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*Review*

# *Listeria monocytogenes* in Milk: Occurrence and Recent Advances in Methods for Inactivation

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**Abstract:** Milk is one of the most important food items consumed by humans worldwide. In addition to its nutritional importance, milk is an excellent culture medium for microorganisms, which may include pathogens such as *Listeria monocytogenes (L. monocytogenes)*. Traditional processing of milk for direct consumption is based on thermal treatments that efficiently eliminate pathogens, including pasteurization or sterilization. However, the occurrence of *L. monocytogenes* in milk as a consequence of failures in the pasteurization process or postpasteurization contamination is still a matter of concern. In recent years, consumer demand for minimally processed milk has increased due to the perception of better sensory and nutritional qualities of the products. This review deals with the occurrence of *L. monocytogenes* in milk in the last 10 years, including regulatory aspects, and recent advances in technologies for the inactivation of this pathogen in milk. The results from studies on nonthermal technologies, such as high hydrostatic pressure, pulsed electric fields, ultrasounds, and ultraviolet irradiation, are discussed, considering their potential application in milk processing plants.

**Keywords:** *L. monocytogenes*; occurrence; milk; non-thermal treatments; high pressure processing (HPP); pulsed electric fields (PEFs); ultrasound; irradiation

## 1. Introduction

Milk is the fluid secreted by mammals for the nourishment of their offspring [1]. Since humans began to domesticate lactating animals, milk and milk products have been part of the human diet [2]. Milk is considered one of the most complete sources of nutrients for human beings because of its diverse components, such as proteins, vitamins, and minerals that are important in human nutrition [3,4].

However, due to its high nutritional value, neutral pH, and high water activity, raw milk serves as an excellent growth medium for different microorganisms, whose multiplication depends mainly on temperature and on competing microorganisms and their metabolic products [5]. Raw milk also creates good growth conditions for a variety of spoilage and potentially pathogenic microorganisms, such as Shiga toxin-producing *Escherichia coli* (STEC), *Listeria monocytogenes (L. monocytogenes)*, *Salmonella enterica*, *Campylobacter* spp., *Yersinia* spp., and others [6,7].

Due to the adoption of pasteurization in 1938, milk-borne disease outbreaks have decreased [8]. Different heat treatments can be distinguished based on the temperature and time conditions applied, and include subpasteurization, pasteurization, and sterilization, including ultrahigh temperature (UHT) and innovative steam injection (ISI) treatment [9]. Sterilization (110–120 °C/10–20 min), UHT (135–140 °C/6–10 s for indirect and 140–150 °C/2–4 s for direct UHT), or ISI (150–200 °C/<0.1 s) treatments destroy vegetative as well as most sporulating pathogens [9], but high temperatures can cause detrimental effects on milk attributes [9].

Currently, there is a trend to consume raw milk based on the idea that heat destroys the nutritional and health benefits of milk [10]. The consumer demand for raw milk occurs from perceptions of better sensory and nutritional qualities of raw milk over those of pasteurized milk, besides the desire of many consumers to support local and small-scale agriculture [11,12]. It is important to observe that epidemiological data have demonstrated microbiological health risks associated with raw milk consumption [13]. This enforces the necessity of raw milk consumption being accompanied by a risk of ingesting pathogenic bacteria, which pose an elevated health hazard [14].

In the dairy industry, many problems associated with *L. monocytogenes* contamination are related to minimally processed or postpasteurization contamination from plant environments [15–17]. *L. monocytogenes* is a Gram-positive, rod-shaped, non-spore-forming, and facultative anaerobe bacterium [18]. It is widespread in the environment, and control of *Listeria* in food production facilities requires constant focus by risk managers [19].

*L. monocytogenes* is an important pathogenic bacterium for humans and animals, and causes public health problems [20]. *L. monocytogenes* is also a transitory resident of the intestinal tract in humans, with 2%–10% of the hosts not presenting any apparent health consequences [19]. Although rare, listerial contamination of dairy products can cause listeriosis, a serious illness [21]. The pasteurization of raw milk does not eliminate further risks of dairy product contamination by *L. monocytogenes* [22]. In addition, the presence of *L. monocytogenes* in food has important economic consequences, such as the withdrawal of products from the consumer marketplace and a decrease in sales of the incriminated products [23].

To meet the consumer demand for fresher-tasting minimally processed foods, the use of nonthermal technologies such as pulsed electric fields (PEFs) and high hydrostatic pressure (HHP) is on the frontline of the "emerging high-potential technologies for tomorrow" [24]. HHP has emerged [25] as a new preservation method to control, slow, and prevent the growth of foodborne pathogens, therefore extending shelf life with high energy efficiency and minimal food processing [26]. Postprocessing contamination is considerably reduced, as HHP technology can be applied to prepackaged products [27,28]. The combination of PEF with thermal treatment can have a beneficial use in improving microbial inactivation [29]. PEF processing of foods involves applying high-voltage electric fields (5–80 kV/cm) of short electric pulses (1–100 μs) to a product hosted in a treatment chamber in either batch or continuous mode [30]. Therefore, the objective of this review is to describe the occurrence of *L. monocytogenes* in milk and advances in new nonthermal inactivation techniques published in the last 10 years (2008–present).

## 2. Occurrence of *L. monocytogenes* in Fluid Milk

*L. monocytogenes* has been detected in milk from several countries, with incidences varying from 0% to 50%, as presented in Table 1. In America, all samples have been from raw milk and from cow milk. Values in the United States have varied from 0% to 19.7%, and in South America values have been lower and have varied from 0% to 3.7%. In Europe, the values have varied from 0% to 28.6%. Most of the studies have used analyzed raw milk, but one in Austria analyzed pasteurized milk and found 0.0% of samples contaminated. In Italy, the sale of raw milk for human consumption in vending machines has been allowed since 2004 [31]. The same authors did two large surveys and found incidences of 0.54% and 0.1% of *L. monocytogenes* in raw milk [31,32].

**Table 1.** Worldwide occurrence of *Listeria monocytogenes* in milk.

| Country | Type of Milk | Samples Analyzed (*N*) | Positive Samples *n* (%) | Reference |
|---|---|---|---|---|
| *America:* | | | | |
| Brazil | Raw cow milk | 20 | 0 (0.0%) | [33] |
| Brazil | Pasteurized cow milk | 12 | 0 (0.0%) | [33] |
| Brazil | Raw goat milk | 53 | 0 (0.0%) | [34] |
| Brazil | Raw cow milk | 548 | 6 (1.1%) | [35] |
| Brazil | Raw cow milk | 210 | 0 (0.0%) | [36] |
| Brazil | Goat milk | 96 | 0 (0.0%) | [37] |
| Brazil | Raw cow milk | 36 | 0 (0.0%) | [38] |
| Brazil | Cow milk kept in cooler tank | 27 | 1 (3.7%) | [39] |
| Brazil | Raw cow milk | 165 | 0 (0.0%) | [40] |
| Colombia | Raw cow milk | 85 | 13 (16%; traditional method)<br>21 (26% Real Time-PCR) | [41] |
| United States | Raw cow milk<br>Raw goat milk<br>Raw sheep milk | 12<br>5<br>4 | 0 (0.0%) | [42] |
| United States | Raw cow milk | 172 | 34 (19.7%) | [12] |
| United States | Raw cow milk | 1412 | 184 (13.0%) | [43] |
| United States | Raw cow milk | 536 | 24 (4.5%) | [44] |
| *Europe:* | | | | |
| Austria | Cow milk and products | 230 | 0 (0.0%) | [45] |
| Czech Republic | Raw goat milk<br>Pasteurized goat milk | 48<br>40 | 0 (0.0%) | [46] |
| Czech Republic | Raw cow milk | 12 | 1 (8.3%) | [47] |
| Estonia | Raw cow milk | 14 | 4 (28.6%) | [48] |
| Estonia | Raw cow milk | 105 | 19 (18.1%) | [49] |
| Finland | Raw cow milk | 115 | 2 (1.7%) | [13] |
| Finland | Retailed raw cow milk bottles | 105 | 5 (4.8%) | [13] |
| Finland | Raw cow milk | 183 | 10 (5.5%) | [50] |
| Italy | Raw cow milk | 8716 | 145 (1.7%) | [51] |
| Italy | Raw cow milk From vending machines | 60,907 | 83 (0.1%) | [52] |
| Italy | Raw cow milk From vending machines | 15,264 | 83 (0.54%) | [32] |
| Italy | Raw cow milk | 27 | 1 (3.7%) | [39] |
| Portugal | Raw milk | 179 | 7 (3.9%) | [52] |
| Republic of Cyprus | Raw cow milk | 205 | 2 (1.0%) | [53] |
| Turkey | Raw milk of:<br>Cow<br>Sheep<br>Goat | <br>50<br>75<br>15 | <br>1 (2.0%)<br>2 (2.7%)<br>0 (0.0%) | [54] |
| Turkey | Raw cow milk | 175 | 1 (0.6%) | [55] |
| *Africa:* | | | | |
| Botswana | Raw cow milk | 278 | 3 (1.1 %) | [56] |
| Egypt | Raw camel milk | 185 | 2 (1.1%) | [57] |
| Egypt | Raw cow milk | 30 | 0 (0.0%) | [58] |
| Egypt | Sheep and goat milk | 102<br>107 | 1 (0.9%)<br>2 (1.9%) | [59] |
| Ethiopia | Raw cow milk | 60 | 2 (3.4%) | [60] |
| Ethiopia | Raw cow milk<br>Pasteurized cow milk | 50<br>50 | 2 (4.0%)<br>0 (0.0%) | [61] |
| Ethiopia | Raw cow milk | 100 | 22 (22.0%) | [62] |
| Ethiopia | Raw cow milk | 343 | 7 (2.0%) | [63] |
| Morocco | Raw cow milk | 96 | 8 (8.33%) | [64] |
| Morocco | Raw cow milk | 120 | 1 (0.8%) | [65] |
| Nigeria | Raw cow milk | 192 | 17 (22.4%) | [66] |
| *Asia and Oceania:* | | | | |
| China | Raw cow milk | 5211 | 19 (0.36%) | [67] |
| India | Raw cow milk | 2060 | 105 (5.1%) | [68] |
| India | Raw cow milk | 195 | 11 (5.6%) | [69] |
| India | Raw cow milk | 5 | 2 (25.0%) | [1] |
| India | Pasteurized milk | 5 | 0 (0.0%) | [1] |
| India | Raw cow milk | 50 | 3 (6.0%) | [70] |

**Table 1.** *Cont.*

| Country | Type of Milk | Samples Analyzed (*N*) | Positive Samples *n* (%) | Reference |
|---|---|---|---|---|
| India | Raw cow milk | 137 | 4 (2.91%) | [71] |
| India | Raw cow milk | 292 | 4 (1.5%) | [72] |
| India | Raw cow milk | 457 | 5 (1.1%) | [73] |
| India | Raw cow milk<br>Pasteurized cow milk | 120<br>48 | 7 (5.8%)<br>0 (0%) | [74] |
| Iran | Raw cow milk | 100 | 5 (5.0%) | [75] |
| Iran | Raw cow milk | 91 | 1 (1.1%) | [76] |
| Iran | Raw cow milk | 59 | 0 (0%) | [77] |
| Iran | Raw cow milk | 100 | 4 (4.0%) | [78] |
| Iran | Raw cow milk | 8 | 0 (0%) | [79] |
| Iran | Raw cow milk<br>Raw sheep milk<br>Raw goat milk | 240<br>166<br>41 | 13 (5.4%)<br>4 (2.4%)<br>1 (2.4%) | [80] |
| Iran | Raw cow milk | 120 | 3 (2.5%) | [81] |
| Iran | Raw cow milk | 18 | 9 (50.0%) | [19] |
| Iran | Raw cow milk<br>Raw sheep milk<br>Raw goat milk<br>Raw camel milk | 90<br>62<br>60<br>48 | 1 (1.1%)<br>4 (6.5%)<br>1 (1.7%)<br>0 (0.0%) | [82] |
| Iran | Pasteurized cow milk<br>Raw cow milk | 100<br>100 | 0 (0%) | [83] |
| Iraq | Raw cow milk<br>Raw sheep milk<br>Raw buffalo milk | 100<br>100<br>100 | 11 (11.0%)<br>8 (8.0%)<br>3 (3.0%) | [84] |
| Jordan | Raw sheep milk | 165 | 19 (11.5%) | [85] |
| Syria | Raw cow milk | 766 | 35 (4.6%) | [86] |
| Uganda | Raw cow milk | 40 | 5 (13 %) | [87] |
| New Zealand | Raw cow milk | 297 | 2 (0.7%) | [7] |

In African countries, *L. monocytogenes* incidence has varied from 0% to 22.0%. Most samples have been from raw cow milk, but pasteurized cow milk, camel, sheep, and goat milk have also been analyzed. Only one study from New Zealand was found and is presented in this review. The authors recovered *L. monocytogenes* in 2 samples (0.7%) of all 297 milk samples analyzed [6]. In the Middle East, *L. monocytogenes* incidence has varied from 0% to 50%. Most samples have been from raw cow milk, but pasteurized cow milk, camel, sheep, buffalo, and goat milk have also been analyzed. It is important to observe that Reference [19] studied only 18 samples, but still the incidence of 9 (50.0%) was very high. Finally, in Asia, *L. monocytogenes* incidence has varied from 0% to 25%. It is also relevant to notice that Reference [1] analyzed only 5 raw milk samples, but still the incidence of 25% was high.

## 3. Technological Approaches for Inactivation of *L. monocytogenes* in Fluid Milk

Although traditional preservation processes (such as pasteurization and sterilization) are efficient in the production of microbiologically safe food, these processes result in degradations and undesirable changes in the nutritional (bioactive compounds, vitamins, and pigments) and sensory (texture, taste, flavor, and color) properties of foods, reducing their acceptability by consumers [88,89]. In this context, nonthermal emergent technologies (also known as mild processing methods) such as high pressure processing (HPP), high isostatic pressure (HIP) pulsed electric fields (PEFs), ultraviolet (UV) light (10–400 nm), and ultrasound (US) have been addressed with the objective of producing safe foods, reducing and eliminating these undesirable changes. Despite the advantages related to mild treatments, the main difficulties that limit the industrialization of these technologies are high costs, incomplete control of variable processes, and lack of regulatory approval. In addition, a study evaluating the acceptance of these products by consumers presented a crucial point for the commercial success of these technologies [90].

Table 2 shows the fundamentals, main principles, and mechanisms of inactivation of microorganisms in HPP, PEFs, UV, and US. According to Reference [90], nonthermal processes such as HPP, PEFs, and UV are the main promising technologies for the dairy sector. Hydrostatic

high-pressure processing (HHPP) is a nonthermal technology applied to packaged foods, either solid or liquid. This technique has great potential to inactivate pathogenic and deteriorating microorganisms, producing microbiologically safe products with a long shelf life and with better nutritional and sensorial attributes [88]. The process basically consists of the application of isostatic pressures, transmitted uniformly and instantaneously to food. The isostatic principle ensures that the applied pressure and pressure within the food are equal, thus avoiding deformations in the food, for example [89].

**Table 2.** The fundamentals, main process parameters, and mechanisms of microbial inactivation by emergence nonthermal processes. PEFs: Pulsed electric fields; US: Ultrasound.

| Technologies | Fundamentals of Technology | Main Process Parameters | Main Mechanisms of Microbial Inactivation |
|---|---|---|---|
| HIP | Application of high isostatic pressures (100–1000 MPa) under mild temperatures (20–60 °C) | - Pressure (Mpa)<br>- Temperature (°C)<br>- Time (s or min) | Combinations of factors such as changes in cell membranes, increased cell wall permeability, and leakage of intracellular material, phospholipid crystallization, protein denaturation, and destruction of vital complexes [88] |
| PEFs | Application of high intensities of pulsed electric fields (5–80 kV/cm) for a short time (s or ms) under mild temperatures (<50 °C) | - Electric Field (kV/cm)<br>- Pulse waveform<br>- Exponential and square wave (mono or bipolar)<br>- Pulse width (ms)<br>- Temperature (°C)<br>- Time (ms or µs) | Induction of electroporation in microbial cells, which changes membrane permeability (temporarily or permanently), resulting in intracellular material extravasation and losses in cell viability [89] |
| UV | Application of an electromagnetic spectrum with wavelengths between 100 and 400 nm | - UV dose (mJ/cm$^2$)<br>- Wavelength (nm)<br>- Power UV-lamp (W) | Formation of lesions in the genomic DNA of organisms, by UV-B and UV-C radiation, inhibiting DNA replication [89] |
| US | Application of sonic waves with frequencies exceeding 16–18 kHz | - Power (W)<br>- Frequency (kHz)<br>- Treatment time (s or min) | Cavitation phenomenon. This phenomenon results in the explosion of bubbles, causing the molecules to collide violently and produce shock waves. These shock waves promote the generation of high temperatures and pressures in the cell, which are the main factors that result in microbial inactivation [90]. |

PEF technology consists of the application of pulsed electric fields of high intensities (5–80 kV/cm) for short periods of time (ms or µs), with the potential to pasteurize liquid foods in mild temperatures (<50 °C). This treatment can be an alternative to traditional thermal processes such as pasteurization, because it presents efficiently in the inactivation of pathogenic microorganisms and some enzymes, maintaining the nutritional and sensory properties of the product. The efficiency of the process depends on several factors, such as electric field strength, treatment time, food temperature, and type of microorganism or enzyme [88,89]. Another promising technology for the dairy industry is ultraviolet (UV) light, which consists of the application of the electromagnetic spectrum, which has wavelengths between 100 and 400 nm. UV light is divided into three regions: Short-wave ultraviolet (UV-C) from 200 to 280 nm, UV-wave (UV-B) from 280 to 320 nm, and UV-wave (UV-A) from 320 to 400 nm. This technology promotes lesions in the genomic DNA of organisms, inhibiting DNA replication and consequently resulting in the inactivation of the microorganisms. Due to the high presence of suspended solids (such as proteins, fats), UV-light has limited penetration depth in milk. Therefore, to avoid subprocessing, one must work with thin films or capillaries, thus avoiding the application in large volumes of the product [88,89,91].

Ultrasound (US) is a technique that uses high-power soundwaves (about 20 kHz). If the amplitude of the ultrasonic wave is high enough, cavitation will occur, which is the formation and breaking of microscopic bubbles. When the bubbles explode, shock waves are generated, promoting the generation of high temperatures and pressures, resulting in the inactivation of the microorganisms. The effects of cavitation on microbial suspensions include dispersion of microbial agglomerates, puncturing of the cell wall, modification of cellular activity, and greater sensitivity to heat. The main processing parameters are power, frequency, and treatment time. In addition to the advantage of maintaining the nutritional and sensory compounds of the product, US promotes the homogenization of milk fats, removes gases, and can better antioxidant activity [92].

A recent study applying US technology in semi-skimmed sheep milk proposed that the parameters tested were promising in achieving bacterial inactivation, eliminating or maintaining low-temperature processing, which is acceptable for drinking milk. The findings presented showed similar bacterial inactivation when US-treated milk was compared to conventional pasteurization, with the advantage of using small temperature processing. In addition, no relevant change was noted on protein or free amino acid profiles of pasteurized or US-treated semi-skimmed sheep milk, proving the technology could be used in milk to produce sheep milk products such as cheeses, maintaining the product quality [93].

Each of these emerging technologies has a specific mechanism of microbial inactivation, and its knowledge is of primary importance for the development of the technique and for the production of safe food. Thus, an understanding of the mechanisms and factors that affect microbial inactivation is of fundamental importance to ensure the microbiological safety of food. In general, most studies involving inactivation of pathogens by mild technologies have been based on the inactivation of *Listeria* spp. This may reflect major concerns about the potential presence of *L. monocytogenes*, mainly because several foods treated by these technologies require storage at low temperatures, which does not impair the growth of *L. monocytogenes* [93]. Several studies have used *Listeria innocua* as a surrogate of *L. monocytogenes* for the determination of kinetic parameters in mild treatments [88,94–96].

Regarding the kinetics of microbial inactivation by these technologies, possible deviations from linearity by some processes may be observed, with very relevant practical implications. To solve this problem, mathematical models (predictive models) are necessary to describe the kinetic parameters of the survival curve. The most-used nonlinear predictive models are the Weibull, log-logistic, Baranyi, and Gompertz models, which can be used through software such as GINAFit and DMFit. A detailed review of the models, as well as the definition of the estimated parameters, can be found in the literature [97–99]. Table 3 shows the kinetics of microbial inactivation of *L. monocytogenes* emerging in the milk during the different treatments.

Several factors may affect the resistance of pathogens such as *L. monocytogenes* in emerging nonthermal processes. According to Reference [89], these factors can be divided into factors that act before (physiological state of the microbial cells), during (product parameters and processing factors), and after (recovery conditions) treatment. This last factor (recovery conditions) presents a great challenge for the production of safe food by mild treatments. The presence of damaged cells due to sublethal inactivation during treatment may allow the microorganisms to repair sublethal damage and redevelop in the food, if they find adequate environmental conditions for their growth. This fact demonstrates the importance of evaluating the robustness of the formulations through challenge tests and the development of combined processes based on the use of these technologies together with additional preservation agents (hurdles) capable of interfering with the maintenance of cellular homeostasis [88,89]. Therefore, the determination of microbial inactivation parameters and the recovery conditions of injured microorganisms are crucial factors for the development and definition of process parameters for safe food production.

*L. monocytogenes* may be present in several places in the dairy environment, with contamination sources such as dairy ingredients or due to ineffective cleaning and sanitation, poor design or condition of food equipment or environment, or insufficient controls in the dairy factory [19]. The use of raw milk is often cited as a major factor for the contamination of *L. monocytogenes* in dairy products. However, this approach is very elementary. In this sense, to understand the survival of *L. monocytogenes* in dairy products during processing, challenge tests can be performed, which means the inoculation of the pathogen during the processing and testing its growth, which can determine the point at which the growth reaches unacceptable levels in a specific product [100]. The use of quantitative food microbiology tools, such as predictive microbiology, constitutes an interesting approach and should be encouraged for the dairy industry, mainly to establish the protective role of lactic bacteria regarding the survival and growth of *L. monocytogenes* in dairy products [101]. A recent study reported the presence of indigenous lactic bacteria with antilisterial activity in artisanal cheese [102], and future challenges

include the study of their behavior in the processing and ripening of these products to increase safety for consumers.

**Table 3.** Recent studies on nonthermal technologies for the inactivation of *L. monocytogenes* in milk. HHP: High hydrostatic pressure.

| Type of Milk | Technologies | Operational Parameters | | | Reduction Effect ($n$ = $\log_{10}$) or Inactivation Kinetics ** | References |
|---|---|---|---|---|---|---|
| | | Doses * | Time (unit) | Temperature (°C) | | |
| Whole Raw Milk | HHP | 300–600 MPa | 1–105 (min) | 25 | $D_{300\ MPa}$ = 10.99 min<br>$D_{400\ MPa}$ = 6.00 min<br>$D_{600\ Mpa}$ = 2.43 min | [103] |
| Human milk | HHP | 400 MPa | 0–50 (min) | 31 | $n$ = 8.0 $\log_{10}$ (2 min) | [104] |
| Raw milk | HHP | 150–400 MPa | 10–120 (min) | 25 | $D_{150\ MPa}$ = 84.4 min<br>$D_{250\ MPa}$ = 46.0 min<br>$D_{300\ MPa}$ = 26.6 min<br>$D_{350\ MPa}$ = 13.9 min | [105] |
| Whole milk | HHPP | 300–500 MPa | <10 (min) | 30 | $D_{300\ MPa}$ = 9.56 min | [106] |
| UHT whole milk | HHPP | 400–600 MPa | 0–30 (min) | 27–60 | $D_{400\ MPa/27\ °C}$ = 592.1 s<br>$D_{400\ MPa/43\ °C}$ = 238.4 s<br>$D_{400\ MPa/60\ °C}$ = 15.4 s<br>$D_{500\ MPa/27\ °C}$ = 75.5 s<br>$D_{500\ MPa/43\ °C}$ = 52.7 s<br>$D_{600\ MPa/27\ °C}$ = 19 s<br>$D_{600\ MPa/43\ °C}$ = 12 s | [107] |
| UHT whole milk | HHPP | 350–600 MPa | 0–40 (min) | 25 | $D_{350\ MPa}$ = 14.53 min<br>$D_{450\ MPa}$ = 7.71 min<br>$D_{550\ MPa}$ = 2.05 min<br>$D_{600\ MPa}$ = 1.46 min | [108] |
| Milk | HHPP | 345 MPa | 5 (min) | 50 | $n$ > 8 $\log_{10}$ | [109] |
| Milk | HHPP | 550 Mpa | 5 (min) | 25 | $n$ ~7 $\log_{10}$ | [110] |
| Whole (W), low-fat (LF) and skim (S) milk | PEF | 25–35 kV/cm; 1700 Hz; 1.5 µs; (square waves) | 100–600 (µs) | 10–50 | $n$ ~ 2.5 $\log_{10}$ (W, LF, S; 30 kV/cm; 600 µs 25 °C)<br>$n$ ~ 4 $\log_{10}$ (W, 30 kV/cm; 600 µs 50 °C)<br>$n$ ~ 3 $\log_{10}$ (W, 30 kV/cm; 300 µs 50 °C)<br>$n$ $n$ ~ 1.5 $\log_{10}$ (W, 30 kV/cm; 300 µs 25 °C) | [111] |
| Milk | PEF | 15–30 kV/cm; 200 Hz; 2 µs (square waves) | 0–600 (µs) | <35 | $n$ ~ 1 $\log_{10}$ (15 kV/cm; 200 µs)<br>$n$ ~ 2.1 $\log_{10}$ (25 kV/cm; 200 µs)<br>$n$ ~ 3.5 $\log_{10}$ (30 kV/cm; 200 µs)<br>$n$ ~ 5.2 $\log_{10}$ (30 kV/cm; 600 µs) | [112] |
| Sweet whey | PEF | 25 kV/cm; 1000 Hz; 3 µs (bipolar waves) | 48 (µs) | 23 | $n$ ~ 1.8–3.6 $\log_{10}$ *** | [94] |
| Skim milk | PEF | 15–30 kV/cm; 3.25 µs | 5–50 (µs) | 0– 60 | $n$ ~ 0.75 $\log_{10}$ (30 kV/cm; 10 µs; 35 °C)<br>$n$ ~ 0.85 $\log_{10}$ (20 kV/cm; 50 µs; 35 °C)<br>$n$ ~ 4.5 $\log_{10}$ (20 kV/cm; 10 µs; 55 °C) | [113] |
| Milk | US | 20 kHz; 750 W; 124 µm | 2.5–10 (min) | <26 | $D_{750\ W}$ = 5.1 min<br>$n$ ~ 2 $\log_{10}$ (10 min) | [92] |
| Nonfat; low-fat; whole milk | US | 28–100 kHz **; 600 W | 50 (min) | 60 (max) | $D_{600\ W}$ = 3.22 min (nonfat)<br>$D_{600\ W}$ = 2.71 min (low-fat)<br>$D_{600\ W}$ = 4.24 min (whole) | [114] |
| Whole (W), skim (S) milk | US | 24 kHz; 100 µm | 50 (min) | <35 | $D$ = 9.31 min (W)<br>$D$ = 8.61 min (S) | [115] |
| Skim milk | UV Light | 0–40 mJ/cm$^2$ | NI | 22 | $n$ = 5 $\log_{10}$ (20 mJ/cm$^2$)<br>$D$ = 2.46 mJ/cm$^2$ | [116] |
| Raw goat milk | UV Light | 0–20 mJ/cm$^2$ | NI | NI | $n$ = 5 $\log_{10}$ (15.8 mJ/cm$^2$) | [117] |
| Human breast milk | UV light | 0–5000 mJ/cm$^2$ | 0–60 (min) | NI | $D_{630.51\ mJ/cm^2}$ = 7.67 min ****;<br>$n$ = 4.51 $\log_{10}$ (60 min) | [118] |
| Milk | UV Light | 21.3 mJ/cm$^2$ | 60 (min) | 25 | $n$ ~ 6 $\log_{10}$ (60 min) | [91] |

* HPP (MPa); PEF (kV/cm; Hz; µs; type of wave); US (kHz; W; µm); UV-light (mJ/cm$^2$). ** Log cycles of *L. monocytogenes* reduction; d is a scale parameter denoting the time for the first decimal reduction by Weibull models; $D$ = time required to reduce the number of survivors by 90%. *** For nine species of *L. monocytogenes*. **** Ultrasonic oscillations where frequencies are switched between 28, 45, and 100 kHz at 1-ms time intervals. NI: Not informed. NA: Not applied. UHT: Ultrahigh temperature.

All of these topics should be tested, considering the intrinsic parameters involved with emerging technologies, to establish synergic actions among the conventional and heat treatments used in raw milk. It is important to emphasize that there are several protocols for manufacturing dairy foods, in particular typical or artisanal ones, hence indicating the necessity of continuous and strict studies to generate safe conclusions for each type of process.

## 4. Conclusions and Future Trends

The occurrence of *L. monocytogenes* is worldwide, and emerging technologies can be used for its inactivation and to guarantee the safety of processed products. Despite the development of technological approaches for the treatment of milk, the worldwide incidence of *L. monocytogenes* in milk for human consumption is still a public health concern. The problems associated with *L. monocytogenes* contamination in the dairy industry are related to minimally processed or postpasteurization contamination from plant environments due to this bacterium being widely spread in the environment and the difficult control constant focus by risk managers. Considering the current demand for minimally processed milk, the recent advances in nonthermal technologies, such as HHP, PEFs, US, and UV, have great potential for applications in milk processing plants aiming to reduce the health risks associated with contamination of milk from *L. monocytogenes*. However, more studies should be conducted concerning the inactivation kinetic determination to establish how the process conditions for microbiological safety should be done.

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