated into the coating, and the significant reduction in the amount of EOs (10–20 times) needing to be employed against *L. monocytogenes* overcomes the problem of their limited use due to the undesirable organoleptic impact when added to foods in high concentration [49–51]. Hence, the inclusion of the EOs within the coating not only ensures the anti-*Listeria* activity, increasing the shelf-life of the food products, but it also mitigates the strong smell of EOs, improving the sensory properties of the food, as already reported [45]. These results have also shown the capability of the bacteriocin bacLP17 to maintain unaltered antibacterial activity against *L. monocytogenes* inside the coatings, with a gradual release from coating to food. Among active packaging applications, the incorporation and subsequent slow release of antimicrobials is receiving much attention as a means to extend the bacterial lag phase and slow down the growth of microorganisms to

maintain food quality and safety over time [52–54]. The combined use, inside an edible coating, of antimicrobial natural substances of different origin and endowed with peculiar characteristics can overcome some of the drawbacks associated with the use of chemical additives, as well as some limitations due to features of EOs and bacLP17, namely that bacteriocins are not usually characterized by a broad host range, and the use of EOs is often limited by undesirable organoleptic impact. The synergistic effect observed in the present investigation led to obtaining a more enhanced anti-*Listeria* activity of EOs at lower MIC values (that permits the use of EOs as preservative without affecting the sensory quality of foods) when combined with each other and/or with bacLP17, whereas the presence of EOs in the mixture ensures the bacteriocin a broader spectrum of activity (which also includes resistant strains). Ghrairi et al. also reported that the combination of bacteriocin (enterocin A) with EO (thyme essential oil) exhibited synergistic activities against *L. monocytogenes* and *E. coli* O157:H7 even at low concentration [55]. Several studies have shown that combined use of EOs with bacteriocins is advantageous in inhibiting the growth of *L. monocytogenes* and other food pathogens in meat and minced meat [56–58].

Further studies will be necessary to improve the prospects of active edible coatings for future application in the food industry. More knowledge of the spatial distribution of both natural compounds and their interactions with other microbial species sharing the same habitat with *L. monocytogenes* in different types of foods is also necessary. Both these natural antimicrobials, in fact, present limits such as the reduced sensitivity of Gram-negative bacteria to LAB bacteriocins and the strong smell of EOs. The synergism obtained with the combined use of EOs and bacteriocin may be a promising natural way to overcome both the narrow range of activity and the unpleasant sensory impact. Lastly, it is important to underline that the use of edible coatings obtained from food by-products is a great advantage for the environment because such coatings are biocompatible and eco-friendly material.

5. Conclusions

The use of EOs and bacteriocins in combination, and added to this type of edible packaging, will allow the exploration of promising opportunities for the development of novel strategies based on active edible coatings effective in controlling *L. monocytogenes* growth in seafood products and in other minimally processed RTE foods. The use of an edible and green coating will also allow the mitigation of post-production losses, a valuable contribution to respect for the environment.

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