Italian Journal of Food Safety

https://www.pagepressjournals.org/index.php/ijfs/index

eISSN 2239-7132

Please cite this article as:
Savini F, Tomasello F, Indio V, et al. **Effect of infrared technology on the behavior of *Listeria monocytogens*, *Salmonella* spp. and Enterobacteriaceae in homogenized raw vaccine milk: preliminary results**. *Ital J Food Saf* doi:10.4081/ijfs.2024.12379

Submitted: 13-02-2024
Accepted: 11-03-2024

Effect of infrared technology on the behavior of *Listeria monocytogens*, *Salmonella* spp. and Enterobacteriaceae in homogenized raw vaccine milk: preliminary results

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Key words: infrared, raw milk, treatment, reduction, bacteria.

Contributions: all the authors made a substantial intellectual contribution, read and approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare no potential conflict of interest.

Ethics approval and consent to participate: not applicable.

Funding: this work was funded by the project *Applicazioni di un approccio di sistema, dall'allevamento alla tavola, per identificare strategie per migliorare la sostenibilità della filiera delle bovine da latte, del latte e dei prodotti derivati – Sustmilk4IT* Programma di filiera*: "Stalla Modello" inserito nella graduatoria definitiva, approvata con Decreto n. 633056 del 15 novembre 2023, relativamente al V Avviso MASAF n. 0182458 del 22/04/2022 e s.m. per la selezione dei Contratti di filiera e di distretto di cui al DM n. 0673777 del 22/12/2021*.

Availability of data and materials: data and materials are available from the corresponding author upon request.

Conference presentation: this paper was presented at the XXXII National Conference of the Italian Association of Veterinary Food Hygienists (AIVI), September 13-15, 2023, Maierato, Italy.

Abstract
Traditional heat treatments in the dairy industry are known for their high water and energy consumption, and more economically and environmentally friendly solutions are being sought. Infrared (IR) technology offers advantages in energy efficiency and environmental sustainability; however, its effectiveness in milk processing, particularly in pathogen inactivation, remains relatively unexplored. In this study, homogenized raw milk was subjected to IR treatment, and its impact on *Listeria monocytogenes*, *Salmonella* spp., and Enterobacteriaceae was assessed. Results indicate that the IR treatment effectively reduces the microbial load, achieving levels of inactivation comparable to conventional pasteurization methods (around 6 Log_{10} CFU/mL). Moreover, the treatment maintains milk pH levels, suggesting minimal alteration to its composition. Further research is needed to explore the full extent of IR treatment on milk sanitation efficacy, deeply exploring IR technology to fully assess its applicability and integration into dairy processing practices. Despite regulatory challenges, the Wir System Milk shows promise as a cost-effective and eco-friendly alternative for raw milk treatment.

Introduction
Milk is a food liquid defined by Royal Decree 994/9 May 1929, article 15, as "the product obtained from the regular, uninterrupted, and complete milking of the udder of animals in good health and nutrition" (Royal Decree, 1929). Commission Regulation 853/2004 details that "raw milk is produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40°C or undergone any treatment that has an equivalent effect" (European Parliament and Council of the European Union, 2004). Section IX "Raw milk, colostrum, dairy products and colostrum-based products" explains the health requirements for raw milk and colostrum production in primary production, the hygiene of milk and colostrum production holdings as well as the criteria for raw milk, namely somatic cell and total bacteria (at 30°C) counts, and residues of antibiotic substances (European Parliament and Council of the European Union, 2004). Nevertheless, given the process hygiene standards and the control of environment and animals (even if laudable) are not critical control points, the control of the finished product alone does not ensure a significant microbial risk reduction and, therefore, a safe food. In this context, milk intended for human consumption requires post-milking treatments to prevent both the inactivation and the proliferation of contaminating microorganisms that could compromise both the safety and the shelf life of the product. Several milk treatments can be carried out following different strategies that result in products with different nutritional, sensory, and preservation characteristics. Traditional milk heat treatments are low temperature-long time, high temperature-short time and ultra-high temperature, in which milk is held respectively at 63°C for 30 min, 72°C for 15 seconds and 138-145°C for 2 seconds (Varnam and Sutherland, 1994). The thermal treatments recognized by Commission Regulation 853/2004 are pasteurization and sterilization (European Parliament and Council of the European Union, 2004), but dairy industries could use alternative combinations of time and temperature associated with other treatments. In the regulation, it is established that the efficacy of the treatment is to be evaluated, for pasteurization by the inactivation of alkaline phosphatase, and for sterilization by the stability of the product after incubating for 15 days at 30°C in closed containers or for seven days at 55°C in closed containers or after any other method demonstrating that the appropriate heat treatment has been applied.
All these treatments, although effective, are related to high water and energy consumption, with the consequence that the dairy industry is considered one of the highest water and energy consuming sectors. In fact, more than 80% of the energy consumed in dairies is related to process heating, pasteurization, sterilization, drying and cleaning operations and 98% of the freshwater used is of drinking quality (Escobet *et al*., 2019). In Italy, depending on the type of milk, the expiration date or minimum shelf life is set either by the producer or by current regulations (Italian Republic, 2004 - *Legge n. 204*; Italian Republic, 2017 - *Decreto legislative n. 231*). For pasteurized fresh milk and high-quality pasteurized fresh milk, the expiration date is set by regulations at six days after the heat

treatment whereas for microfiltered pasteurized milk at ten days after the treatment undergone (Italian Republic, 2003). For all other types of milk, both the expiration date and the minimum shelf life are determined by the producer, namely food business operators. In parallel, to reduce food waste as well as to meet the diverse habits of consumers, who prefer to buy milk with a longer expiration date, both consumers and the milk processing industry and distributors have a strong interest in an extended shelf life of products. Innovative technologies or the application of these in several raw matrices of animal origin play a pivotal role in this changing, but the interaction between a specific process and a specific product needs coherent experiments and should be experimentally investigated.

Heat is transmitted by conduction, convection, and radiation. Infrared (IR) radiation is part of the electromagnetic spectrum that is located between the visible region and microwaves, and its wavelength ranges from 0.5 to 100 μm. IR penetration causes vibrating movement of water molecules and, thereby, heating. In the food industry, IR technology is considered promising because it is highly energy-efficient, less water-consuming, and environmentally friendly compared to conventional heating (Aboud *et al*., 2019). In addition, the Food and Drug Administration has indicated that IR radiation can be used in food processing. Recently, there has been an increasing interest in the applicability of IR radiation to food processing for the inactivation of pathogens (Shavandi *et al*., 2019; Choi *et al*., 2022). The potential use of IR heating as a decontamination agent in various food applications is well known. IR radiation can be used to inhibit bacteria, spores, yeasts, and mold in liquid and solid foods. The effectiveness of IR inhibition depends on the amount of IR energy, food temperature, wavelength, wave width, food depth, moisture content, food material type, and microorganism type. *Listeria monocytogenes* and Enterobacteriaceae are microbiological criteria laid down in Annex I, Commission Regulation 2073/2005 on microbiological criteria for foodstuffs, and respectively a food safety criterion for ready-to-eat (RTE) products and a process hygiene criterion for milk and dairy products (European Commission, 2005). *Salmonella* spp. is comprised within the Enterobacteriaceae family and is a foodborne pathogen that is not included in Regulation CE 2073/2005 for this food category(European Commission, 2005), but it is listed as one of the more widely distributed microbiological hazards in the EU in raw milk (EFSA BIOHAZ Panel, 2015). In the literature, numerous studies reported the efficacy of IR technology in many food manufacturing processes, but few studies were in milk process (Giraffa and Bossi, 1984; Krishnamurthy *et al*., 2008) and no studies were on these pathogens in milk. Therefore, the aim of this study was to assess, for the first time, the effect of the IR technology on the behavior of *L. monocytogenes*, *Salmonella* spp., and Enterobacteriaceae in homogenized vaccine raw milk.

Materials and Methods

Challenge test

For this study, a total of 20 L of raw vaccine milk was collected from a dairy farm classified by Veterinary Authority as *Mycobacterium bovis* and *Brucella abortus* free and transported to the laboratory to be artificially contaminated within 12 hours from purchase. Milk was sampled before the inoculation procedure to verify the initial absence of inhibitory substances in milk by Delvotest SP (Tecnomilk®). Three replicate tests were performed on the investigated batch. Milk, homogenized with an industrial homogenizer at a pressure of 200 bar, was experimentally inoculated with *L. monocytogenes* and *Salmonella* spp., whereas the assessment on the reduction of Enterobacteriacae was performed considering the natural level of contamination of raw milk. A mix of three strains each for the two pathogens was experimentally inoculated in the milk, namely *L. monocytogenes* ATCC 15313, and the ANSES wild strains 105 and 106 isolated from dairy products, and *Salmonella* Typhimurium ATCC 118174, and the field isolates and serotypes *Salmonella* Dublin and *Salmonella* Anatum, most isolated in Europe in healthy cattle (Gutema *et al*., 2019). For each stock culture, the inoculum was prepared following the procedures reported in ISO 20976-2 (ISO, 2022). Each milk replicate, cooled at room temperature, was experimentally spiked with the tested pathogens to reach a concentration in the milk of 10^6 CFU/ml before the IR treatment. The IR treatment was performed using a portable laboratory prototype (Wir System Milk, patent number 102020000007867). The

setup utilizes three tubular quartz ducts, each with a diameter of 8 mm and a length of 1250 mm. A single emitter from Infrared S.r.l in Rho, Italy, capable of delivering a maximum power of 7000W at 400V, radiates over a span of 1100mm. Milk is pumped into the ducts externally at a rate of 1.5 L/min, completely surrounding the quartz tubes and exposed to the IR radiation. Positioned adjacent to the duct and aligned with the milk flow, the IR source typically reaches a temperature of 800°C. It emits IR radiation within the 3-5 μm wavelength range, adjustable through the control panel provided by Infrared S.r.l in Rho, Italy.

Microbiological analysis and pH measurements

Microbiological analyses were performed on vaccine milk to establish the effectiveness of the treatment on the reduction of *L. monocytogenes*, *Salmonella* spp., and Enterobacteriacae. Samples were collected and analyzed before and after the IR treatment of each replicate; a total of 12 samples were performed, 6 for the three replicates (pre- and post-treatment) and 6 for control. Milk was tested, according to ISO 11290-1 (ISO, 2017a) and ISO 6579-1 (ISO, 2020), for the presence of *L. monocytogenes* and *Salmonella* spp. before inoculation and after the inoculation and the IR treatment. Enumeration of *L. monocytogenes* was performed according to the ISO method 11290-2 (ISO, 2017b), whereas enumeration of *Salmonella* spp. was performed in xylose lysine deoxycholate agar. Enumeration of Enterobacteriaceae was performed before and after the treatment according to the ISO method 21528-2 (ISO, 2017c). The pH value was determined using a pH-meter (Mettler-Toledo, Columbus, USA) in accordance with ISO 2917 (ISO, 1999).

Results and Discussion

No *L. monocytogenes* and *Salmonella* spp. and no inhibitory substances were found in any sample of raw milk used for the challenge test. The findings of the challenge test and of the reduction of Enterobacteriaceae as well as of the values of pH after the IR treatment are shown in Table 1. Our findings clearly show how IR treatment can inactivate *L. monocytogenes* and different serotypes of *Salmonella* spp. experimentally spiked in homogenized raw milk at concentrations as high as 6 Log_{10} CFU/ml as well as reduce of >3 Log CFU/ml the levels of Enterobacteriaceae to below the enumeration limit. Further, it should be detailed the absence of *L. monocytogenes* and *Salmonella* spp. using both detection and enumeration analytical methods. A slight decrease of pH was determined after the IR treatment even if the values remain at similar levels of those observed for raw milk. When food is exposed to IR radiation, it is absorbed, reflected, or scattered. Absorption intensities at different wavelengths by food components differ. As the IR radiation is absorbed by food components, the vibration and rotation of the molecules change because of a decrease or increase in the distance between atoms, movement of atoms, or vibration of molecules (Skjoldebrand, 2001). In detail, when heating is done by radiation, the heat is transferred by convection and conduction. Electromagnetic radiation causes thermal movements of the molecules, but conversion efficiency is highly dependent on the frequency (energy) of the radiation (Aboud *et al*., 2019). The rate of heat transfer to food material depends on several factors such as the composition, temperature of the IR lamp, moisture content of the food material, shape, and surface characteristics of the food material. In detail, electromagnetic waves reach the body, are absorbed by it, and then transformed from radiated energy into thermal energy, which predominantly propagates along the surface (Demirci and Ngadi, 2012). The effect of IR heating in the inactivation of bacteria present in foods is due to the performed damaging versus intracellular components such as DNA, RNA, ribosome, cell envelope and/or proteins in the cell (Sawai *et al*., 1995). Absorption of IR energy by water molecules in microorganisms is one of the important factors for microbial inactivation because water absorbs readily in the IR region and results in rapid temperature increase (Hamanaka *et al*., 2006). In this study, the exposure of the milk to the IR-radiation induces a fast change of the amount of energy inside the liquid that lasts for the entire duration of the IR exposure and that is followed by a quick return to the initial status quo once the radiation stops. Such irradiation sequence generates an energetic shock in the liquid that devitalizes the present microorganisms.

In the literature, the efficacy of IR heating on microbial reduction in milk was investigated in only two studies. Giraffa and Bossi (1984) reported that IR heat treatment of milk at 65°C and 1.000 L/h flow rate resulted in reductions of total bacterial count, psychotropic bacteria, coliforms, Enterococci, lactic acid bacteria, and spores by 63%, 96% 99.9%, 80%, 71 and 60% (grown in different agars), and 21%, respectively. Krishnamurthy *et al.* (2008) investigated the efficacy of IR heat treatment for inactivation of *Staphylococcus aureus* in milk: reductions ranging from a minimum of 0.10 to a maximum of 8.41 Log_{10} CFU/mL were obtained depending of IR lamp temperatures (536°C and 619°C), volumes of treated milk samples (3, 5 and 7 mL) and treatment times (1, 2 and 4 minutes). A complete inactivation was obtained at 619 °C lamp temperature within 4 min (with a sample volume of 3 or 5 mL, but not in case of 7 mL) but, after an enrichment step, the presence of *S. aureus* was reported almost always at 536°C whereas no growth was reported at 619°C in case of IR treatment for 5, 10 and 15 minutes (with only one exception). The authors stated that a growth during enrichment indicated that some of the cells were injured because of IR heating and were able to repair themselves. This aspect must be investigated more (Krishnamurthy *et al*., 2008). In conclusion, these studies show that IR has the potential to be utilized for microbial inactivation, but it has to be noted that the findings of all these studies are difficult to compare given the different IR technologies used. In fact, IR heating depends not only on the spectrum (because the energy emitted from the emitter consists of different wavelengths and part of the radiation depends on the source temperature and the lamp emission) but also on the direction, given electromagnetic radiation is weakened as a result of absorption by the medium as well as scattering (Aboud *et al*., 2019).

In the dairy sector, it is well known that improper handling and management practices during milking could result in contamination of the udder (Bramley and Mckinnon, 1990) and contamination of milk by pathogenic microorganisms. Raw milk can easily get contaminated by feces during milking, and therefore, effective inactivation is central to assuring milk safety. The microorganisms belonging to the family Enterobacteriaceae are of great importance in food safety, given some of these are involved in food spoilage, some are foodborne pathogens (for example, *Salmonella* spp.), and some are indicators of fecal contamination of food products. In milk production, Enterobacteriaceae have been used as indicators of microbial quality and hygiene because they are naturally present. In fact, Enterobacteriaceae are used as process hygiene criteria in Chapter 2, section 2.2.1 of the EU Regulation 2073/2005 for the food category “pasteurised milk and other pasteurised liquid dairy products” given they could be classified as indicators of an adequate heat treatment and a fast chilling/cooling after the treatment (European Commission, 2005). Concerning the behavior of *L. monocytogenes* and *Salmonella* spp., the level of inactivation observed by IR treatment, namely equal to or higher than the 6 Log reduction, is comparable with the results obtained by pasteurization (Farber *et al*., 1988; Bean *et al*., 2012) as well as in line with the 6 Log reduction requested for *L. monocytogenes* for pasteurized milk (Rouweler, 2015).

Conclusions

The findings of this study clearly show that the investigated IR treatment appears to be comparable to pasteurization treatment as well as suitable to obtain homogenized raw milk with safety criteria and process hygiene criteria compliant with Commission Regulation 2073/2005 (European Commission, 2005). However, hitherto, based on the regulation in force (EC Regulation 853/2004), milk subjected to IR treatment cannot be classified as either raw milk or pasteurized milk due to the treatment it undergoes (European Parliament and Council of the European Union, 2004). Commission Regulation 853/2004 specifies alkaline phosphatase activity determination ISO 11816-1 (ISO, 2024) as the method to control the efficacy of pasteurization, specifically for milk.

Currently, there is a lack of data on the effect of IR treatment on alkaline phosphatase activity. Therefore, at the moment, it cannot be used as a parameter to evaluate the efficacy of IR treatment on milk sanitation. From a legislative standpoint, there are two potential approaches to address this issue: i) conduct research to determine the effect of IR treatment on alkaline phosphatase and peroxidase activity and potentially establish new parameters for evaluating the efficacy of IR-treated

milk; ii) follow the approach used for other food commodities and leave it to producers to validate their processes to ensure compliance with legislation. Each approach has its own implications and considerations, and further research may be needed to determine the most appropriate course of action.

In conclusion, based on these preliminary results, the Wir System Milk appears to offer an efficient alternative to traditional raw milk heat treatments, also in consideration of the fact that IR treatment has an excellent cost-to-production ratio and low environmental impact. Given that the thermal efficiency of IR is high, it is considered a valuable energy source, and finally, it could allow for the integration of food waste reduction strategies.

Further studies investigating the effect of IR technology in different raw milk batches with different quality conditions, in terms of initial total bacterial counts and somatic cell counts, are needed to evaluate the overall microbiological inactivation performances of this treatment.

Moreover, studies on other pathogenic microorganisms will be necessary since these results are not directly applicable to them and D-values were not yet calculated for this process. Finally, further challenge studies and shelf-life assessment studies are essential to understand how IR can truly be used as a treatment for raw milk for food production, as a substitute for the thermal treatments currently in use.

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Table 1. Values of the investigated microorganisms (Log_{10} CFU/ml) and pH values observed in milk before and after the IR treatment.

	Raw milk			IR treated milk		
	Replicate 1	**Replicate 2**	**Replicate 3**	**Replicate 1**	**Replicate 2**	**Replicate 3**
L. monocytogenes	6.46	6.10	6.36	n.d.[2]	n.d.[2]	n.d.[2]
Salmonella spp.	6.17	6.14	6.11	n.d.[2]	n.d.[2]	n.d.[2]
Enterobacteriaceae	>3[1]	>3[1]	>3[1]	n.d.[3]	n.d.[3]	n.d.[3]
pH	6.62	6.58	6.58	6.56	6.51	6.54

IR, infrared; [1]values estimated from counts above the enumeration limit; [2]not detected in 25 g; [3]values below the enumeration limit (15 CFU/mL) of the ISO method and with no colonies detected in any plate.