Biocontrol of *L. monocytogenes* in Meat Products

Subjects: Food Science & Technology

Contributor: Leontina Grigore-Gurgu, Florentina Ionela Bucur, Octavian Augustin Mihalache, Anca Ioana Nicolau

Listeria monocytogenes is a foodborne pathogen that causes listeriosis, a group of human illnesses that appear more frequently in countries with better-developed food supply systems. Meat and meat products, especially the ready-to-eat (RTE) ones, have been reported as being a major food vehicle for *L. monocytogenes* transmission to humans. The cause of this phenomenon is mainly attributed to the contamination during processing or post-processing steps, such as slicing and packaging, followed by the growth of the pathogen during storage to numbers that endanger the consumers' health. In the attempt to satisfy the consumers' demand with respect to both healthy and safe foods, studies have focused on biocontrol methods, including bacteriophages, antagonistic microbial interactions, and plant- or microbe-derived substances having antilisterial activity.

Keywords: bacteriophage ; lactic acid bacteria ; bacteriocins ; endolysins ; antilisterial ; listeriosis ; active packaging

1. Introduction

Listeria monocytogenes is a foodborne pathogen responsible for listeriosis, the fifth most reported zoonosis in humans in the European Union (EU). In 2022, in Europe, 2.738 listeriosis cases were reported, with a notification rate of 0.62/100,000 individuals leading to 1.330 hospitalizations (48.6%) and 286 deaths (10.4%). *L. monocytogenes* was also responsible for 35 foodborne outbreaks and 296 foodborne illnesses-related to the outbreaks, with 242 hospitalizations (81.8%) and 28 deaths (9.5%) [1]. Listeriosis mainly affects vulnerable consumer groups such as children, pregnant women, the elderly, and individuals with immunocompromised systems. This is also validated by the fact that most of the foodborne illness cases were reported in the age group of over 64 years old [1]. While in healthy individuals listeriosis manifests itself as mild influenza and gastroenteritis, in vulnerable consumers, it results in severe symptoms like septicemia, meningitis, and miscarriage/stillbirth [2]. *L. monocytogenes* is often associated with pig meat and products thereof, fish and fish products, mixed food, vegetables and juices, and dairy products other than cheese. This is confirmed by the Rapid Alert System for Food and Feed (RASFF), in which 340 alerts were reported in the last three years, mainly with *L. monocytogenes* in fish, meat, and dairy products [1]. *L. monocytogenes* is a food safety threat due to its ubiquitous nature and ease of entering the processing environment through raw ingredients. Some strains of *L. monocytogenes* can survive for many years and serve as a source of ongoing cross-contamination due to their ability to cling to a range of abiotic surfaces [3][4][5]. Out of 37,779 samples tested at the manufacturing stage, 578 (1.53%) were positive for *L. monocytogenes.* In 2022, the highest notification rate and number of cases of listeriosis were recorded since 2007, indicating the need for ongoing research and mitigation strategies for the reduction in *L. monocytogenes* [1]. However, *L. monocytogenes* is known to be a difficult organism to eradicate even when the best safety management plans are implemented [6][7].

2. The Use of Bacteriophages in Meat Products

Nowadays, two phage biocontrol products against L. monocytogenes are commercially available: ListShield™ (formerly known as LMP-102™) produced by Intralytix Inc. (Baltimore, MD, USA) and PhageGuard Listex™ (formerly known as ListexTM or P100) produced by Micreos Food Safety (Wageningen, The Netherlands). The LMP-102™ is a mixture of six purified phages with specific activity against the pathogen that could be applied on the surface of the meat products by spraying at a level not exceeding 1 mL per 500 cm^2 [8]. Unlike the ListShield phage product, the PhageGuard Listex contains only one phage, P100 [9].

Table 1 summarizes studies regarding the efficacy of commercial antilisterial bacteriophages aimed to control *L. monocytogenes* in meat and meat products. The degree of *L. monocytogenes* reduction has been shown to depend on several factors: the ratio between bacteriophages titer and contamination level [10], diversity of pathogenic strains [10], the contact between the phages and the host [11], occurrence of host resistance to phages, products' chemical composition and characteristics, and storage conditions [10].

Table 1. Studies exploring the application of bacteriophages as biocontrol tools against *L. monocytogenes* in meat and meat products.

Meat or Meat Products	Contamination Procedure	Antimicrobial Agent	Treatment Conditions	Storage Conditions and Results	References
Fresh beef	Surface inoculation with *L. monocytogenes* LM-94 at 6.2 log CFU/g	ListShield™	1×10^9 PFU/mL spot inoculation followed by incubation at RT for 2.5 h	Reduction by 2.3 log CFU/g after storage at 4 ± 1 °C for 15 days	[12]
Spanish dry-cured ham	Surface inoculation with *L. monocytogenes* S2 at 10^5 CFU/cm^2, 10^4 CFU/cm^2, and 10^3 CFU/cm^2	ListShield™	10^7 PFU/cm^2	Reduction below the detection limit (10 CFU/cm^2) for lower contamination level (10^4 CFU/cm^2 and 10^3 CFU/cm^2) and by 3.5 log units for high contamination level (10^5 CFU/cm^2) after storage at 4 °C for 14 days	[10]
		Listex™	10^9 PFU/cm^2	Reduction below the detection limit (10 CFU/cm^2) for all contamination levels after storage at 4 and 12 °C for 24 h	
Fermented meat sausage (*Alheira*)	Contamination with *L. monocytogenes* Scott A and *L. monocytogenes* 1942 at 10^5 CFU/g	Listex™ P100	10^8 PFU/g	Reduction below the detection limit of both strains after storage at 4 °C for 14 days	[13]

Meat or Meat Products	Contamination Procedure	Antimicrobial Agent	Treatment Conditions	Storage Conditions and Results	References
Cooked turkey and roast beef	Surface contamination with a four-strain cocktail (*L. monocytogenes* 08-5578, Li0512, Li0529, and ATCC19115) at 10^3 CFU/cm^2	Listex™ P100	10^7 PFU/cm^2	Reduction by 2.1 $\log_{10}$ CFU/cm^2 and 1.7 $\log_{10}$ CFU/cm^2 in cooked turkey and roast beef, respectively, compared to the control (non-treated samples) during storage at 4 °C for 28 days	[14]
RTE pork ham	Surface contamination with a two-strain cocktail (*L. monocytogenes* B7, AL48/15, and *L. monocytogenes* Scott A) at ~2.5 log CFU/g	Listex™ P100	5×10^5 PFU/g	Reduction to undetectable level after storage at 6–8 °C for 72 h	[15][16][17]

PFU—plaque-forming units; CFU—colony-forming units; and RT—refrigeration temperature.

Regarding the contact between bacteriophages and *L. monocytogenes* cells contaminating the meat products, one study tested the efficiency of *Listeria* bacteriophage A511 in a cooked-meat model system under multiple scenarios: both bacteriophage and pathogen in the meat, bacteriophage in the meat and pathogen on its surface, pathogen in the meat and bacteriophage on its surface, and both bacteriophage and pathogen on the meat surface. The research revealed that the phages' ability to control the growth of *L. monocytogenes* on the meat product is limited because their direct contact with the targeted bacterial cells is limited [11].

3. Endolysins in Meat Products

There is no sufficient data available in the scientific literature regarding the use of endolysins to control *L. monocytogenes* in meat and meat products. The inactivation of *L. monocytogenes* by endolysins in combination with high-pressure processing (HPP) was described by Nassau et al. [18]. Three strains of *L. monocytogenes* (ATCC 15313, WSLC 11043, WSLC 11048) were co-incubated with different endolysin concentrations, ranging from 0.16 mg/mL to 20 mg/mL for PlyP40 and Ply511, respectively, and 100 mg/mL for PlyP825. The enzyme activity was assessed at 90 and 180 min before HPP treatments. The HPP parameter level of 200 MPa maintained for 2 min is usually too low to kill the pathogenic cells when this type of treatment is applied without any other cell sensibilization. Interestingly, the results obtained highlighted a good reduction in *Listeria* spp. cells (up to 5 log CFU/mL), when a synergic effect between endolysins and the HPP treatment (200 MPa/2 min/30 °C) was obtained. The use of endolysins not only significantly enhanced the bactericidal impact of HPP but also facilitated the deactivation of bacterial cells at considerably lower pressure thresholds [18]. A similar strategy by combining endolysin PlyP825 and HHP processing was applied to inactivate *L. monocytogenes* artificially inoculated in smoked salmon, in a concentration of 10^7 CFU/g. The results showed a reduction of only 1.6 log cycles even when a higher level of endolysin (34 µg/mL) and HPP treatment (500 MPa/10 min/25 °C) were used [19].

4. Lactic Acid Bacteria (LAB) in Meat Products

Another biocontrol method used to prevent the proliferation of *L. monocytogenes* in meat products is fermentation either occurring naturally, as a result of indigenous LAB presence, or stimulated by adding starter cultures. Fermentation results

in a pH decrease through the formation of lactic acid. Following fermentation, meat products need to be subjected to a drying step, so that the final water activity drops below the limit that allows *L. monocytogenes* to grow. On the other hand, the inhibitory effect of LAB against the pathogen during fermentation may be caused by the production of antimicrobial peptides called bacteriocins.

Several studies evaluated the behavior of *L. monocytogenes* in fermented meat products in terms of interaction between the pathogen and LAB. Huang et al. [15] showed that LAB addition at a concentration of ~7 log CFU/g to meat sausages subjected to simultaneous fermentation and drying (incubation at 30 °C and relative humidity RH of 76% for 5 days) caused the inhibition of the *L. monocytogenes* population (initially inoculated at ~5 log CFU/g) growth. Moreover, the number of pathogenic cells indicated a slow decrease during the process, by ~0.5 log CFU/g. A similar experiment was reported by Giello and colleagues [20] who co-cultured *L. monocytogenes* OH and Scott A (10^4 CFU/g) and *Lactobacillus curvatus* 54M16 (10^7 CFU/g), a strain-producing bacteriocin, in sausages ripened for three days at 20 °C (RH: 75–85%) followed by other 25 days at 15 °C (RH: 65–70%). Their results showed that the number of *L. monocytogenes* decreased under the detection limit within the 5 days of co-incubation at 15 °C. Moreover, after 48 h at 15 °C, the only surviving strain was the OH strain, as this was demonstrated by the RAPD-PCR profile [20].

The effect of the product's changing pH on *L. monocytogenes* capacity to multiply during fermentation was also assessed. Kamiloğlu and co-workers [21] concluded that the reduction in *L. monocytogenes* population (2.74 log CFU/g) during the ripening of suçuk (Turkish sausages), for 11 days, was especially due to the fast acidification (pH below 5) caused by the autochthonous *L. plantarum* S50, added as starter culture. The authors did not exclude the antagonistic activity of LAB against the pathogen as a supplementary inhibitory factor, as the strain was confirmed to produce bacteriocins by in vitro tests [21].

An innovative approach to benefit from LAB biopreservation potential is to incorporate postbiotics, namely, the substances released during their growth, such as bacteriocins, organic acids, carbon dioxide, and di-acetylene, into polymeric films, which are then used as active packaging materials. In this regard, Beristain-Bauza and co-workers [22] supplemented whey protein films with *L. sakei* cell-free supernatant and used them to wrap beef cubes artificially contaminated with *L. monocytogenes* (~3 log CFU/g). The antimicrobial film reduced *L. monocytogenes* population by 1.4 log CFU/g during refrigerated storage (4 °C) for 120 h [22]. More recently, Shafipour et al. [23] obtained an antimicrobial meat wrapping paper based on bacterial nanocellulose that contained postbiotics produced by *L. plantarum*. The nanopaper proved to have a strong antilisterial activity, as it was shown to reduce *L. monocytogenes* counts in ground meat by ~5 log CFU/g after storage at 4 °C for 9 days [23].

5. Bacteriocins in Meat Products

Nisin's efficacy in reducing *Listeria* spp. cells in meat and meat products has been demonstrated in various studies [24][25][26]. However, the occurrence of nisin resistance in *L. monocytogenes* cells after exposure to this peptide is not an uncommon phenotype [27], and this fact led to the necessity of combining it with other hurdles, represented by either antimicrobial substances or physical treatments. Wongchai et al. showed that nisin (62.5 µg/mL) combined with salts of organic acids could overcome this problem, as the synergism with citric acid (1000 µg/mL) prevented the growth of *L. monocytogenes* on pork ham during storage at 4 °C for 4 days [28]. Hammou et al. also noticed that nisin (200 µg/g) combined with NaCl (salt; 12%) can significantly inhibit the growth of the pathogen on natural sheep casing during 90 days of storage at 6 °C [29].

Other researchers focused on the synergism between nisin and EOs as a preventive strategy regarding *L. monocytogenes* proliferation in meat and meat products. Raeisi and colleague [30] investigated the fate of *L. monocytogenes* (at an initial concentration of 3.2 log CFU/g) during storage at 4 °C for 15 days on chicken meat coated with sodium alginate that contained either nisin (N) alone or in combination with *Cinnamomum zeylanicum* EO (CEO + N) and rosemary EO (REO + N). The results of the study indicated a better efficiency in controlling *L. monocytogenes* growth of the coatings supplemented with CEO + N (final concentration of 6.4 log CFU/g) and REO + N (final concentration of 6.6 log CFU/g) than that containing only N (final concentration of 7.5 log CFU/g) [30]. Carvacrol, the main constituent of thyme or oregano EOs, was shown to affect *L. monocytogenes* cells by inducing irreversible damage to their cell wall and cellular membranes [31]. Therefore, it is considered a good candidate in combating the occurrence of *L. monocytogenes* resistance against nisin. Indeed, the pathogen's growth on sliced bologna sausage was significantly decreased in samples treated with nisin (25 µg/mL) together with carvacrol (62.5 µg/mL) compared to those treated with these antimicrobial substances separately. Moreover, due to the synergism between the two additives and, as such, the side effects concerning the sensorial properties, consumers' acceptance of meat products treated with EOs can be increased [31].

Nisin was shown to increase the *L. monocytogenes* inactivation rate in meat products by high-pressure processing (HPP) [32][33]. Teixeira et al. [34] obtained a reduction in *L. monocytogenes* counts on ham by more than 5 log CFU/g when the meat product was subjected to a combined treatment consisting of HPP (500 MPa, 5 °C, 3 min) and nisin (~2 μg/cm^2). Moreover, after 4 weeks of refrigerated storage of ham, *L. monocytogenes* cells remained undetectable [34]. By achieving microbial safety, synergism with nisin may also contribute to the reduction in the HPP expenses at a level comparable to that of the traditional methods of meat product processing, such as heat treatments [32]. This bacteriocin was also successful in the enhancement of gamma radiation treatments against *L. monocytogenes* [35][36][37]. The study conducted by Mohamed and co-workers combined gamma radiation with nisin and showed an antimicrobial additive effect against *L. monocytogenes*, during the first 24 h after treatment, and a synergistic one, during the next 48 h of storage at 4 °C. The authors suggested as a potential strategy for *L. monocytogenes* elimination the combined treatment consisting of nisin (10^3 IU/g) and gamma radiation applied at 1.5 kGy [38].

The next most studied bacteriocin in *L. monocytogenes* biocontrol research is pediocin. The pediocin-like peptides belong to class IIa of bacteriocins, being produced by *Pediococcus* spp. and described as biologically active against *Listeria* spp. [39][40]. Their effectiveness in the reduction of *L. monocytogenes* load in meat products by direct addition or produced by LAB strains has been demonstrated by former studies [41][42][43]. More recently, the biocontrol of *L. monocytogenes* by pediocin was evaluated by its incorporation in active packaging materials. Such materials are intended to inhibit or delay the growth of undesired microorganisms while minimizing preservatives' addition to food products [44]. The in vivo approach showed encouraging results [45]. For instance, Woraprayote [46] developed an antilisterial polylactic acid/sawdust particle biocomposite film incorporated with pediocin PA-1/AcH. The highest anti-listeria activity was achieved by pediocin adsorption to the coating at 11.63 ± 3.07 μg protein/cm^2. The authors indicated that the obtained material's potential of *L. monocytogenes* inhibition in contact with the contaminated raw sliced pork could be of 99%, as suggested by a model study [46]. Another study assessed the efficacy of a film based on cellulose-containing 25% or 50% pediocin against *Listeria* spp. on sliced ham. While the film containing 25% pediocin could not prevent the growth of *L. innocua*, the one containing 50% pediocin reduced the bacterium by 2 log cycles after 15 days of storage at an abusive temperature (12 ± 1 °C) compared to the control (without bacteriocins) [44]. Pediocin was shown to enhance the inactivation of *Listeria* in meat products treated with HPP. The HPP (300 MPa, 10 °C, 5 min) in conjunction with the *ex* or *in situ* pediocin bacHA-6111-2 production was applied in the study of Castro et al. [47] to inactivate *L. innocua* inoculated in fermented meat sausages and to evaluate the survival of the bacterium during 60 days of storage at 4 °C. Considering a contamination level more likely to occur during the sausages' processing (~10^4 CFU/g), it was shown that both ways of pediocin production resulted in a synergistic effect with HPP, as the counts of *Listeria* spp. after the combined treatment decreased by >2 log CFU/g. However, the analysis of bacterium behavior during the storage of sausages revealed that in situ bacteriocin production was more efficient regarding the control of its growth [47].

6. Essential Oils in Meat Products

Many studies evaluated EOs' potential to control *L. monocytogenes* in meat and meat products. The aromatic oils have been applied either as such [37][48][49][50], encapsulated [51], or incorporated into edible coatings [52] (**Table 2**). The last two delivery systems were reported to be more acceptable to consumers in terms of meat products' organoleptic properties after being applied. Besides this, the encapsulation of EOs and their addition to various active packaging materials have been shown to solve the inconveniences regarding EOs' instability to external factors [53] and poor solubility in foods with low-fat content [54]. Also, due to the need for relatively high amounts of EOs to achieve a satisfactory degree of pathogens' inactivation, the treated meat products can become inappropriate for consumption as a result of altered sensorial characteristics and possible toxicity [55]. Lower concentrations of EOs can be used if combining them with other natural antimicrobial substances, such as bacteriocins [56] or physical treatments, results in a synergistic effect. Bearing in mind that minced beef supplemented with 0.9% thyme oil was unacceptable in terms of organoleptic properties, to achieve a sufficient inactivation degree of *L. monocytogenes*, Solomakos et al. instead recommended a combined treatment consisting of 0.6% thyme oil and 1000 IU/g nisin [56]. In the study by Huq et al. [37], it was shown that during storage at 4 °C, the synergism between oregano (250 μg/mL) or cinnamon (250 μg/mL) EOs and nisin (16 μg/mL) determined slower growth rates of *L. monocytogenes* on cooked ham (0.20 and 0.11 ln CFU/g/day, respectively) compared to EOs alone (0.21 and 0.18 ln CFU/g/day, respectively) [37]. Moreover, the encapsulation of cinnamon EO combined with nisin and the treatment of the RTE ham with the obtained capsule resulted in a much lower growth rate of the bacterium, of 0.05 ln CFU/g/day. The increased efficiency was attributed to better preservation of the antimicrobials' biological activity when entrapped in the biopolymeric matrix and their better distribution on the meat product's surface [37][57].

The nanoemulsion of EOs has been shown to generate better results in terms of *L. monocytogenes* biocontrol in comparison with emulsions. Some of the advantages of using EOs in this form are the improved stability and increased

physical resistance of the EOs, and better transferability of the hydrophobic bioactive compounds. Kazemeini [58] compared the antimicrobial activity of an alginate edible coating containing the nanoemulsion of *Trachyspermum ammi* EO to that of the coating containing the emulsion of the same EO against *L. monocytogenes* inoculated on turkey fillets. By the end of the contaminated meat product's storage (4 ± 1 °C for 12 days), the counts of *L. monocytogenes* were lower in the meat treated with *T. ammi* EO nanoemulsion (7.12 ± 0.09 log CFU/g) compared to that treated with *T. ammi* emulsion (5.53 ± 0.13 log CFU/g) [58].

Dini et al. [59] assessed the efficacy of a chitosan film containing 1% nanoemulsion of cumin EO combined with low-dose gamma irradiation (2.5 kGy) against *L. monocytogenes* on beef loan during refrigerated storage. While the edible film alone did not exert good control on the pathogenic bacterium's growth, its combination with the physical treatment generated an enhanced antilisterial effect, which might become an effective strategy to ensure the microbiological safety and improved shelf-life of the meat product [59]. Khaleque et al. [50] showed that the reduction in *L. monocytogenes* population in ground beef treated with 5% clove EO was more accelerated at refrigeration (8 °C) and chill (0 °C) temperatures compared to the storage at freezing temperatures (−18 °C) [50]. Solomakos and colleagues noticed that the antilisterial effect of EOs was also influenced by the storage temperature, and a stronger antimicrobial activity of thyme EO against the pathogen in minced beef was found when stored at 10 °C than at 4 °C [56].

Table 2. Studies exploring the application of essential oils as biocontrol tools against *L. monocytogenes* in meat and meat products.

Meat or Meat Products	Application of EOs	Contamination Procedure	Storage Conditions and Results	References
Beef meatballs	Addition of *O. vulgare*, *R. officinalis,* and *T. vulgaris* at concentrations of 0.5%, 1%, or 2% (*v*/*w*)	Inoculation with a five-strain cocktail (*L. monocytogenes* HSD 2434, HSD3261, HSD 3705, HSD 3948, HSD 4210) at 10, 10^2, 10^3, and 10^4 CFU/g	Concentrations of 2% and 1% restricted the growth of *L. monocytogenes*, regardless of the initial microbial loading, during storage at 4 °C for 14 days, but affected the meatballs' flavor. The concentration of 0.5% restricted the growth of *L. monocytogenes* at initial counts of $<10^2$, and the taste of meatballs was acceptable.	[48]
Italian mortadella	Addition of combined *T. vulgaris* and *R. officinalis* at concentrations of 0.025% and 0.05% during manufacturing	Contamination of mortadella slices with a three-strain cocktail (*L. monocytogenes* ATCC 19111, ATCC 13932, and ATCC 19117) at ~2.5 log CFU/g	Compared to the untreated contaminated mortadella, the addition of combined EOs to the concentrations of 0.025% and 0.05% led to a reduction in *L. monocytogenes* by 2.29 log CFU/g and 2.79 log CFU/g by the end of storage at 4 °C for 30 days.	[60]

Meat or Meat Products	Application of EOs	Contamination Procedure	Storage Conditions and Results	References
Ground beef	Addition of crude and commercial *C. cassia* and *S. aromaticum* EOs at concentrations of 5% and 10%, and 2.5% and 5%, respectively	Inoculation with a five-strain cocktail (*L. monocytogenes* ATCC 43256, ATCC 49594, JCM 7676, JCM 7672, and JCM 7671)	The ground beef was stored at 8 °C and 0 °C for 7 days and at −18 °C for 60 days. A 10% concentration of clove EO (both crude and commercial) completely inactivated *L. monocytogenes* within 3 days of storage, irrespective of temperature. A 5% concentration of clove EO (both crude and commercial) reduced *L. monocytogenes* gradually throughout storage, irrespective of temperature, without achieving complete inactivation. The 2.5% and 5% concentrations of crude and commercial cinnamon EO did not inactivate *L. monocytogenes* throughout storage. Consumers did not find the ground beef treated with 10% clove EO acceptable, while some of them found the meat treated with 5% clove EO acceptable.	[16][30][50]
Dry-cured ham-based medium	Addition of *C. cassia* EO in dry-cured ham-based medium with water activity of 0.93 or 0.95 at a concentration of 10%	Inoculation with a serotype 4 *L. monocytogenes* strain at ~4 log CFU/mL	During storage at 7 °C for 7 days, 10% cinnamon EO completely inhibited *L. monocytogenes* growth irrespective of the ham-based medium's a_w.	[16]
Fresh chicken meat	Corn starch edible coating containing *Zataria multiflora* EO nanoemulsion alone and fortified with cinnamaldehyde	Contamination of the meat with *L. monocytogenes* to a final concentration of ~10^4 CFU/g followed by its immersion in the corn starch solutions	The coating with fortified nanoemulsion was more effective in controlling *L. monocytogenes* than that with the nanoemulsion alone during storage at 4 ± 1 °C for 20 days, with a growth difference between the treatments of ~1 log CFU/g.	[52]

Meat or Meat Products	Application of EOs	Contamination Procedure	Storage Conditions and Results	References
Fresh beef	Soy protein edible coatings containing 1%, 2%, or 3% thyme or oregano EOs	Contamination with *L. monocytogenes* at 5.59 log CFU/g followed by beef pieces immersion in the coating solutions	At the end of storage (14 days at 4 °C) period, compared to the uncoated beef pieces, coating with 1, 2, and 3% thyme and oregano EOs reduced *L. monocytogenes* by 1.02, 1.73, and 1.97 log CFU/g and 0.91, 1.66, and 1.90 log CFU/g, respectively. The treatments improved the color of beef, and its organoleptic properties were acceptable.	[61]
Spiced beef	Chitosan films incorporated with apricot (*Prunus armeniaca*) kernel EO at 0%, 0.125%, 0.25%, 0.5%, and 1% (*v*/*v*)	The beef slices were inoculated with *L. monocytogenes* to 10^4 CFU/g and placed in contact with the antimicrobial films	After 15 days of storage at 4 °C, compared to the control samples (film without EO addition), the chitosan films containing 0.5 and 1% apricot kernel EO reduced *L. monocytogenes* by 3.3 and 4.1 log CFU/g. After 24 days of storage, the sensorial attributes (taste, color, texture, and overall acceptance) of the spiced beef packed with the chitosan film containing 1% apricot kernel oil were significantly improved compared to those of the unpacked one.	[62]

References

1. European Food Safety Authority (EFSA); European Centre for Disease Prevention and Control (ECDC). The European Union One Health 2022 Zoonoses Report. EFSA J. 2023, 21, e8442.
2. Thakur, M.; Asrani, R.K.; Patial, V. Listeria monocytogenes: A Food-Borne Pathogen. In Foodborne Diseases; Elsevier: Amsterdam, The Netherlands, 2018; pp. 157–192. ISBN 978-0-12-811444-5.
3. Fox, E.; Hunt, K.; O'Brien, M.; Jordan, K. Listeria monocytogenes in Irish Farmhouse Cheese Processing Environments. Int. J. Food Microbiol. 2011, 145, S39–S45.
4. Coughlan, L.M.; Cotter, P.D.; Hill, C.; Alvarez-Ordóñez, A. New Weapons to Fight Old Enemies: Novel Strategies for the (Bio)Control of Bacterial Biofilms in the Food Industry. Front. Microbiol. 2016, 7, 1641.
5. Colagiorgi, A.; Bruini, I.; Di Ciccio, P.A.; Zanardi, E.; Ghidini, S.; Ianieri, A. Listeria monocytogenes Biofilms in the Wonderland of Food Industry. Pathogens 2017, 6, 41.
6. Drew, C.A.; Clydesdale, F.M. New Food Safety Law: Effectiveness on the Ground. Crit. Rev. Food Sci. Nutr. 2015, 55, 689–700.
7. Tompkin, R.B. Control of Listeria monocytogenes in the Food-Processing Environment. J. Food Prot. 2002, 65, 709–725.

8. Lang, L.H. FDA Approves Use of Bacteriophages to Be Added to Meat and Poultry Products. Gastroenterology 2006, 131, 1370.
9. Kawacka, I.; Olejnik-Schmidt, A.; Schmidt, M.; Sip, A. Effectiveness of Phage-Based Inhibition of Listeria monocytogenes in Food Products and Food Processing Environments. Microorganisms 2020, 8, 1764.
10. Gutiérrez, D.; Rodríguez-Rubio, L.; Fernández, L.; Martínez, B.; Rodríguez, A.; García, P. Applicability of Commercial Phage-Based Products against Listeria monocytogenes for Improvement of Food Safety in Spanish Dry-Cured Ham and Food Contact Surfaces. Food Control 2017, 73, 1474–1482.
11. Ahmadi, H.; Barbut, S.; Lim, L.-T.; Balamurugan, S. Examination of the Use of Bacteriophage as an Additive and Determining Its Best Application Method to Control Listeria monocytogenes in a Cooked-Meat Model System. Front. Microbiol. 2020, 11, 779.
12. Ishaq, A.; Ebner, P.D.; Syed, Q.A.; Ubaid Ur Rahman, H. Employing List-Shield Bacteriophage as a Bio-Control Intervention for Listeria monocytogenes from Raw Beef Surface and Maintain Meat Quality during Refrigeration Storage. LWT 2020, 132, 109784.
13. Komora, N.; Maciel, C.; Amaral, R.A.; Fernandes, R.; Castro, S.M.; Saraiva, J.A.; Teixeira, P. Innovative Hurdle System towards Listeria monocytogenes Inactivation in a Fermented Meat Sausage Model—High Pressure Processing Assisted by Bacteriophage P100 and Bacteriocinogenic Pediococcus Acidilactici. Food Res. Int. 2021, 148, 110628.
14. Chibeu, A.; Agius, L.; Gao, A.; Sabour, P.M.; Kropinski, A.M.; Balamurugan, S. Efficacy of Bacteriophage LISTEXTMP100 Combined with Chemical Antimicrobials in Reducing Listeria monocytogenes in Cooked Turkey and Roast Beef. Int. J. Food Microbiol. 2013, 167, 208–214.
15. Huang, L.; Hwang, C.-A.; Liu, Y.; Renye, J.; Jia, Z. Growth Competition between Lactic Acid Bacteria and Listeria monocytogenes during Simultaneous Fermentation and Drying of Meat Sausages—A Mathematical Modeling. Food Res. Int. 2022, 158, 111553.
16. Dos Santos, L.R.; Alía, A.; Martin, I.; Gottardo, F.M.; Rodrigues, L.B.; Borges, K.A.; Furian, T.Q.; Córdoba, J.J. Antimicrobial Activity of Essential Oils and Natural Plant Extracts against Listeria monocytogenes in a Dry-cured Ham-based Model. J. Sci. Food Agric. 2022, 102, 1729–1735.
17. Figueiredo, A.C.L.; Almeida, R.C.C. Antibacterial Efficacy of Nisin, Bacteriophage P100 and Sodium Lactate against Listeria monocytogenes in Ready-to-Eat Sliced Pork Ham. Braz. J. Microbiol. 2017, 48, 724–729.
18. Van Nassau, T.J.; Lenz, C.A.; Scherzinger, A.S.; Vogel, R.F. Combination of Endolysins and High Pressure to Inactivate Listeria monocytogenes. Food Microbiol. 2017, 68, 81–88.
19. Misiou, O.; Van Nassau, T.J.; Lenz, C.A.; Vogel, R.F. The Preservation of Listeria-Critical Foods by a Combination of Endolysin and High Hydrostatic Pressure. Int. J. Food Microbiol. 2018, 266, 355–362.
20. Giello, M.; La Storia, A.; De Filippis, F.; Ercolini, D.; Villani, F. Impact of Lactobacillus curvatus 54M16 on Microbiota Composition and Growth of Listeria monocytogenes in Fermented Sausages. Food Microbiol. 2018, 72, 1–15.
21. Kamiloğlu, A.; Kaban, G.; Kaya, M. Effects of Autochthonous Lactobacillus plantarum Strains on Listeria monocytogenes in Sucuk during Ripening. J. Food Saf. 2019, 39, e12618.
22. Beristain-Bauza, S.D.C.; Mani-López, E.; Palou, E.; López-Malo, A. Antimicrobial Activity of Whey Protein Films Supplemented with Lactobacillus sakei Cell-Free Supernatant on Fresh Beef. Food Microbiol. 2017, 62, 207–211.
23. Shafipour Yordshahi, A.; Moradi, M.; Tajik, H.; Molaei, R. Design and Preparation of Antimicrobial Meat Wrapping Nanopaper with Bacterial Cellulose and Postbiotics of Lactic Acid Bacteria. Int. J. Food Microbiol. 2020, 321, 108561.
24. Ruiz, A.; Williams, S.K.; Djeri, N.; Hinton, A.; Rodrick, G.E. Nisin Affects the Growth of Listeria monocytogenes on Ready-to-Eat Turkey Ham Stored at Four Degrees Celsius for Sixty-Three Days. Poult. Sci. 2010, 89, 353–358.
25. Kara, R.; Yaman, H.; Gök, V.; Akkaya, L. The Effect of Nisin on Listeria monocytogenes in Chicken Burgers. Indian J. Anim. Res. 2014, 48, 171.
26. Martín, I.; Rodríguez, A.; Delgado, J.; Córdoba, J.J. Strategies for Biocontrol of Listeria monocytogenes Using Lactic Acid Bacteria and Their Metabolites in Ready-to-Eat Meat- and Dairy-Ripened Products. Foods 2022, 11, 542.
27. Zhou, H.; Fang, J.; Tian, Y.; Lu, X.Y. Mechanisms of Nisin Resistance in Gram-Positive Bacteria. Ann. Microbiol. 2014, 64, 413–420.
28. Wongchai, M.; Churklam, W.; Klubthawee, N.; Aunpad, R. Efficacy of a Combination of Nisin and Citric Acid against Listeria monocytogenes 10403S In Vitro and in Model Food Systems. Sci. Technol. Asia 2018, 23, 5359.
29. Hammou, F.N.; Skali, S.N.; Idaomar, M.; Abrini, J. Combinations of Nisin with Salt (NaCl) to Control Listeria monocytogenes on Sheep Natural Sausage Casings Stored at 6C. Afr. J. Biotechnol. 2010, 9, 1190–1195.

30. Raeisi, M.; Tabaraei, A.; Hashemi, M.; Behnampour, N. Effect of Sodium Alginate Coating Incorporated with Nisin, Cinnamomum Zeylanicum, and Rosemary Essential Oils on Microbial Quality of Chicken Meat and Fate of Listeria monocytogenes during Refrigeration. Int. J. Food Microbiol. 2016, 238, 139–145.
31. Churklam, W.; Chaturongakul, S.; Ngamwongsatit, B.; Aunpad, R. The Mechanisms of Action of Carvacrol and Its Synergism with Nisin against Listeria monocytogenes on Sliced Bologna Sausage. Food Control 2020, 108, 106864.
32. Aras, S.; Kabir, M.N.; Chowdhury, S.; Fouladkhah, A.C. Augmenting the Pressure-Based Pasteurization of Listeria monocytogenes by Synergism with Nisin and Mild Heat. Int. J. Environ. Res. Public. Health 2020, 17, 563.
33. Martillanes, S.; Rocha-Pimienta, J.; Llera-Oyola, J.; Gil, M.V.; Ayuso-Yuste, M.C.; García-Parra, J.; Delgado-Adámez, J. Control of Listeria monocytogenes in Sliced Dry-Cured Iberian Ham by High Pressure Processing in Combination with an Eco-Friendly Packaging Based on Chitosan, Nisin and Phytochemicals from Rice Bran. Food Control 2021, 124, 107933.
34. Teixeira, J.S.; Repková, L.; Gänzle, M.G.; McMullen, L.M. Effect of Pressure, Reconstituted RTE Meat Microbiota, and Antimicrobials on Survival and Post-Pressure Growth of Listeria monocytogenes on Ham. Front. Microbiol. 2018, 9, 1979.
35. Jin, T.; Liu, L.; Sommers, C.H.; Boyd, G.; Zhang, H. Radiation Sensitization and Postirradiation Proliferation of Listeria monocytogenes on Ready-to-Eat Deli Meat in the Presence of Pectin-Nisin Films. J. Food Prot. 2009, 72, 644–649.
36. Turgis, M.; Stotz, V.; Dupont, C.; Salmieri, S.; Khan, R.A.; Lacroix, M. Elimination of Listeria monocytogenes in Sausage Meat by Combination Treatment: Radiation and Radiation-Resistant Bacteriocins. Radiat. Phys. Chem. 2012, 81, 1185–1188.
37. Huq, T.; Vu, K.D.; Riedl, B.; Bouchard, J.; Lacroix, M. Synergistic Effect of Gamma (γ)-Irradiation and Microencapsulated Antimicrobials against Listeria Monocytogenes on Ready-to-Eat (RTE) Meat. Food Microbiol. 2015, 46, 507–514.
38. Mohamed, H.M.H.; Elnawawi, F.A.; Yousef, A.E. Nisin Treatment to Enhance the Efficacy of Gamma Radiation against Listeria monocytogenes on Meat. J. Food Prot. 2011, 74, 193–199.
39. Foegeding, P.M.; Thomas, A.B.; Pilkington, D.H.; Klaenhammer, T.R. Enhanced Control of Listeria monocytogenes by in Situ-Produced Pediocin during Dry Fermented Sausage Production. Appl. Environ. Microbiol. 1992, 58, 884–890.
40. Papagianni, M.; Anastasiadou, S. Pediocins: The Bacteriocins of Pediococci. Sources, Production, Properties and Applications. Microb. Cell Factories 2009, 8, 3.
41. Schlyter, J.H.; Glass, K.A.; Loeffelholz, J.; Degnan, A.J.; Luchansky, J.B. The Effects of Diacetate with Nitrite, Lactate, or Pediocin on the Viability of Listeria monocytegenes in Turkey Slurries. Int. J. Food Microbiol. 1993, 19, 271–281.
42. Goff, J.H.; Bhunia, A.K.; Johnson, M.G. Complete Inhibition of Low Levels of Listeria monocytogenes on Refrigerated Chicken Meat with Pediocin AcH Bound to Heat-Killed Pediococcus acidilactici Cells. J. Food Prot. 1996, 59, 1187–1192.
43. Chen, C.; Sebranek, J.G.; Dickson, J.S.; Mendonca, A.F. Use of Pediocin (Alta Tm 2341) For Control of Listeria monocytogenes on Frankfurters. J. Muscle Foods 2004, 15, 35–56.
44. Santiago-Silva, P.; Soares, N.F.F.; Nóbrega, J.E.; Júnior, M.A.W.; Barbosa, K.B.F.; Volp, A.C.P.; Zerdas, E.R.M.A.; Würlitzer, N.J. Antimicrobial Efficiency of Film Incorporated with Pediocin (ALTA® 2351) on Preservation of Sliced Ham. Food Control 2009, 20, 85–89.
45. Espitia, P.J.P.; Otoni, C.G.; Soares, N.F.F. Pediocin Applications in Antimicrobial Food Packaging Systems. In Antimicrobial Food Packaging; Elsevier: Amsterdam, The Netherlands, 2016; pp. 445–454. ISBN 978-0-12-800723-5.
46. Woraprayote, W.; Kingcha, Y.; Amonphanpokin, P.; Kruenate, J.; Zendo, T.; Sonomoto, K.; Benjakul, S.; Visessanguan, W. Anti-Listeria Activity of Poly(Lactic Acid)/Sawdust Particle Biocomposite Film Impregnated with Pediocin PA-1/AcH and Its Use in Raw Sliced Pork. Int. J. Food Microbiol. 2013, 167, 229–235.
47. Castro, S.M.; Silva, J.; Casquete, R.; Queirós, R.; Saraiva, J.A.; Teixeira, P. Combined Effect of Pediocin bacHA-6111-2 and High Hydrostatic Pressure to Control Listeria innocua in Fermented Meat Sausage. Int. Food Res. J. 2018, 25, 553–560.
48. Pesavento, G.; Calonico, C.; Bilia, A.R.; Barnabei, M.; Calesini, F.; Addona, R.; Mencarelli, L.; Carmagnini, L.; Di Martino, M.C.; Lo Nostro, A. Antibacterial Activity of Oregano, Rosmarinus and Thymus Essential Oils against Staphylococcus aureus and Listeria monocytogenes in Beef Meatballs. Food Control 2015, 54, 188–199.
49. Gouveia, A.R.; Alves, M.; Silva, J.A.; Saraiva, C. The Antimicrobial Effect of Rosemary and Thyme Essential Oils against Listeria monocytogenes in Sous Vide Cook-Chill Beef During Storage. Procedia Food Sci. 2016, 7, 173–176.

50. Khaleque, M.A.; Keya, C.A.; Hasan, K.N.; Hoque, M.M.; Inatsu, Y.; Bari, M.L. Use of Cloves and Cinnamon Essential Oil to Inactivate Listeria monocytogenes in Ground Beef at Freezing and Refrigeration Temperatures. LWT 2016, 74, 219–223.
51. Radünz, M.; Dos Santos Hackbart, H.C.; Camargo, T.M.; Nunes, C.F.P.; De Barros, F.A.P.; Dal Magro, J.; Filho, P.J.S.; Gandra, E.A.; Radünz, A.L.; Da Rosa Zavareze, E. Antimicrobial Potential of Spray Drying Encapsulated Thyme (Thymus vulgaris) Essential Oil on the Conservation of Hamburger-like Meat Products. Int. J. Food Microbiol. 2020, 330, 108696.
52. Abbasi, Z.; Aminzare, M.; Hassanzad Azar, H.; Rostamizadeh, K. Effect of Corn Starch Coating Incorporated with Nanoemulsion of Zataria Multiflora Essential Oil Fortified with Cinnamaldehyde on Microbial Quality of Fresh Chicken Meat and Fate of Inoculated Listeria monocytogenes. J. Food Sci. Technol. 2021, 58, 2677–2687.
53. Sharma, S.; Barkauskaite, S.; Jaiswal, A.K.; Jaiswal, S. Essential Oils as Additives in Active Food Packaging. Food Chem. 2021, 343, 128403.
54. Reis, D.R.; Ambrosi, A.; Luccio, M.D. Encapsulated Essential Oils: A Perspective in Food Preservation. Future Foods 2022, 5, 100126.
55. Ju, J.; Chen, X.; Xie, Y.; Yu, H.; Guo, Y.; Cheng, Y.; Qian, H.; Yao, W. Application of Essential Oil as a Sustained Release Preparation in Food Packaging. Trends Food Sci. Technol. 2019, 92, 22–32.
56. Solomakos, N.; Govaris, A.; Koidis, P.; Botsoglou, N. The Antimicrobial Effect of Thyme Essential Oil, Nisin, and Their Combination against Listeria monocytogenes in Minced Beef during Refrigerated Storage. Food Microbiol. 2008, 25, 120–127.
57. Wagh, A.M.; Jaiswal, S.G.; Bornare, D.T. A Review: Extraction of Essential Oil from Lemon Grass as a Preservative for Animal Products. J. Pharmacogn. Phytochem. 2021, 10, 26–31.
58. Kazemeini, H.; Azizian, A.; Adib, H. Inhibition of Listeria monocytogenes Growth in Turkey Fillets by Alginate Edible Coating with Trachyspermum Ammi Essential Oil Nano-Emulsion. Int. J. Food Microbiol. 2021, 344, 109104.
59. Dini, H.; Fallah, A.A.; Bonyadian, M.; Abbasvali, M.; Soleimani, M. Effect of Edible Composite Film Based on Chitosan and Cumin Essential Oil-Loaded Nanoemulsion Combined with Low-Dose Gamma Irradiation on Microbiological Safety and Quality of Beef Loins during Refrigerated Storage. Int. J. Biol. Macromol. 2020, 164, 1501–1509.
60. Giarratana, F.; Muscolino, D.; Ragonese, C.; Beninati, C.; Sciarrone, D.; Ziino, G.; Mondello, L.; Giuffrida, A.; Panebianco, A. Antimicrobial Activity of Combined Thyme and Rosemary Essential Oils against Listeria monocytogenes in Italian Mortadella Packaged in Modified Atmosphere: Thyme & Rosemary EOs vs L. monocytogenes. J. Essent. Oil Res. 2016, 28, 467–474.
61. Yemiş, G.P.; Candoğan, K. Antibacterial Activity of Soy Edible Coatings Incorporated with Thyme and Oregano Essential Oils on Beef against Pathogenic Bacteria. Food Sci. Biotechnol. 2017, 26, 1113–1121.
62. Wang, D.; Dong, Y.; Chen, X.; Liu, Y.; Wang, J.; Wang, X.; Wang, C.; Song, H. Incorporation of Apricot (Prunus Armeniaca) Kernel Essential Oil into Chitosan Films Displaying Antimicrobial Effect against Listeria monocytogenes and Improving Quality Indices of Spiced Beef. Int. J. Biol. Macromol. 2020, 162, 838–844.