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ORIGINAL ARTICLE

The effectiveness of radiant catalytic ionization in inactivation of *Listeria monocytogenes* planktonic and biofilm cells from food and food contact surfaces as a method of food preservation

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biofilm, food contact surfaces, *Listeria monocytogenes*, microbicidal effectiveness, radiant catalytic ionization.

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Abstract

Aims: The aim of the study was to evaluate the microbicidal effectiveness of radiant catalytic ionization (RCI) against *Listeria monocytogenes* strains in the form of planktonic cells and biofilm on food products and food contact surfaces as a method of food preservation.
Methods and Results: The study material comprised six strains of *L. monocytogenes*, isolated from food. Samples of different types of food available by retail (raw carrot, frozen salmon filets, soft cheese) and the fragments of surfaces (stainless steel AISI 304, rubber, milled rock tiles, polypropylene) were used in the experiment. The obtained results showed the effectiveness of RCI in the inactivation of both forms of the tested *L. monocytogenes* strains on all the surfaces. The effectiveness of RCI for biofilm forms was lower as compared with planktonic forms. The PRR value ranged from 18·19 to 99·97% for planktonic form and from 3·92 to 70·10% for biofilm.
Conclusions: The RCI phenomenon induces the inactivation of *L. monocytogenes* on surfaces of food and materials used in the processing industry to a varying degree, depending on the manner of surface contamination, the properties of the contaminated materials as well as on the origin of the strain and the properties of surrounding dispersive environment in which the micro-organisms were suspended.
Significance and Impact of the Study: Searching of new actions aimed at the reduction of the microbial contamination of food and food contact surfaces are extremely important. RCI method has been already described as an effective technique of microbial and abiotic pollution removal from air. However, our studies provide new, additional data related to evaluation the RCI efficacy against microbes on different surfaces, both in planktonic and biofilm form.

Introduction

Listeria monocytogenes is a short, Gram-positive, pleomorphic, nonspore forming bacterium (Gezali *et al.* 2016). They multiply in a wide range of temperature (0–45°C) and pH (4·5–9·0). They also tolerate a high salinity of the environment (up to 10% NaCl). *Listeria monocytogenes* are commonly found in the soil, fresh and salt waters and in sewage. Due to their common occurrence in the natural environment, there is a risk of contamination of the raw materials, of plant and animal origin, with *L. monocytogenes* (Lundén *et al.* 2002; Strawn *et al.* 2013).

The main route of the spread of *L. monocytogenes* is the intake of contaminated food (Camargo *et al.* 2017). In spite of a possible primary contamination of materials of animal and plant origin, in most cases, the stage of processing and the way of storage of the final products determine reaching an infectious *L. monocytogenes* dose (Kramarenko

et al. 2013). The studies conducted in recent years indicate that the frequency of occurrence of these micro-organisms in final articles is considerably higher as compared to the original materials. Moreover, analyses indicate cross-contamination of products. Often, the strains isolated from production surfaces and from final products present different genotypes than the strains isolated from raw materials before the technological process (Kathariou 2002; Jemmi and Stephan 2006). The main cause of the secondary contamination of food products is bacterial biofilm formation on many process surfaces made from different materials (containers, conveyor belts, cutting surfaces), as well as on the walls or ceilings of industrial plants (Poimenidou *et al.* 2016; Dhowlaghar *et al.* 2017). Biofilm of *L. monocytogenes* forming in the processing environment comprises the most pathogenic serotypes of those bacteria: 1/2a, 1/2b and 4b, but the mechanism promoting this tendency has not been known so far (Kramarenko *et al.* 2013). Micro-organisms forming biofilm have a higher resistance to unfavourable external factors as well as to disinfectants, antibiotics and surfactants, as compared with micro-organisms remaining in the form of plankton (Czaczyk and Myszka 2007; Flemming and Wingender 2010). Horizontal exchange of genes taking place within the biofilm and transmission of plasmids contribute to the spread of resistance to antibiotics and disinfectants. Moreover, it has been proved that the resistance of bacterial cells forming biofilm depends largely on the specific character of a surface on which it was formed and on its contamination (Simoes *et al.* 2010).

Various methods of elimination of *L. monocytogenes* from the environment of processing plants are used to ensure microbiological safety during food production. They include physical, chemical and biological methods.

The photocatalytic oxidation depends on creating reactive oxygen species. High energy electrons and photons react with particles in the air or surfaces. This process is going on without heating and producing too much ozone (Daniels 2007). The different steps of this process have been presented in Fig. 1.

Technology of removing pollutants from the environment with the use of heterogeneous photocatalysis has been the subject of research for almost 50 years. Following the introduction of structural innovations aiming at increasing the effectiveness of the process, the technology was defined as radiant catalytic ionization (RCI) (Space Fundation, Radiant Catalytic Ionization Air & Water Purification 2014).

RCI is defined as an active air purification technology. Devices generating RCI are intended for installation in heating, ventilation and air conditioning systems or for use in the standalone form, independent of existing systems. It has been shown that the reactive forms of oxygen and ozone molecules produced by the process will reduce levels of unpleasant odours and fumes and eliminate microbiological impurities (Grinshpun *et al.* 2007). Emitted reactive oxygen forms generate oxidative damage to the viral genetic material and impair the functionality of

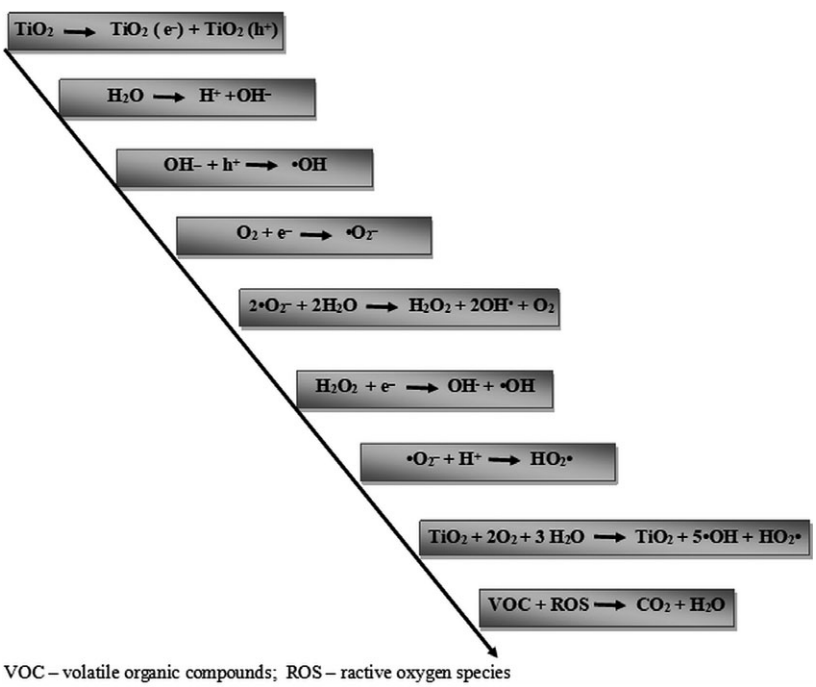

Figure 1 Scheme of photocatalytic reaction (acc. Daniels 2007).

capsid proteins. In bacterial cells, the coenzyme A molecules are oxidized which results in inhibition of cellular respiration pathways, oxidation of unsaturated phospholipids and destruction of the outer cell membrane, as well as accumulation of harmful changes in DNA or RNA (Sunada *et al.* 1998; Maness *et al.* 1999; Vohra *et al.* 2005). Nonliving particles are removed from the air by electrostatic precipitation induced by ionization process (Tepper and Kessick 2008).

In 2007, the first study was published describing the inactivation of different bacterial species (including *L. monocytogenes*) from the surface of stainless steel using the RCI technology, which forecasts the potential use of the method to disinfect surfaces in the industrial sector and medical care (Ortega *et al.* 2007).

The aim of the study was to evaluate the microbicidal effectiveness of RCI against *L. monocytogenes* strains in the form of planktonic cells and biofilm on food products and food contact surfaces as a method of food preservation.

Materials and methods

The study included six strains of *L. monocytogenes*, isolated from different types of food: two strains derived from frozen mixed vegetables (V1 and V2), two strains from fresh salmon filets (S1 and S2) and two strains from soft cheese type Camembert (CH1 and CH2). Selected strains belong to two different groups: susceptible to all tested antibiotics (V1, S1, CH1) and resistant to at least one (V2, S2, CH2) according to EUCAST ver. 7.0. The characteristics of the tested strains (based on previous studies) are presented in Table 1. The isolates used came from the collection of the Department of Microbiology, L. Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń.

RCI device (Induct 750) description

The RCI cell has a structure optimized for purified air flow (Fig. 2). In Induct 750 (ActivTek Sp. z o.o., Kielce, Poland), air flow is forced by a fan with a diameter of 120 mm and a power of 3W. The maximum air capacity of the Induct 750 is 233 $m^3 \times h^{-1}$. RCI device consists of a matrix of elongated tubular components made of polycarbonate, arranged parallel in a honeycomb-like pattern. In Induct 750 (ActivTek) there are two matrixes consisting of 406 tubular components each. Each tubula has a diameter of 4 mm and a length of 15 mm. The total active surface of both matrixes is 763·28 cm^2. The coating of basic elements of the matrix shows hydrophilic properties and contains titanium dioxide. Opposite to the system is a 8W power UV lamp GPH118T5L/4, which is a source of ultraviolet radiation of a wide spectrum. The UV lamp uses argon gas with mercury and carbide fibres with a spectrum of 100 and 367 nm. This UV lamp generates UV ray with wavelengths of 185 and 254 nm. As a result of catalytic oxidation, stimulated by UV radiation, at the boundary of heterogeneous phases (gas-solid), reactive oxygen forms are generated: hydroxyl radicals, superoxide ions, hydroxide ions. A total number of generated ions is about $5{\cdot}0 \times 10^4$ ions $\times$ cm^{-3} of air. A small amount of ozone (≤0·02 ppm) is also produced. Detailed information about RCI technology is included in the patent no. US 8,585,979 B2.

Preparation of the tested surfaces

Samples of different types of food available by retail, raw carrot, frozen salmon filets, soft cheese (Camembert type), were used in experiment. The studied food products reflect food groups being the main source of *L. monocytogenes*—fish, dairy products and vegetables.

The food was cut in slices with an area of about 1 cm × 2 cm and a thickness of 2–3 mm. The slices were

Table 1 The characteristics of the tested *Listeria monocytogenes* strains

Strains	Serogroup (based PCR method)	Genetic distance (PFGE method) (%)	Virulence genes	Antibiotic resistance profile	Persistence strains
V1	1/2a-3a	94	*actA, fbpA, hlyA, iap, inlA, inlB, mpl, plcA, plcB, prfA*	R: — S: P, AM, MEM, E, SXT	No
V2	4b-4d-4e		*actA, fbpA, hlyA, iap, inlA, inlB, plcA, plcB, prfA*	R: P S: AM, MEM, E, SXT	No
S1	1/2a-3a	72	*actA, fbpA, hlyA, iap, inlA, inlB, mpl, plcA, plcB, prfA*	R: — S: P, AM, MEM, E, SXT	No
S2	1/2a-3a		*actA, fbpA, hlyA, iap, inlA, inlB, mpl, plcA, plcB, prfA*	R: E S: P, AM, MEM, SXT	No
CH1	1/2a-3a	84	*actA, fbpA, hlyA, iap, inlA, inlB, mpl, plcA, plcB, prfA*	R: — S: P, AM, MEM, E, SXT	No
CH2	1/2a-3a		*actA, fbpA, hlyA, iap, inlA, inlB, mpl, plcA, plcB, prfA*	R: E, SXT S: P, AM, MEM	No

R—resistant; S—susceptible; P—penicillin; AM—ampicillin; MEM—meropenem; E—erythromycin; SXT—co-trimoxazole.

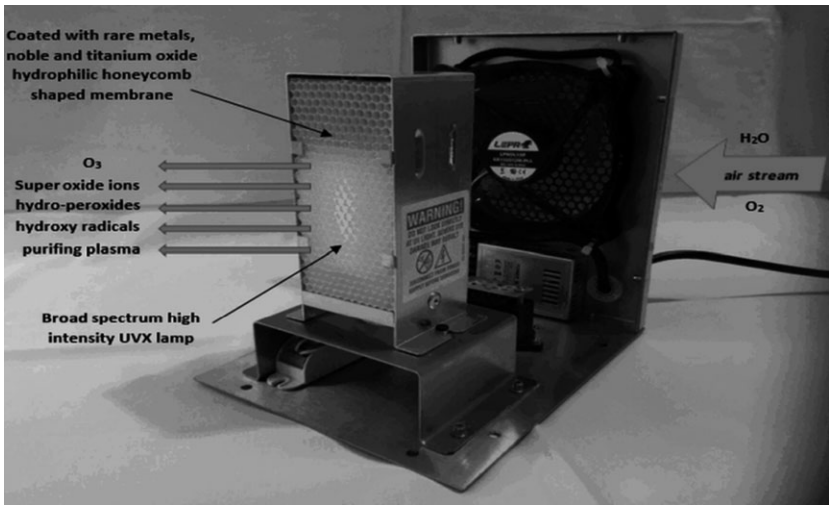

Figure 2 Diagram of the structure and function of RCI device.

disinfected in 70% ethanol solution for 5 min and then sterilized with ultraviolet radiation type C, emitted by an UV-C lamp (Philips TUV 36W/G36 T8, Philips, Amsterdam, Holand) for 15 min on each side of the piece of food. At the next stage, the samples of particular products were divided into two parts which were placed in separate sterile sacks with string closure. From one part, pulps were made —vegetable, fish and cheese respectively—in 100 ml of phosphate-buffered saline (PBS) (Avantor, Gliwice, Poland). Prepared pulps were pipetted to sterile test tubes. The other part of the material was used as tested surfaces in the next stages of the experiment. The sterility control of tested food fragments and the appropriate pulps prepared for them was carried out. For this purpose, the food fragments after sterilization were sonicated, and 100 μl of the obtained washings was transferred on Columbia Agar with 5% sheep blood (bioMérieux, Marcy l'Etoile, France). Also, 100 µl of each type of pulp, was transferred on the same agar. Prepared cultures were incubated for 24 h at 37°C.

In the study, the fragments (1 cm × 2 cm) of four types of surfaces commonly found in food processing plants and food storage areas—stainless steel AISI 304, rubber, milled rock tiles, polipropylene—were also used. The all types of coupons were initially soaked in acetone or ethanol (in case of rubber) (overnight) to remove any debris and grease. Then tested surfaces were washed in commercial detergent solution, rinsed thoroughly twice with distilled water, air-dried at room temperature, and finally sterilized by autoclaving (121°C, 1 atm., 20 min). In each experiment the new surface fragments were used.

Preparation of bacterial suspensions and contamination of surface

For each of the *L. monocytogenes* strains, two types of bacterial suspensions in sterile saline were made based on bacterial cultures in early stationary phase: the first with an optical density of 0·5 on McFarland's scale (McF), the other with a density of 1·0 McF. Then each of the bacterial suspensions with a density of 1·0 McF was mixed with the appropriate pulp in a volume ratio of 1 : 1. Vegetable pulp was used for strains derived from frozen mixed vegetables, fish pulp for strains isolated from salmon filets, cheese pulp for isolates from soft cheese Camembert type (Fig. 3). Bacterial suspensions with a density of 0·5 McF were used for contamination of the surfaces of food products and those with a density of 1·0 McF mixed with a pulp, or contamination of the tested abiotic surfaces.

The experiment was conducted in two variants—for planktonic cells and a biofilm of *L. monocytogenes*.

In order to obtain planktonic forms of the bacteria, 50 μl of the previously prepared bacterial suspension of *L. monocytogenes* in saline with a density of 0·5 McF was applied on each of the prepared slices of food. Each strain was used for the contamination of four pieces of the suitable type of food products; strains V1 and V2—contamination of slices of carrot, strains S1 and S2—fragments of salmon, strains CH1 and CH2—slices of soft cheese. At the same time, 50 μl of a bacterial suspension of *L. monocytogenes* prepared in a mixture of saline and a suitable type of pulp was applied on each of the tested abiotic surfaces. Each of the prepared bacterial suspensions with an addition of pulp was applied on four single fragments of all the tested materials: steel, rubber, tile, polypropylene. The samples were left to dry at the room temperature in a sterile laminar chamber, which prevented secondary contamination of the tested surfaces. As a result, we obtained four identical sets of samples for each of the strains, consisting of one slice of food, one fragment of steel, one fragment of rubber, one fragment of milled rock tile and one fragment of polypropylene (Fig. 4).

In order to form biofilm by *L. monocytogenes* on the tested surfaces, slices of food and fragments of abiotic surfaces were placed directly in the prepared suspensions and incubated at 37°C for 24 h. After incubation, tested surfaces were washed twice in sterile PBS to eliminate the planktonic bacterial cells loosely adherent to the surfaces. In each of the bacterial suspensions with a density of 0·5 McF in saline, four fragments of the appropriate type of food products were left. For strains V1 and V2, slices of carrot; S1 and S2, fragments of salmon filets and CH1 and CH2, pieces of cheese. In each mixture of the suspension in saline and appropriate pulp, in turn, four single fragments of all the tested materials, steel, rubber, tile, polypropylene, were placed. We obtained four identical sets of samples for each of the strains, consisting of one slice of food, one fragment of steel, one fragment of rubber, one fragment of tile and one fragment of polypropylene. The experiment was made in two replication for each strain and method of bacterial cells applying on the surfaces.

In the case of applying the cells in the planktonic form on the tested surfaces, two of the four sets prepared in this variant were used as tested samples (subjected to the action of the RCI device), the other two constituted positive control samples. In a similar way—two of the four sets of samples, in which *L. monocytogenes* formed biofilm, were exposed to RCI, the other two were used as positive control samples. The negative sample comprised fragments of the tested surfaces not contaminated with bacterial suspensions.

Subjecting the material to the action of a RCI device

All sets of the tested samples were exposed to the action of the device Induct 750 generating the phenomenon of radial catalytic ionization. The exposure was conducted for 30 min in a closed chamber (temperature 25°C, RH = 49%), limiting the possibility of additional surface contamination with other microorganisms. The tested materials were placed at a distance of about 20 cm from the device. The control samples were not exposed to the action of the RCI device.

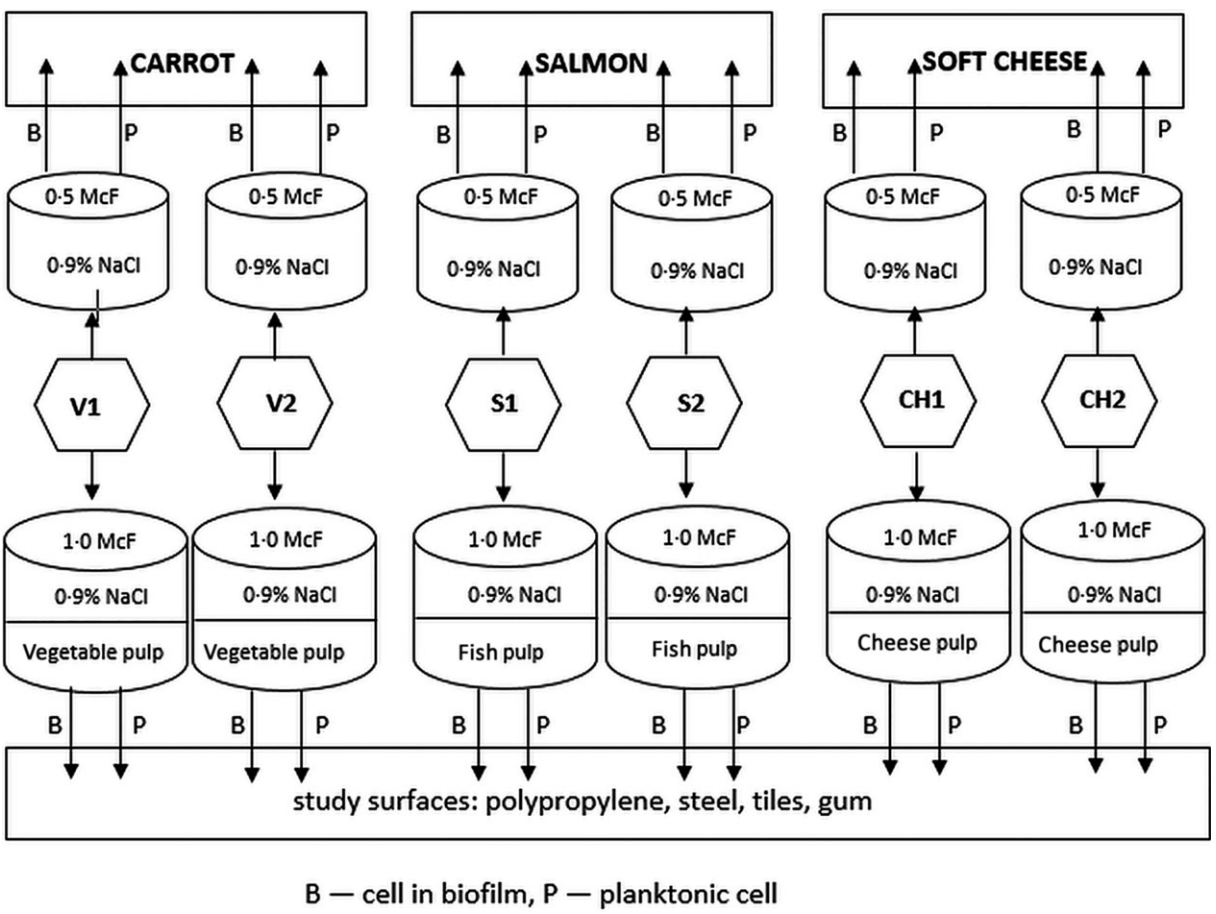

Figure 3 Method for the preparation of bacterial suspensions and their applying on food and tested surfaces.

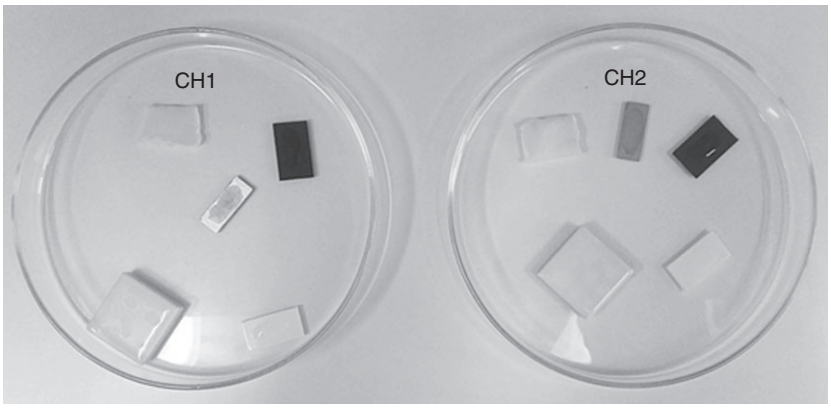

Figure 4 Sets of tested samples for bacterial strains CH1 and CH2 (a suspension applied in the form of planktonic cells on slices of food and fragments of surfaces).

Determination of the number of *Listeria monocytogenes* in the control and tested samples

At the next stage of the study, all fragments of food and surfaces were placed separately in sterile containers containing 25 ml of PBS (Avantor), and then subjected to 10-min sonication in a Ultrasonic DU-4 (operating frequency—30 kHz, sonic power—150 W) (Nickel-Electro, North Somerset, UK) sonicator and shaking (10 min, 400 rev min^{-1}) to release the bacterial cells present on the surfaces of the solution (Webber *et al.* 2015). Two independent series of 10-fold dilutions with PBS (Avantor) were made from the suspensions obtained in the containers. From each dilution, a surface culture of a suspension with a volume of 100 μl was made on Columbia Agar with 5·0% sheep blood (bioMérieux). Prepared cultures were incubated for 24 h at 37°C.

After incubation, the grown colonies of *L. monocytogenes* were counted and expressed as the number of colony-forming units (CFU) of bacteria per 1 cm^2 of the surface.

Based on the growth of *L. monocytogenes* derived from the tested samples (subjected to RCI) obtained from the cultures and the control samples (not subjected to RCI), the percentage reduction rate of the bacteria count (PRR) was calculated for all the tested strains on different materials used in this study, according to the formula:

$$\mathrm{PRR}\,(\%) = \frac{a-b}{a} \times 100\%$$

where: *a*—the number of micro-organisms in the control sample; *b*—the number of micro-organisms in the tested sample.

Statistical analysis

The results obtained concerning the number of bacteria isolated from the tested surfaces and the calculated PRR were analysed statistically in the STATISTICA 13.0 PL software (StatSoft, Palo Alto, CA, USA).

For all of the tested strains, both for the number and for PRR, the arithmetic mean and standard deviation were calculated from all replications performed for the same surfaces and the same method of applying the micro-organisms.

For the calculated PRR values, a multivariate analysis of variance was performed, treating the strain, its origin, the type of surface and the method of applying micro-organisms, as independent variables. Based on *post hoc* Tukey's test, taking into account the above variables, the significance of differences between PRR values was checked at the significance level $\alpha = 0{\cdot}05$.

Results

The sterility control of tested food fragments and pulps showed that they were free from microbial contamination.

The obtained results showed the effectiveness of RCI in the inactivation of both planktonic and biofilm forms of the tested *L. monocytogenes* strains on all the surfaces (Tables 2–4).

In the case of planktonic form, in all the control variants, the smallest number of *L. monocytogenes* were isolated from carrot—$2{\cdot}15 \times 10^5$ CFU × cm^{-2} (a strain isolated from frozen mixed vegetables) (Table 2). In contrast, most *L. monocytogenes* were isolated from steel—$1{\cdot}84 \times 10^7$ CFU × cm^{-2} for planktonic forms (a strain isolated from salmon) (Table 3). From the biofilm formed on the tested surfaces, the smallest number of bacteria in the control variants were isolated from a piece of cheese, for a strain isolated from cheese ($3{\cdot}13 \times 10^6$ CFU × cm^{-2}) (Table 4). The largest number of bacteria in the control variants were re-isolated from the surface of polypropylene, for a strain derived from salmon ($6{\cdot}85 \times 10^7$ CFU × cm^{-2}) (Table 3). The calculated percentage reduction rate for planktonic cells was the highest for a strain derived from frozen mixed vegetables applied on the rubber surface (99·97%),

Table 2 Influence of radial catalytic ionization on inactivation *Listeria monocytogenes* strains isolated from vegetables on the surface of carrots and tested materials

	Planktonic form			Biofilm		
	Number of bacteria (CFU × cm^{-2})			Number of bacteria (CFU × cm^{-2})		
Strains	Positive control	Tested sample	PRR (%)	Positive control	Tested sample	PRR (%)
Carrot						
V1	$2{\cdot}15 \times 10^5$ ($\pm 2{\cdot}12 \times 10^4$)*	$2{\cdot}58 \times 10^4$ ($\pm 1{\cdot}13 \times 10^3$)	$87{\cdot}97^{d}$ ($\pm 0{\cdot}66$)†	$1{\cdot}24 \times 10^7$ ($\pm 6{\cdot}36 \times 10^5$)	$9{\cdot}60 \times 10^6$ ($\pm 4{\cdot}24 \times 10^5$)	$22{\cdot}25^{f}$ ($\pm 0{\cdot}57$)
V2	$2{\cdot}75 \times 10^5$ ($\pm 3{\cdot}54 \times 10^4$)	$1{\cdot}76 \times 10^4$ ($\pm 2{\cdot}55 \times 10^3$)	$93{\cdot}61^{b}$ ($\pm 0{\cdot}10$)	$1{\cdot}91 \times 10^7$ ($\pm 3{\cdot}46 \times 10^6$)	$1{\cdot}11 \times 10^7$ ($\pm 1{\cdot}48 \times 10^6$)	$41{\cdot}74^{a}$ ($\pm 2{\cdot}80$)
Rubber						
V1	$1{\cdot}95 \times 10^6$ ($\pm 2{\cdot}83 \times 10^5$)	$7{\cdot}85 \times 10^4$ ($\pm 4{\cdot}95 \times 10^4$)	$95{\cdot}95^{c}$ ($\pm 0{\cdot}33$)	$6{\cdot}00 \times 10^7$ ($\pm 2{\cdot}83 \times 10^6$)	$2{\cdot}69 \times 10^7$ ($\pm 3{\cdot}32 \times 10^6$)	$55{\cdot}33^{b,c,d}$ ($\pm 3{\cdot}43$)
V2	$1{\cdot}69 \times 10^6$ ($\pm 2{\cdot}62 \times 10^5$)	$5{\cdot}75 \times 10^2$ ($\pm 8{\cdot}13 \times 10^2$)	$99{\cdot}97^{a}$ ($\pm 0{\cdot}04$)	$2{\cdot}68 \times 10^7$ ($\pm 1{\cdot}98 \times 10^6$)	$1{\cdot}34 \times 10^7$ ($\pm 1{\cdot}56 \times 10^6$)	$50{\cdot}08^{a,b,c}$ ($\pm 2{\cdot}12$)
Polypropylene						
V1	$6{\cdot}08 \times 10^5$ ($\pm 9{\cdot}55 \times 10^4$)	$2{\cdot}80 \times 10^3$ ($\pm 4{\cdot}24 \times 10^2$)	$99{\cdot}53^{a}$ ($\pm 0{\cdot}14$)	$5{\cdot}25 \times 10^7$ ($\pm 2{\cdot}47 \times 10^7$)	$2{\cdot}76 \times 10^7$ ($\pm 1{\cdot}33 \times 10^7$)	$47{\cdot}57^{a,b}$ ($\pm 0{\cdot}61$)
V2	$1{\cdot}62 \times 10^6$ ($\pm 1{\cdot}63 \times 10^5$)	$8{\cdot}68 \times 10^4$ ($\pm 1{\cdot}52 \times 10^4$)	$94{\cdot}55^{b,c}$ ($\pm 1{\cdot}49$)	$1{\cdot}61 \times 10^7$ ($\pm 4{\cdot}10 \times 10^6$)	$9{\cdot}50 \times 10^6$ ($\pm 1{\cdot}41 \times 10^6$)	$40{\cdot}17^{a}$ ($\pm 6{\cdot}46$)
Steel						
V1	$1{\cdot}35 \times 10^6$ ($\pm 4{\cdot}88 \times 10^5$)	$8{\cdot}35 \times 10^2$ ($\pm 1{\cdot}20 \times 10^2$)	$99{\cdot}94^{a}$ ($\pm 0{\cdot}01$)	$3{\cdot}00 \times 10^7$ ($\pm 1{\cdot}48 \times 10^6$)	$8{\cdot}95 \times 10^6$ ($\pm 2{\cdot}12 \times 10^5$)	$70{\cdot}10^{e}$ ($\pm 0{\cdot}77$)
V2	$1{\cdot}53 \times 10^6$ ($\pm 3{\cdot}89 \times 10^5$)	$6{\cdot}70 \times 10^2$ ($\pm 2{\cdot}40 \times 10^2$)	$99{\cdot}96^{a}$ ($\pm 0{\cdot}01$)	$3{\cdot}53 \times 10^7$ ($\pm 1{\cdot}01 \times 10^7$)	$9{\cdot}55 \times 10^6$ ($\pm 3{\cdot}04 \times 10^6$)	$72{\cdot}99^{e}$ ($\pm 0{\cdot}28$)
Tiles						
V1	$4{\cdot}98 \times 10^5$ ($\pm 3{\cdot}89 \times 10^4$)	$1{\cdot}14 \times 10^3$ ($\pm 1{\cdot}91 \times 10^2$)	$99{\cdot}77^{a}$ ($\pm 0{\cdot}06$)	$2{\cdot}63 \times 10^7$ ($\pm 1{\cdot}77 \times 10^6$)	$1{\cdot}06 \times 10^7$ ($\pm 9{\cdot}90 \times 10^5$)	$59{\cdot}65^{c,d}$ ($\pm 1{\cdot}05$)
V2	$1{\cdot}71 \times 10^6$ ($\pm 6{\cdot}43 \times 10^5$)	$6{\cdot}75 \times 10^2$ ($\pm 2{\cdot}47 \times 10^2$)	$99{\cdot}96^{a}$ ($\pm 0{\cdot}01$)	$2{\cdot}66 \times 10^7$ ($\pm 4{\cdot}24 \times 10^6$)	$9{\cdot}45 \times 10^6$ ($\pm 1{\cdot}77 \times 10^6$)	$64{\cdot}55^{d,e}$ ($\pm 0{\cdot}99$)

a, b, c, . . .—values marked with different letters differ statistically significant ($P \leq 0{\cdot}05$).
*Standard error for bacteria number.
†Standard deviation for PRR values.

Table 3 Influence of radial catalytic ionization on inactivation of *Listeria monocytogenes* strains isolated from fish on the surface of salmon filet and tested materials

	Planktonic form			Biofilm		
	Number of bacteria (CFU × cm^{-2})			Number of bacteria (CFU × cm^{-2})		
Strains	Positive control	Tested sample	PRR (%)	Positive control	Tested sample	PRR (%)
Salmon						
S1	$2{\cdot}90 \times 10^6$ ($\pm 1{\cdot}41 \times 10^5$)*	$1{\cdot}38 \times 10^6$ ($\pm$ 3·89 × 10^5)	$52{\cdot}86^{a}$ (±11·11)†	$4{\cdot}65 \times 10^7$ ($\pm 7{\cdot}78 \times 10^6$)	$4{\cdot}40 \times 10^7$ ($\pm 7{\cdot}07 \times 10^6$)	$5{\cdot}32^{a}$ (±0·63)
S2	$1{\cdot}56 \times 10^6$ ($\pm 2{\cdot}26 \times 10^5$)	$1{\cdot}27 \times 10^6$ ($\pm 9{\cdot}90 \times 10^4$)	$18{\cdot}19^{c}$ (±5·52)	$3{\cdot}90 \times 10^7$ ($\pm 2{\cdot}83 \times 10^6$)	$3{\cdot}75 \times 10^7$ ($\pm 3{\cdot}54 \times 10^6$)	$3{\cdot}92^{a}$ (±2·10)
Rubber						
S1	$8{\cdot}65 \times 10^6$ ($\pm 4{\cdot}95 \times 10^5$)	$2{\cdot}49 \times 10^6$ ($\pm 3{\cdot}75 \times 10^5$)	$71{\cdot}35^{b}$ (±2·69)	$6{\cdot}05 \times 10^7$ ($\pm 3{\cdot}54 \times 10^6$)	$5{\cdot}60 \times 10^7$ ($\pm 2{\cdot}83 \times 10^6$)	$7{\cdot}42^{a}$ (±0·74)
S2	$6{\cdot}93 \times 10^6$ ($\pm 1{\cdot}17 \times 10^6$)	$1{\cdot}73 \times 10^6$ ($\pm 4{\cdot}60 \times 10^5$)	$75{\cdot}30^{b}$ (±2·48)	$4{\cdot}70 \times 10^7$ ($\pm 8{\cdot}49 \times 10^6$)	$4{\cdot}35 \times 10^7$ ($\pm 9{\cdot}19 \times 10^6$)	$7{\cdot}71^{a}$ (±2·90)
Polypropylene						
S1	$7{\cdot}70 \times 10^6$ ($\pm 1{\cdot}13 \times 10^6$)	$5{\cdot}65 \times 10^6$ ($\pm 2{\cdot}62 \times 10^6$)	$28{\cdot}35^{c}$ (±23·45)	$6{\cdot}85 \times 10^7$ ($\pm 6{\cdot}36 \times 10^6$)	$6{\cdot}45 \times 10^7$ ($\pm 4{\cdot}95 \times 10^6$)	$5{\cdot}77^{a}$ (±1·53)
S2	$9{\cdot}65 \times 10^6$ ($\pm 4{\cdot}95 \times 10^5$)	$7{\cdot}68 \times 10^6$ ($\pm 1{\cdot}52 \times 10^6$)	$20{\cdot}77^{c}$ (±11·69)	$8{\cdot}40 \times 10^7$ ($\pm 4{\cdot}24 \times 10^6$)	$8{\cdot}00 \times 10^7$ ($\pm 4{\cdot}24 \times 10^6$)	$4{\cdot}77^{a}$ (±0·24)
Steel						
S1	$1{\cdot}84 \times 10^7$ ($\pm 4{\cdot}38 \times 10^6$)	$5{\cdot}73 \times 10^6$ ($\pm 7{\cdot}42 \times 10^5$)	$68{\cdot}47^{a,b}$ (±3·48)	$3{\cdot}31 \times 10^7$ ($\pm 2{\cdot}62 \times 10^6$)	$1{\cdot}97 \times 10^7$ ($\pm 1{\cdot}63 \times 10^6$)	$40{\cdot}55^{c}$ (±0·21)
S2	$7{\cdot}90 \times 10^6$ ($\pm 8{\cdot}49 \times 10^5$)	$5{\cdot}65 \times 10^6$ ($\pm 1{\cdot}20 \times 10^6$)	$28{\cdot}89^{c,d}$ (±7·58)	$2{\cdot}83 \times 10^7$ ($\pm 3{\cdot}11 \times 10^6$)	$1{\cdot}37 \times 10^7$ ($\pm 2{\cdot}83 \times 10^5$)	$51{\cdot}35^{d}$ (±4·35)
Tiles						
S1	$1{\cdot}49 \times 10^7$ ($\pm 4{\cdot}03 \times 10^6$)	$5{\cdot}33 \times 10^6$ ($\pm 8{\cdot}13 \times 10^5$)	$63{\cdot}54^{a,b}$ (±4·42)	$3{\cdot}95 \times 10^7$ ($\pm 2{\cdot}12 \times 10^6$)	$3{\cdot}25 \times 10^7$ ($\pm 2{\cdot}12 \times 10^6$)	$17{\cdot}75^{b}$ (±0·95)
S2	$8{\cdot}40 \times 10^6$ ($\pm 8{\cdot}49 \times 10^5$)	$5{\cdot}00 \times 10^6$ ($\pm 1{\cdot}76 \times 10^6$)	$39{\cdot}17^{d}$ (±27·10)	$2{\cdot}97 \times 10^7$ ($\pm 4{\cdot}24 \times 10^5$)	$2{\cdot}44 \times 10^7$ ($\pm 6{\cdot}36 \times 10^5$)	$18{\cdot}02^{b}$ (±0·97)

a, b, c, … —values marked with different letters differ statistically significant ($P \leq 0{\cdot}05$).
*Standard error for bacteria number.
†Standard deviation for PRR values.

Table 4 Influence of radial catalytic ionization on inactivation *Listeria monocytogenes* strains isolated from cheese on the surface of soft cheese and tested materials

	Planktonic form			Biofilm		
	Number of bacteria (CFU × cm^{-2})			Number of bacteria (CFU × cm^{-2})		
Strains	Positive control	Tested sample	PRR (%)	Positive control	Tested sample	PRR (%)
Soft cheese						
CH1	$1{\cdot}81 \times 10^6$ ($\pm 1{\cdot}63 \times 10^5$)*	$6{\cdot}70 \times 10^5$ ($\pm 4{\cdot}24 \times 10^4$)	$62{\cdot}84^{b}$ ($\pm 1{\cdot}00$)†	$3{\cdot}13 \times 10^6$ ($\pm 9{\cdot}12 \times 10^5$)	$2{\cdot}74 \times 10^6$ ($\pm 7{\cdot}99 \times 10^5$)	$12{\cdot}48^{a}$ ($\pm 0{\cdot}02$)
CH2	$1{\cdot}69 \times 10^6$ ($\pm 7{\cdot}99 \times 10^5$)	$1{\cdot}19 \times 10^6$ ($\pm 2{\cdot}69 \times 10^5$)	$24{\cdot}69^{c}$ ($\pm 19{\cdot}77$)	$4{\cdot}72 \times 10^6$ ($\pm 3{\cdot}04 \times 10^5$)	$4{\cdot}25 \times 10^6$ ($\pm 2{\cdot}12 \times 10^5$)	$9{\cdot}82^{a}$ ($\pm 1{\cdot}32$)
Rubber						
CH1	$1{\cdot}98 \times 10^6$ ($\pm 8{\cdot}13 \times 10^5$)	$1{\cdot}31 \times 10^4$ ($\pm 1{\cdot}34 \times 10^3$)	$99{\cdot}26^{a}$ ($\pm 0{\cdot}37$)	$5{\cdot}64 \times 10^6$ ($\pm 6{\cdot}58 \times 10^5$)	$4{\cdot}15 \times 10^6$ ($\pm 3{\cdot}54 \times 10^5$)	$26{\cdot}22^{b,c,d}$ ($\pm 2{\cdot}34$)
CH2	$6{\cdot}43 \times 10^5$ ($\pm 1{\cdot}45 \times 10^5$)	$1{\cdot}79 \times 10^4$ ($\pm 5{\cdot}44 \times 10^3$)	$97{\cdot}25^{a}$ ($\pm 0{\cdot}23$)	$3{\cdot}51 \times 10^6$ ($\pm 5{\cdot}52 \times 10^5$)	$2{\cdot}53 \times 10^6$ ($\pm 3{\cdot}46 \times 10^5$)	$27{\cdot}95^{b,c,d}$ ($\pm 1{\cdot}45$)
Polypropylene						
CH1	$9{\cdot}15 \times 10^5$ ($\pm 7{\cdot}78 \times 10^4$)	$1{\cdot}83 \times 10^5$ ($\pm 3{\cdot}18 \times 10^4$)	$80{\cdot}13^{a,b}$ ($\pm 1{\cdot}79$)	$6{\cdot}55 \times 10^6$ ($\pm 4{\cdot}95 \times 10^5$)	$5{\cdot}43 \times 10^6$ ($\pm 4{\cdot}60 \times 10^5$)	$17{\cdot}20^{a,b}$ ($\pm 0{\cdot}76$)
CH2	$6{\cdot}00 \times 10^5$ ($\pm 2{\cdot}83 \times 10^4$)	$1{\cdot}65 \times 10^4$ ($\pm 1{\cdot}23 \times 10^4$)	$97{\cdot}30^{a}$ ($\pm 1{\cdot}92$)	$5{\cdot}40 \times 10^6$ ($\pm 2{\cdot}83 \times 10^5$)	$4{\cdot}36 \times 10^6$ ($\pm 1{\cdot}84 \times 10^5$)	$19{\cdot}24^{a,b,c}$ ($\pm 0{\cdot}83$)
Steel						
CH1	$4{\cdot}95 \times 10^5$ ($\pm 1{\cdot}06 \times 10^5$)	$2{\cdot}47 \times 10^4$ ($\pm 6{\cdot}65 \times 10^3$)	$95{\cdot}04^{a}$ ($\pm 0{\cdot}28$)	$6{\cdot}95 \times 10^6$ ($\pm 3{\cdot}54 \times 10^5$)	$3{\cdot}70 \times 10^6$ ($\pm 4{\cdot}24 \times 10^5$)	$46{\cdot}85^{e}$ ($\pm 3{\cdot}40$)
CH2	$2{\cdot}55 \times 10^5$ ($\pm 7{\cdot}78 \times 10^4$)	$1{\cdot}92 \times 10^4$ ($\pm 4{\cdot}45 \times 10^3$)	$92{\cdot}40^{a}$ ($\pm 0{\cdot}57$)	$5{\cdot}95 \times 10^6$ ($\pm 2{\cdot}12 \times 10^5$)	$2{\cdot}65 \times 10^6$ ($\pm 5{\cdot}16 \times 10^5$)	$55{\cdot}67^{e}$ ($\pm 7{\cdot}10$)
Tiles						
CH1	$4{\cdot}80 \times 10^5$ ($\pm 1{\cdot}13 \times 10^5$)	$1{\cdot}15 \times 10^4$ ($\pm 2{\cdot}12 \times 10^3$)	$97{\cdot}59^{a}$ ($\pm 0{\cdot}13$)	$4{\cdot}50 \times 10^6$ ($\pm 4{\cdot}24 \times 10^5$)	$2{\cdot}94 \times 10^6$ ($\pm 3{\cdot}82 \times 10^5$)	$34{\cdot}78^{d}$ ($\pm 2{\cdot}34$)
CH2	$1{\cdot}79 \times 10^5$ ($\pm 3{\cdot}04 \times 10^4$)	$3{\cdot}95 \times 10^4$ ($\pm 2{\cdot}12 \times 10^3$)	$77{\cdot}44^{a,b}$ ($\pm 5{\cdot}03$)	$3{\cdot}29 \times 10^6$ ($\pm 4{\cdot}17 \times 10^5$)	$2{\cdot}31 \times 10^6$ ($\pm 3{\cdot}25 \times 10^5$)	$29{\cdot}74^{c,d}$ ($\pm 0{\cdot}98$)

a, b, c, ...—values marked with different letters differ statistically significant ($P \leq 0{\cdot}05$).
*Standard error for bacteria number.
†Standard deviation for PRR values.

(Table 2). The lowest PRR for planktonic cells was recorded for a strain isolated from salmon applied on the surface of salmon filet (18·19%), (Table 3). In the case of the biofilm, for all the tested isolates, the effectiveness of RCI was clearly lower as compared with planktonic forms. The lowest effectiveness of RCI was recorded for a strain isolated from salmon applied on the surface of salmon (3·92%), (Table 3). The highest effectiveness of RCI was shown for the strain isolated from frozen mixed vegetables applied on steel (70·10%) (Table 2). This study allowed for indicating certain differences in susceptibility of strains isolated from vegetables, salmon and soft cheese to RCI technologies. The PRR values on some tested surfaces differed statistically significantly between strains (Tables 2–4). The highest recovery of micro-organisms from the tested surfaces, in the control variant, was recorded for strains derived from salmon, irrespectively whether they occurred in the form of plankton or biofilm (Tables 2–4).

Mean percentage reduction rates of the numbers of *L. monocytogenes* for strains of different origin, depending on the form of contamination of different surfaces used in the study is presented in Fig. 5. The value of PRR rate for cells applied on the surfaces in planktonic form ranged from 24·56 (isolates from salmon on polypropylene) to 99·95% (isolates from frozen mixed vegetables on steel). For the bacteria-forming biofilm it ranged from 4·62 (isolates from salmon on a salmon filet) to 71·54% (isolates from vegetables on steel). In most cases, the effectiveness of inactivation of micro-organisms applied on the tested surfaces in the form of planktonic cells was statistically significantly higher as compared with the same strains on the analogous surfaces, forming biofilm (Fig. 5).

The origin of strains of *L. monocytogenes* and the associated type of pulp added to the bacterial suspension significantly influenced the resistance of the tested rods to RCI technologies. Among the bacterial cells, both applied in the form of plankton and forming biofilm, the highest percentages of elimination were obtained for isolates derived from frozen mixed vegetables, whereas the lowest was for isolates from salmon filets. The PRR values from the planktonic forms of strains isolated from vegetables reached the level from 90·79 (isolates from vegetables on carrot) to 99·95% (isolates from frozen mixed vegetables on steel), whereas from those forming biofilm, they reached the level from 32·00 (isolates from frozen mixed vegetables on carrot) to 71·54% (isolates from frozen mixed vegetables on steel) (Fig. 5). Among isolates derived from salmon filets, applied on the tested surfaces,

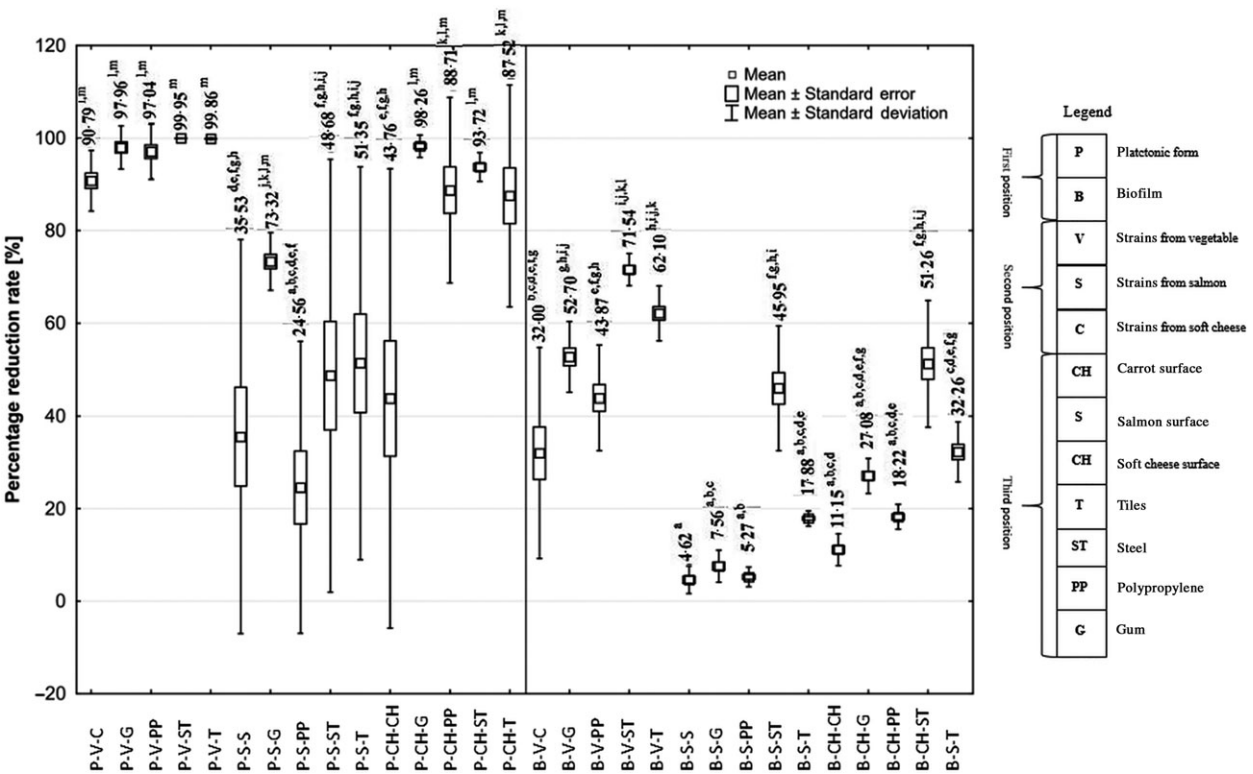

Figure 5 Percentage reduction rate of *Listeria monocytogenes* for all tested samples (a, b, c, ...—values marked with different letters differ statistically significant ($P \leq 0{\cdot}05$)) mean; mean ± SE; mean ± SD.

the reduction rate ranged from 24·56 (isolates from salmon on polypropylene) to 73·32% (isolates from salmon on rubber), whereas for those strains forming biofilm, from 4·62 (isolates from salmon on a salmon filet) to 45·95% (isolates from salmon on steel) (Fig. 5). The differences found in PRR values between both groups of strains in most cases were statistically significant (Fig. 5).

In groups of bacterial strains derived from the same type of food and applied with identical way on the tested materials (as plankton or biofilm), differences in inactivation depending on the type of surface were not statistically significant (Fig. 5). However, the analysis of the PRR values allowed for indicating certain tendencies. Among most *L. monocytogenes* strains, the effectiveness of inactivation of bacterial cells caused by RCI was the lowest on surfaces of the tested fragments of food—90·79% (plankton) and 32·00% (biofilm), respectively, for isolates from mixed vegetables; 4·62% (biofilm) for isolates from salmon filets as well as 43·76% (plankton) and 11·15% (biofilm) for isolates from soft cheese. The exceptions were planktonic forms of *L. monocytogenes* strains derived from salmon, among which the lowest value of the PRR rate was achieved on the surface of polypropylene (24·56%) (Fig. 5). Among micro-organisms applied on the tested abiotic surfaces in the form of suspensions, the process of bacteria elimination was the most effective on the surfaces of rubber (for isolates derived from salmon filets and cheese) and steel (for isolates from frozen mixed vegetables), and the least effective on the surface of polypropylene (Fig. 5). In the group of strains forming biofilm on abiotic surfaces, the highest effectiveness of *L. monocytogenes* inactivation was achieved on the surfaces of steel (45·95–71·54%), and then tile (17·88–62·10%), whereas the lowest was achieved on the surface of polypropylene (5·27–43·87%) (Fig. 5).

Discussion

Effectiveness of the process of photocatalytic heterogeneous oxidation using titanium dioxide in inactivation of microbial contaminants was documented for the first time by Matsunaga *et al.* (1985). Grinshpun *et al.* (2007) published results of experiments evaluating the effectiveness of portable devices generating the phenomenon of radial catalytic ionization used in the active air purification technology. Also, our previous study (Skowron *et al.* 2018) has shown the effectiveness of RCI on airborne micro-organisms. The full elimination of micro-organisms from the air was obtained in the case of *Escherichia coli* and *Candida albicans*. Unfortunately, the mechanism by which RCI induces microbial death and the genomic or proteomic effects is poorly understood. Little is known about initial oxidative attack on the cell membrane and actives of enzyme depended on coenzyme A and damage DNA triggered by hydroxyl radicals (Sunada *et al.* 1998; Maness *et al.* 1999; Vohra *et al.* 2005; Gogniat and Dukan 2007). Kubacka *et al.* (2014) indicated that photocatalytic action causes decreased expression of gene-encoding proteins responsible for regulation, signalization and growth of *Pseudomonas aeruginosa* cells. After exposure to TiO_2 photocatalysis, bacterial cells try to improve the mechanisms of DNA protection and repair, promote the coenzyme-dependent respiratory chain and β-oxidation of fatty acid. Bacterial cells mobilize the carbon resources. Moreover, cells react to TiO_2 photocatalysis by improving membrane integrity to compensate for the strong alterations caused in the cell wall. Additionally, the activation of metabolic pathways involving production and utilization of antioxidant coenzymes with the corresponding attenuation of those producing oxidative subproducts, such as H_2O_2 are observed (Kubacka *et al.* 2014).

The results of the studies have shown that the RCI generated by the Induct 750 portable device induces the inactivation of *L. monocytogenes* on the surfaces of food and abiotic materials of the order of 4·62–99·95%, depending on the origin of the tested strain, properties of the contaminated surface and the form of contamination. Ortega *et al.* (2007) recorded more than 90% reduction in *L. monocytogenes* applied in the form of planktonic cells on the fragments of the surface of stainless steel, after 2-h exposure to the effect of a device emitting RCI in a closed chamber with controlled air flow. Our previous study (Skowron *et al.* 2018) confirmed the effectiveness of RCI towards many microbial species, including *L. monocytogenes*, applied in planktonic form on abiotic surfaces. The effectiveness of photo-oxidation towards *L. monocytogenes* on the steel surface was also shown by Saini *et al.* (2014) (4·37 log CFU/coupon on stainless steel after 15-min treatment).

To the best of our knowledge, there are no data about the efficacy of RCI towards bacterial cells in biofilm. The results obtained in the experiment indicate a significantly higher resistance to the eliminating action of RCI on the cells of *L. monocytogenes* grouped in the form of biofilm on the tested materials, as compared with bacterial cells applied in the planktonic form.

The relationship observed in the present study correlates with the results obtained in a study comparing the resistance of *L. monocytogenes* in the form of biofilm and plankton to chemical disinfectants. Robbins *et al.* (2005) demonstrated the bactericidal efficacy of ozone, chlorine and hydrogen peroxide in relation to *L. monocytogenes,* both forming biofilm on the surface of stainless steel and applied in the form of planktonic cells, but the bacteria-forming biofilm showed a considerably higher resistance

to each of the applied agents. Increased resistance of *L. monocytogenes* forming the structure of biofilm on the tested materials in relation to the bactericidal effect of RCI most probably results from the presence of extracellular polymeric substance on the surface of biofilm that physically limits the diffusion of generated ions into the system, protecting the bacterial cells from inactivation.

The results obtained in the experiment additionally indicate the relationship between the degree of inactivation of *L. monocytogenes* under the effect of RCI and the properties of the abiotic materials used. The effectiveness of inactivation of *L. monocytogenes* from abiotic materials was the highest on the surface of steel, rubber and tiles, and the lowest on the surface of polypropylene. Similar results were obtained by Krysinski *et al.* (1992) who studied the effect of different disinfectants and cleaning agents on the biofilm of *L. monocytogenes* formed on surfaces used in food processing plants. Moreover, Krysinski *et al.* (1992) ruled out morphological differences between the materials used, such as the presence of micro-cracks, which could provide an additional shelter for micro-organisms from the harmful effects of disinfectants, indicating the presence of other factors determining the observed relationship.

The degree of inactivation of *L. monocytogenes* under the effect of RCI on the food surface observed in the study was significantly lower in comparison with the bacteria reduction level on the surfaces of abiotic materials used in the experiment. The differences are most probably due to the completely different properties of food products, which allow the penetration of bacteria suspended in aqueous solution into the raw material, and thus the protection against bactericidal ions generated during radial catalytic ionization. Saini *et al.* (2014) showed the effectiveness of photo-oxidation towards *L. monocytogenes* applied on food fragments. They showed reductions in *L. monocytogenes*: 1·39 and 1·63 log CFU/sample after 120 s and 2·16 and 2·52 log CFU/sample after 5 min were seen on American cheese and ready-to-eat turkey respectively.

In the present study, we observed differences in the degree of inactivation of *L. monocytogenes* depending on the origin of the tested strains. The effectiveness of inactivation of *Listeria* sp. was the highest for strains isolated from frozen mixed vegetables, situated in the environment of carrot pulp, and the lowest for strains derived from salmon filets, situated in the environment of fish pulp. The observed differences appear to be the result of different physical properties of each of the dispersive environments used. Carrot pulp was characterized by the lowest density and degree of raw material fragmentation; fish pulp—on the contrary—presented the highest density and concentration of fragmented product particles. Cheese pulp showed average properties. The increased density of the loading suspension and the high concentration of the raw material particles seem to protect the cells of *L. monocytogenes*, suspended in it. Probably these factors prevent the penetration of ions emitted during RCI and, consequently, limit the elimination of microbial contamination.

Summarizing the results of this study, it can be stated, that the RCI phenomenon induces the inactivation of *L. monocytogenes* on surfaces of food and materials used in the processing industry to a varying degree, depending on the manner of surface contamination, the properties of the contaminated materials as well as on the origin of the strain and the properties of surrounding dispersive environment in which the micro-organisms were suspended. Resistance of biofilm-forming *L. monocytogenes* to the action of RCI on the process surfaces used in the study corresponds to the results of the analysis of resistance of the tested micro-organism to other disinfection methods. The effectiveness of RCI in the inactivation of *L. monocytogenes* derived from food is largely determined by the physical properties of organic dispersing environment, whose increased density and concentration limit the bactericidal action of the method.

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Conflict of Interest

No conflict of interest declared.

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