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Reduction of *Listeria monocytogenes* on Various Food Contact Surfaces by *Listeria* Phage Suspension or Electrolyzed Oxidizing Water

Reduktion von Listeria monocytogenes auf verschiedenen Lebensmittel-Kontaktflächen mit Listeria Phagensuspension oder elektrolysiertem oxidierenden Wasser

Evren Burcu Şen Yılmaz[1]), S. Benjakul[2]), Şükran Çaklı[1]), Kitiva Vongkamjan[2])

Summary

Listeria monocytogenes often contaminates food processing areas, including food contact surfaces (FCS), thus leading to occurrence of re- and cross-contamination of finished products. Effective sanitizing agents are needed to reduce *Listeria* contamination. The phage suspension (broad host-range phage, LP-124) and electrolyzed oxidizing water (EOW, pH 2.5 and oxidation-reduction potential, ORP 1080 mV) were evaluated against *L. monocytogenes* on three food contact surfaces (polyethylene – PE cutting board (n = 3), stainless steel – SS spoon (n = 3) and SS sieve (n = 3), and cotton towel (n = 3) – directly used on ready-to-eat squid sushi). The initial *L. monocytogenes* concentration of 4.3 to 5.0 $\log_{10}$CFU/100 cm^2 was reduced by 1.2 to 2.4 log units and 2.7 to 3.7 log units after treatments with the phage suspension and EOW, respectively. Counts below the detection limit (< 2 $\log_{10}$CFU/100 cm^2) were observed on all surfaces treated with EOW for 5 min contact time, suggesting a high efficacy of EOW against *L. monocytogenes*. The highest CFU reduction was on SS spoon (2.4 log units) treated with *Listeria* phage and on PE cutting board (3.7 log units) treated with EOW. Both treatments are of interest for applications to inactivate *L. monocytogenes* on different surfaces in the food processing facilities as well as retail operations.

Keywords: *Listeria monocytogenes,* Listeria phage, EO Water, food contact surface, food processing facility

Zusammenfassung

Listeria monocytogenes kann beim Kontakt mit Prozessflächen (inklusive der Lebensmittel-Kontaktflächen) zur Kontamination derselbigen führen und damit zu Re- oder Kreuz-Kontamination des Fertigprodukts. Effektive Desinfektionsmittel sind notwendig um die Kontamination von Prozessflächen mit *Listeria* zu reduzieren. Bakteriophagenhemmer (broad host-range phage, LP-124) und elektrolysiertes oxidierendes Wasser (EO Wasser, pH 2.5 und ORP 1080 mV) wurden getestet auf Wirksamkeit gegen *L. monocytogenes* auf verschiedenen Lebensmittel-Kontaktstellen (Polyethylene – PE Schneidebrett, Löffel aus rostfreiem Stahl, Sieb aus rostfreiem Stahl, Baumwolltuch), die direkt mit Fertigsushi in Berührung kamen. Die Anfangskonzentration von *L. monocytogenes* von 4.3–5.0 $\log_{10}$ KbE/100 cm^2 wurden nach der Behandlung mit Bakteriophagen-Suspension auf 1,2–2,4 $\log_{10}$ Einheiten und EO Wasser auf 2,7–3,7 $\log_{10}$ Einheiten reduziert. Auf allen Oberflächen, die 5 Minuten mit EO Wasser behandelt wurden, war die Anzahl unter der Nachweisgrenze von < 2 $\log_{10}$ KbE/100 cm^2. Damit scheint EO Wasser effektiv gegen *L. monocytogenes* zu sein. Die höchste Reduktion fand beim Löffel aus rostfreien Stahl (2.4 $\log_{10}$ Einheiten) statt, der mit *Listeria* Phagensuspension behandelt wurde sowie beim mit EO Wasser behandeltem Schneidebrett aus PE (3.7 $\log_{10}$ Einheiten). Damit können beide Behandlungen zur Hemmung von *L. monocytogenes* auf verschiedenen Lebensmittel-Prozessoberflächen sowohl in Herstellungsanlagen als auch in Einzelbetrieben berücksichtigt werden.

Schlüsselwörter: *Listeria monocytogenes*, *Listeria* Phagen, EO Wasser, Kontakt mit der Prozessfläche, Lebensmittelverarbeitungsbetriebe

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Introduction

Listeria monocytogenes is an important foodborne pathogen that causes serious public health issues worldwide. *L. monocytogenes* has been linked to several listeriosis outbreaks due to the consumption of food contaminated with this pathogen (CDC, 2014). Food processing environments have been shown as potential sources of *L. monocytogenes* (Tompkin, 2002). Contamination of food products often occurs through post-processing contamination from environmental *Listeria* (Tompkin, 2002). Previous studies have shown that *L. monocytogenes* could be isolated from food contact surfaces (FCS) of seafood processing facilities, e. g., slicers, utensils, knives, sieves, conveyors, towels, employee's gloves (Vongkamjan et al., 2013; Vongkamjan et al., 2015). FCS could be linked with cross-contamination of *L. monocytogenes* in seafood, including ready-to-eat food products (Vongkamjan et al., 2013). In addition, cross-contamination can occur frequently in the environmental areas and surfaces of retail operations, in which *L. monocytogenes* was detected in 60 % of the retail operations in a survey in New York (Hoelzer et al., 2011).

Improved control measures, including cleaning procedures for *L. monocytogenes* are thus essential to reduce bacterial contamination in finished products. Appropriate and effective cleaning and sanitizing agents in the food industry are one of the key-approaches, especially on FCS. Cleaning is a prerequisite for effective sanitization. Cleaning is the removal of organic matter, using appropriate detergent chemicals under recommended conditions. Sanitization follows cleaning. Sanitization is the application of heat or chemicals to a properly cleaned (and thoroughly rinsed) food-contact surface, yielding a 99.999 % reduction of representative pathogenic microorganisms of public health importance. Sanitizing agents that require sufficient anti-microbial effect to kill microorganisms present in the available time, while leaving no residue which could harm the products, equipments, and operators are desired.

Bacteriophages (phages) are viruses that can specifically infect and kill their own bacterial host. Phages have been evaluated in various applications for controlling bacterial pathogens, including *L. monocytogenes* in foods (Guenther et al., 2009; Guenther and Loessner, 2011; Oliveira et al., 2014). *Listeria* phages have also been approved for use as antimicrobial food additives (FDA, 2007). Phage treatments have been proposed for use in many types of foods and food processing environments [reviewed in Endersen et al. (2014) and Sulakvelidze (2013)]. Phage has its natural (non-chemical) properties and the ability to reduce bacterial cells. During phage infection, phage proteins, holins and endolysins are produced within the bacteria, leading to lysis from within or cell lysis of bacteria. Lysis of bacterial cells is normally associated with lytic phages (Young, 1992). Certain phage can thus be considered for use as effective agent to inactivate pathogens on equipment surfaces. For example, a *Listeria* phage preparation (ListShield™) has been previously approved by the Environmental Protection Agency (EPA) for surface decontamination applications (EPA registration number 74234-1). Electrolyzed oxidizing water (EOW) has been studied for its antimicrobial activity against several foodborne pathogens on different surfaces typically involved in the food processing areas such as floor tile and ceramic tile, and processing gloves (Liu and Su, 2006; Phuvasate and Su, 2010). The high ORP of EOW could lead to the modification of metabolic fluxes and ATP production. HOCl generated from the anode side has been hypothesized to provide active chlorine compounds that could kill bacterial cells through interruption of various pathways involved in, for example, carbohydrate metabolism, protein synthesis, induction of DNA lesions [for review see Huang et al., 2008)]. A previous study reported that EOW could effectively reduce *L. monocytogenes* counts on food processing gloves. Soaking inoculated gloves in EOW at room temperature for 5 min could completely eliminate *L. monocytogenes* by greater than 4.46 $\log_{10}$CFU/cm^2 reductions (Liu and Su, 2006). In the current study, two liquid-based agents, *Listeria* phage suspension and EOW were evaluated for the effectiveness against *L. monocytogenes* adhered, in the presence of food residual, on various FCS. Surfaces included in the current study were polyethylene – PE plastic cutting board, stainless steel – SS spoon and SS sieve, and cotton towel – directly used on ready-to-eat squid sushi, in which *L. monocytogenes* has been commonly found. Outcomes from this study can provide opportunity for control of *L. monocytogenes* on FCS and equipment of food processing facilities and retail operations for both organic and inorganic food production.

Materials and methods

Bacterial strains and preparation of inocula

Two isolates of *L. monocytogenes*, PSU-KV-033LM (LM33) previously isolated from a seafood processing plant environment (Vongkamjan et al., 2015) and PSU-KV-120LM (LM120) from ready-to-eat food (Vongkamjan et al., 2016) were used in this study. Overnight culture was prepared by inoculating an isolated colony in 5 ml of Brain Heart Infusion (BHI, Oxoid, Hampshire, UK), followed by shaking (220 rpm) incubation at 37±1 °C for 18±2 h. Bacterial cells were harvested by centrifugation at 5,500 × g (Eppendorf, 5430R, Hamburg, Germany) at 4 °C for 10 min and washed three times with Phosphate Buffered Saline (PBS; Merck, Darmstadt, Germany), pH 7.4. The Pellet was resuspended in PBS. For artificial contamination of *L. monocytogenes* on the surfaces, a mixture of both *L. monocytogenes* isolates was prepared by mixing each isolate suspension at 1:1 (v/v) to obtain approximately 5±0.5 $\log_{10}$ CFU/ml. The concentration of each isolate and a mixture was confirmed by plating serial decimal suspension dilutions on BHI agar in duplicates, followed by incubation at 37±1 °C for 24±2 h.

Preparation of *Listeria* phage suspension

Listeria phage LP-124, previously characterized as a broad host range phage against multiple *L. monocytogenes* strains and showed high similarity to a commercial *Listeria* phage P100 (Denes et al., 2014; Vongkamjan et al., 2012), was used in this study. Lysate of phage LP-124 was prepared on *L. monocytogenes* host FSL J1-208 according to Vongkamjan et al. (2012). The overlay was harvested using 10 ml of PBS, followed by centrifugation at 5,500 × g for 15 min at 4 °C. The supernatant was filtered through a 0.2-µm syringe filter. Phage titers were determined by a spot test on the host lawn according to Vongkamjan et al. (2012). Phage lysate stock was stored at 4 °C. The phage suspension was prepared and diluted with PBS to obtain approximately 8±0.5 $\log_{10}$ plaque-forming units (PFU) in a final volume of 200 ml.

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Preparation of electrolyzed oxidizing water (EOW)

EOW was prepared with 0.2 % NaCl and sterile distilled water using a continuous EOW generator (LABO SCI, Hario Science CO., LTD, Tokyo, Japan) for 15 min, following the manufacturer's instructions. The acidic EOW was collected and measured for pH, free chlorine concentration, and ORP. The acidic EOW used in this study showed a pH of 2.5 with 40 ppm of chlorine concentration, and the ORP of 1080 mV.

Monitoring of *L. monocytogenes* cell suspension in-vitro after treatments with EOW

Cell suspension of each *L. monocytogenes* isolate (LM33 and LM 120) was prepared as described above and diluted in PBS to obtain a final concentration of approximately 5±0.5 $\log_{10}$CFU/ml. For each isolate, 10 ml of the cell suspension was mixed with 1 ml of EOW which was freshly prepared as described in section 2.3. Only *L. monocytogenes* cell suspension in PBS was included as a control for the study. The mixture was incubated at room temperature (23±2 °C) with shaking at 220 rpm. An aliquot of samples (1 ml) was taken from each treatment and control at 0, 4, 8, 12, and 24 h after treatment initiation. To neutralize available chlorine, 3 % $Na_2S_2O_3$ was added to each taken sample [1 % (v/v) final concentration]. The mixture was centrifuged at 5,500 × g at 4 °C for 10 min. The Pellet was resuspended in PBS, followed by plating of 100 µl of appropriate serial decimal dilutions onto BHI agar in duplicates. Plates were incubated at 37±1 °C for 24±2 h.

Preparation of food debris suspension and inoculation of food contact surfaces

To imitate an inadequate cleaning where some food residuals are left on the FCS, sterilized ground seafood was placed on each surface before treatment initiation. Seafood was blended in a food processor (Philips, HR7625/70, Japan), followed by sterilization at 121±2 °C for 15 min using the autoclave (S5-325, Tomy digital biology Co., Ltd., Japan). Sterilized seafood mixture was used to prepare a 10% (w/v) suspension of food debris in PBS. Food debris suspension (1 ml) was evenly placed onto each surface. Three types of surfaces made of three different materials commonly used in the seafood processing plant and previously linked to the presence of *L. monocytogenes* (Vongkamjan et al., 2015) were used in this study: (i) plastic cutting board (25 cm^2, polyethylene, PE), (ii) spoon (22.4 cm^2, stainless steel, SS), (iii) sieve (28 cm^2, stainless steel, SS), and (iv) towel used for absorbing moisture from foods (2-mm thick, 25 cm^2, 100 % cotton). Each surface material was sterilized as mentioned above. A mixture of *L. monocytogenes* culture (100 µl) prepared as described above was transferred onto each surface to obtain a final concentration of 5.6 $\log_{10}$CFU/100 cm^2. Each inoculated surface was allowed to air dry in a biological safety cabinet at room temperature (23 ± 2 °C) for 30 min. For each inoculated surface, the number of *L. monocytogenes* counts was confirmed by plating appropriate serial decimal dilutions onto BHI agar in duplicates.

Treatments of food contact surfaces with *Listeria* phage suspension and EOW

Each inoculated surface was separately submerged into 200 ml of *Listeria* phage suspension (final concentration of 8±0.5 $\log_{10}$PFU), acidic EOW, or sterile distilled water. Each treatment was subjected to 5 min contact time at room temperature (23 ± 2 °C). Treatment with *Listeria* phage on surfaces was achieved with the final concentration of 8.6 $\log_{10}$PFU/100 cm^2, representing the multiplicity of infection (MOI) of 1000, as commonly used in previous study (Vongkamjan et al., 2013). The experiments were conducted in three independent studies. After 5 min, a sterile cotton stick was swabbed on the entire surface from each treatment. Each cotton stick was transferred into a tube containing 5 ml of PBS, vortexted and incubated at 23±2 °C for 1 h to allow some cells to release from the swab to PBS. An aliquot (100 µl) of the PBS suspension was plated directly and after serial dilution onto BHI agar in duplicates. Plates were incubated at 37±1 °C for 24±2 h. *L. monocytogenes* counts were determined manually and transformed into $\log_{10}$CFU/100 cm^2. Reduction of *L. monocytogenes* ($\log_{10}$CFU) on each surface was calculated based on the initial adhesion level (no treatment) and after each treatment.

Monitoring of *L. monocytogenes* counts and *Listeria* phage titers in the used liquid after treatments

To determine the recovery ability of *L. monocytogenes* in each used liquid-based agent as the used liquid has to be eventually drained, growth of *L. monocytogenes* was monitored after 3, 5, and 7 days of storage. An aliquot sample (100 µl) was taken, followed by serial decimal dilution and appropriate dilutions (100 µl) were plated on BHI agar in duplicates.

For the used phage suspension, titer of *Listeria* phage was monitored to determine the phage stability. An aliquot sample (1 ml) was taken, followed by centrifugation at 5,500 x g for 15 min at 4 °C. Supernatant was filtered using a 0.2-µm syringe filter. Ten 10-fold serial decimal dilutions were performed with the filtrate in PBS. Each dilution (5 µl) was spotted onto the *Listeria* host lawn (FSL J1-208) prepared as described in Vongkamjan et al. (2012).

Statistical analysis

L. monocytogenes counts from each treatment obtained from three independent studies were combined and analyzed. Data were subjected to Analysis of Variance (ANOVA) using the statistical software SPSS for Windows, Version 16.0. (SPSS Inc., Chicago). Duncan's Multiple Range Test was used to determine (i) the significant difference of *L. monocytogenes* counts between treatment means and (ii) the significant difference of *L. monocytogenes* counts recovered in various types of liquid after treatments. A significance value was defined as $P < 0.05$.

Results and discussion

In-vitro treatments of EOW against *L. monocytogenes* cell suspension

In the in-vitro challenge studies, two *L. monocytogenes* isolates recovered from food (LM120) and food processing plant environment (LM33) were included to investigate the efficacy of EOW. With the initial cell suspension level of 5.2 and 5.4 $\log_{10}$CFU/ml, EOW showed initial reduction of 1.2 and 1.4 log units for LM33 and LM120, respectively, as compared to the control (no-EOW) at room temperature (23±2 °C) (Tab. 1). After 4 h of treatment initiation, *L. monocytogenes* counts of both isolates were reduced below the detection limit (< 1 $\log_{10}$CFU/ml). Counts continued to be lower than the detection limit from 4 h to 24 h

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TABLE 1: *Reduction of L. monocytogenes cell suspension after treatment with electrolyzed oxidizing water (EOW).*

L. monocytogenes isolate	Treatment	Counts[a] (log_{10}CFU/ml) after treatment for: 0 h	4 h	8 h	12 h	24 h
PSU-KV033LM (LM33)	Control	5.2	5.9	5.7	6.3	6.4
	EOW	4.0	<DL	<DL	<DL	<DL
PSU-KV120LM (LM120)	Control	5.4	5.0	4.7	5.8	6.7
	EOW	4.0	<DL	<DL	<DL	<DL

[a]: Counts of *L. monocytogenes* (mean counts, n = 3) are expressed as log_{10}CFU/ml. Detection limit (DL) represents <1 log_{10}CFU/ml.

after treatment initiation. *L. monocytogenes* counts of the control (no-EOW) increased from 5.2 to 6.4 log_{10}CFU/ml in LM33 and 5.4 to 6.7 log_{10}CFU/ml in LM120 during 24 h of incubation at room temperature. In another in-vitro challenge study against LM33 and LM120, a reduction of *L. monocytogenes* counts (initial cell suspension of 5±0.5 log_{10}CFU/ml) below the detection limit was observed after treatment with 40 ppm chlorine concentration acidic EOW (pH 2.5) at the exposure time of 60 s at room temperature (results not shown). Similar results were reported recently: EOW with up to 50 ppm chlorine concentration resulted in a reduction of *L. monocytogenes* counts by 5.5 log units at the exposure time of 30 s and 60 s at room temperature (Rahman et al., 2012). In addition, in our study, the contact time for EOW treatment was 5 min which could be more relevant to practical purposes during food processing operation, considering less contact time is preferred while maintaining high efficacy against foodborne pathogens.

Reduction of *L. monocytogenes* on various food contact surfaces by *Listeria* phage and EOW

This study evaluated *Listeria* phage and EOW as potential agents for the reduction of *L. monocytogenes* on various surfaces. Four types of FCS (PE cutting board, SS spoon, SS sieve, and cotton) were treated with *Listeria* phage at the MOI of 1000, EOW and sterile distilled water (control). The equipments tested in this study, including cotton towels are commonly used in the food processing facilities as well as retail operations. We have previously found that *L. monocytogenes* could be frequently isolated from these FCS from the seafood processing facilities, as well as in other equipments such as slicers, utensils, knives, conveyors, towels, and employee's gloves (Vongkamjan et al., 2013; Vongkamjan et al., 2015). In addition, cotton towel was included as FCS because it is used as a surface material to absorb moisture directly from ready-to-eat sushi squid.

The recovery of *L. monocytogenes* cells on each surface before phage or EOW treatment was between 77.0 % and 88.8 %, representing the counts of 4.3 to 5.0 log_{10}CFU/100 cm^2 (Tab. 2). For all three types of surfaces, EOW treatment showed greater reduction of *L. monocytogenes* cells than treatment using *Listeria* phage. Counts below the detection limit (<2 1 log_{10}CFU/100 cm^2) were observed on all surfaces treated with EOW for 5 min contact time. However, in phage treatment, counts below the detection limit were observed only on SS spoon. For phage application (8.6 log_{10}PFU/100 cm^2), reduction of *L. monocytogenes* cells represented by 1.2 log units on cotton towel to 2.4 log units on SS spoon. The significant reduction of *L. monocytogenes* cells was observed on PE cutting board (1.6 log units) and SS sieve (2.1 log units) treated with *Listeria* phage ($P < 0.05$). Similar to our study, a previous study reported that treatment of *Listeria* phage cocktail (6.5 log_{10}PFU/cm^2) on stainless steel with and without a fish broth layer could decrease *L. monocytogenes* to 0.78 and 0.68 log_{10}CFU/cm^2 after 5 and 10 min, respectively (Arachchi et al., 2013). Complete decontamination of *L. monocytogenes* cells was reported after 15 min of treatment with a three-phage cocktail (LiMN4L, LiMN4p and LiMN17) at > 5.8 log_{10}PFU/cm^2 (Arachchi et al., 2013). Furthermore, Roy et al. (1993) reported that *L. monocytogenes*, with initial counts of approximately 4 to 5 log_{10}CFU/ml adhered on stainless steel and polypropylene, was affected by individual phages and phage cocktail (H387, H387-A and 2671) tested. Counts of *L. monocytogenes* were reduced by 99 to 99.9 % (Roy et al., 1993).

Overall, surfaces of both PE and SS were affected by *Listeria* phage suspension tested in our study. Phage numbers were sufficient to facilitate the infection of *L. monocytogenes* cells attached on the surfaces, while rapid lysis of *L. monocytogenes* cells could be caused by high adsorption capabilities of phages (Abedon, 2011; Gallet et al., 2012). To facilitate an appropriate selection of specific phages with potential applications in pre- or post-harvest as phage-biosanitation treatments, a decision tree suggested by Mahony et al. (2011) can be followed. In addition, the contact time we applied here could affect the number of cell reduction by phages since some phages may take longer than 5 min for adsorption until latent period and cell lysis.

Among the three types of surfaces tested, cotton towel was least affected by *Listeria* phage treatment as compared to other surfaces treated with the phage suspension. *L. monocytogenes* counts remained high as 3.6 log_{10}CFU/100 cm^2 after *Listeria* phage treatment on cotton towel as compared to other surfaces ($P < 0.05$). The control (distilled water treatment) showed *L. monocytogenes* cells on the cotton towel remained high (4.4 log_{10}CFU/100 cm^2). The control did not influence *L. monocytogenes* cell concentration on any surfaces as indicated by high remaining cell counts, 2.9 log_{10}CFU/100 cm^2 on PE cutting board and 3.1 log_{10}CFU/100 cm^2 on SS spoon and SS sieve. Specifically, in our study, this kind of towel is used for absorbing moisture directly on food. While many studies have emphasized cleaning of stainless steel and plastic materials to prevent *L. monocytogenes*, cotton towels have been shown

TABLE 2: *Reduction of L. monocytogenes after treatments with phages and EOW onto three food contact surfaces.*

Food contact surface (material)	Initial inoculum on surfaces (log_{10}CFU/100 cm^2) (% recovery on surfaces)	Mean counts (log_{10}CFU/100 cm^2) on surfaces[a]	Counts[b] (log_{10}CFU/100 cm^2) after various treatments: Listeria phage	Electrolyzed Oxidizing water (EOW)	Sterile distilled water
Plastic cutting board (polyethylene, PE)	5.6 (83.4)	4.7 ± 0.6^{Aa}	3.1 ± 0.1^{Aab}	1.0 ± 0.7^{Ab}	2.9 ± 0.6^{Aab}
Spoon (stainless steel)	5.6 (77.0)	4.3 ± 0.2^{Aa}	1.9 ± 0.7^{Aa}	1.6 ± 0.4^{Aa}	3.1 ± 0.1^{Aa}
Sieve (stainless steel with holes)	5.6 (88.8)	5.0 ± 0.2^{Aa}	2.9 ± 0.1^{Aab}	1.6 ± 0.4^{Ab}	3.1 ± 0.4^{Ab}
Squid-absorbed Towel (100 % cotton)	5.6 (85.9)	4.8 ± 0.8^{Aa}	3.6 ± 0.4^{Ba}	1.9 ± 0.7^{Aa}	4.4 ± 0.3^{Aa}

[a]: Counts of *L. monocytogenes* mixture that adhered on surfaces before treatment. [b]: Counts of *L. monocytogenes* are expressed as log_{10}CFU/100 cm^2 (mean of *L. monocytogenes* mixture ± standard deviation, n = 3). Detection limit (DL) represents <2 log_{10}CFU/100 cm^2. Means within the same column with the same uppercase letter and means within the same row with the same lowercase letter are not significantly different at a significance level of 0.05 ($P > 0.05$).

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to be difficult to eliminate this pathogen. *L. monocytogenes* has been previously isolated from "during-operation-" and "post-cleaning-" cotton towels from seafood processing facilities (data not shown). Our study has shown that *L. monocytogenes* was difficult to be removed from cotton towels with some food residue by the phage suspension. *L. monocytogenes* could still adhered on the cotton matrix after submerging in the phage suspension for 5 min. Phages might not be able to penetrate well into the cotton matrix with the presence of food residue. This study suggests that phage suspension might not be suitable for inactivating *L. monocytogenes* on cotton material, while EOW could reduce *L. monocytogenes* by 2.9 log units on this material. However, increasing contact time, concentration, or treatment approach (combined with hurdle technologies) may yield an improved outcome. Additional sanitizers may be used following the treatment of phage or EOW, if needed.

EOW generated through electrolysis of dilute salt conditions (0.05 % to 0.2 % NaCl) has been introduced as a potential sanitizer with the ability to control various spoilage and foodborne pathogens, including *L. monocytogenes* on different foods and contact surfaces (Phuvasate and Su, 2010; Rahman et al., 2010). In our study, acidic EOW (pH 2.5, 40 ppm of chlorine concentration) treatment for 5 min contact time was effective on all three types of surfaces tested as counts below the detection limit (< 2 $\log_{10}$CFU/100 cm^2) were observed. *L. monocytogenes* cell counts were reduced by 2.7 log units on SS spoon to 3.7 log units on PE cutting board. The significant reduction of *L. monocytogenes* cells was observed on SS sieve (3.4 log units) and PE cutting board (3.7 log units) treated with EOW ($P < 0.05$). Overall, as compared to the controls, counts of *L. monocytogenes* after EOW treatment on PE cutting board and phage treatment on SS sieve were significantly different from the control ($P < 0.05$). Similar results were reported by McCarthy and Burkhardt (2012); EOW with 50 ppm chlorine concentration showed a reduction of multiple *L. monocytogenes* strains and one *Morganella morganii* strain attached to conveyor belt coupons by 1 to 2.5 log10CFU/cm^2 upon exposure to EOW for 5 min as compared to exposure to sterile distilled water.

Overall, our results revealed the effectiveness of EOW on the inactivation of (i) *L. monocytogenes* cell suspension in the in-vitro challenge studies and (ii) *L. monocytogenes* inoculated on various FCS. The mode of action for inactivation of bacterial cells of EOW has continuously been studied. Several previous studies have incorporated the effects from (i) the available chlorine concentration, (ii) the oxidation-reduction potential (ORP, > 1000 mV), and (iii) pH [for review see Huang et al., 2008]. As bacterial cells can typically grow at ORP range between 200 and 800 mV, some studies suggested the antimicrobial mechanisms of the EOW through its high ORP (Liao et al., 2007).

Recovery of *L. monocytogenes* cells and monitoring of phage titers in the used phage suspension and EOW after treatments with various food contact surfaces

After treatment of various food contact surfaces, used phage suspension and EOW were evaluated for the recovery of *L. monocytogenes* during storage at 4 °C for 7 days. Used phage suspension showed *L. monocytogenes* counts of 1.5, 2.2, and 2.9 $\log_{10}$CFU/ml on day 3, 5, and 7 of storage, respectively (Tab. 3). Titers of *Listeria* phages in the used phage suspension after treatment on various surfaces were monitored for 7 days of storage at 4 °C. Phage titers in the used phage suspension were 8.0, 8.4, and 7.9 $\log_{10}$PFU/ml on day 3, 5, and 7 of storage, respectively (Tab. 4). Phage titers remained at approximately 8 $\log_{10}$PFU/ml during storage, suggesting the phage stability in the presence of food soil and other food compositions. *L. monocytogenes* counts during storage were significantly different ($P < 0.05$) than counts observed in the control (used distilled water). Used distilled water could maintain *L. monocytogenes* cells from 4.6 to 5.9 $\log_{10}$CFU/ml during 7 days of storage. pH and ORP of the used EOW remained the same during storage. In the used EOW, *L. monocytogenes* counts were lower than the detection limit (< 1 $\log_{10}$CFU/ml) during 7 days of storage. The presence of recovered *L. monocytogenes* cells in the phage suspension might suggest a potential that some cells can still recovered from phage treatment as phages could not completely eliminate all cells from the surfaces. Also, these cells might be able to reside in the drains of the food processing plants if the used phage suspension is eventually poured into the drain. It is suggested to avoid reusing the used phage suspension and used phage suspension needs appropriate treatments to eliminate surviving cells before discarding. However, this incidence might not happen with EOW treatment since the cell recovery of less than the detection limit was observed.

TABLE 3: *Recovery of L. monocytogenes counts in different liquid-based agents after treatment during 7-day storage.*

Liquid-based agents	Counts[a] of *L. monocytogenes* ($\log_{10}$CFU/ml) in the used agent during storage at 4 °C		
	3 days	5 days	7 days
Listeria phage	1.5 ± 0.4^{Aa}	2.2 ± 0.4^{Ab}	2.9 ± 0.1^{Ac}
Electrolyzed oxidizing water	<DL	<DL	<DL
Distilled water	4.6 ± 0.2^{Ba}	5.0 ± 0.6^{Ba}	5.9 ± 0.1^{Bb}

[a]: Counts of *L. monocytogenes* are expressed as $\log_{10}$CFU/ml (mean of *L. monocytogenes* mixture ± standard deviation, n = 3). Detection limit (DL) represents <1 $\log_{10}$CFU/ml. Means within the same column with the same uppercase letter and means within the same row with the same lowercase letter are not significantly different at a significance level of 0.05 ($P > 0.05$). Counts below the detection limit were not included in the statistical analysis.

Conclusion

Contamination of *L. monocytogenes* in food processing facilities or retail operations continues to be a major issue for the food industry and consumers worldwide. *Listeria* phage suspension and electrolyzed oxidizing water are potential agents for controlling *L. monocytogenes* on food contact surfaces and equipments. The highest reduction was on SS spoon (2.4 log units) treated with *Listeria* phage and on PE cutting board (3.7 log units) treated with EOW. Both treatments are of interest for applications to inactivate *L. monocytogenes* on different food contact surfaces. In our study, high ORP up to 1080 mV was applied, sugge-

TABLE 4: *Monitoring of Listeria phage titers after treatments with food contact surfaces.*

Listeria phage after treatments	Titers[a] of *Listeria* phage LP-124 ($\log_{10}$PFU/ml) during storage at 4 °C		
	3 days	5 days	7 days
Listeria phage suspension after washing:			
– Plastic cutting board (polyethylene, PE)	8.0 ± 0.9	8.4 ± 0.4	7.9 ± 0.1
– Spoon (stainless steel)	8.0 ± 0.9	8.4 ± 0.4	7.9 ± 0.1
– Sieve (stainless steel)	8.0 ± 0.9	8.4 ± 0.4	7.9 ± 0.1
– Towel (100 % cotton)	8.0 ± 0.9	8.4 ± 0.4	7.9 ± 0.1

[a]: Titers of *Listeria* phage are expressed as $\log_{10}$PFU/ml (mean ± standard deviation, n = 3).

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sting suitability ORP for inactivation of *L. monocytogenes* on surfaces. Use of EOW at 5 min contact time is practical for the food industry as EOW can be easily generated before use. A contact time of 5 min is appropriate to reduce most cells on the surfaces of equipments.

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Conflict of interest

The authors declare that no conflict of interest exists.

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