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ORIGINAL PAPER

Inactivation of biofilm cells of foodborne pathogens by steam pasteurization

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Abstract The objective of this study was to evaluate the effect of steam pasteurization on the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* biofilms on stainless steel and polyvinyl chloride (PVC). Biofilms were formed on a stainless steel and PVC coupon by using a mixture of three strains each of three foodborne pathogens. Six-day-old biofilms on stainless steel and PVC coupons were treated with steam at 75 and 85 °C for 5, 10, 20, 30, 40, and 50 s. Biofilm cells of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on stainless steel were reduced by more than 6 log CFU/coupon after exposure to steam at 75 °C for 30, 40, and 30 s, respectively, and at 85 °C for 30, 20, and 20 s, respectively. Steam treatment resulted in less reduction in the levels of biofilm cells on PVC coupons. Biofilm cells of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were reduced by 1.78, 2.04, and 1.29 log CFU/coupon, respectively, after 50 s of exposure to steam at 75 °C. Exposure to steam at 85° for 50 s reduced biofilm cells of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* by 2.53, 3.01, and 1.70 log CFU/coupon, respectively. The results of this study suggest that steam pasteurization has potential as a biofilm control method by the food industry.

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Introduction

Escherichia coli O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* are the most relevant foodborne pathogens [1]. These foodborne pathogens have been known to form biofilms on food processing surfaces such as stainless steel, plastic, polycarbonate, glass, and polyvinyl chloride (PVC) [2–6]. Biofilms can be defined as communities of microorganisms attached to each other and embedded in an organic polymer matrix, adhering to a surface [7, 8]. In the food processing environment, several factors such as flowing water, suitable attachment surfaces, pH, and sufficient nutrients favor biofilm formation [9, 10]. The attachment of bacteria and subsequent development of biofilms on food processing surfaces may cause food spoilage or transmission of diseases [2]. Biofilm cells of microorganisms appear to be more resistant to heat and antimicrobial agents [7, 11–13].

Washing with various sanitizers including peracetic acid [14], chlorine [15], hydrogen peroxide [16], ethylenediaminetetraacetic acid (EDTA) [17], quaternary ammonium chloride [18], and ozone [19] has been tested as control measures for microbial biofilms. Also, various cleaning methods such as electrolyzed water [20], ultrasound [21], and bacteriophages [22] have been evaluated as ways to remove biofilms from food processing surfaces. However, these methods cannot be utilized for cleaning inaccessible areas. It is difficult to clean inaccessible environmental surfaces such as walls and ceilings, whereas product contact surfaces may be easily cleaned [9].

Steam can increase food surface temperatures rapidly. It can transfer large amounts of heat to foods [23]. Steam at 100 °C has a greater heat capacity than the same amount of water at the same temperature. Also, steam can penetrate cavities and crevices effectively [24]. Steam pasteurization for the decontamination of meat products is used extensively in many countries [25]. The existing data suggest that the application of steam effectively reduces the presence of a number of pathogens such as *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes*, and *Campylobacter* [26–28].

The main objective of this study was to evaluate the efficacy of steam pasteurization against biofilms of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* formed on stainless steel and PVC. Different exposure times and treatment temperatures were evaluated to guide appropriate steam pasteurization protocols for biofilms of foodborne pathogens.

Materials and methods

Bacterial cultures and cell suspension

Three isolates each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19586, ATCC 43174, DT 104), and *L. monocytogenes* (ATCC 7644, ATCC 19114, ATCC 19115) were obtained from the Food Hygiene Laboratory Bacterial Culture Collection at Seoul National University (Seoul, Korea) and used in this experiment. Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured in 10 ml of tryptic soy broth (TSB; Difco, Sparks, MD, USA) at 37 °C for 24 h, harvested by centrifugation at $4{,}000\times g$ for 20 min at 4 °C, and washed three times with buffered peptone water (BPW; Difco). The final pellets were resuspended in sterile phosphate-buffered saline (PBS; pH 7.4), corresponding to approximately 10^7–10^8 CFU ml^{-1}.

Preparation of stainless steel and PVC coupons and biofilm formation

Stainless steel (no. 4 grade) and PVC were fabricated into coupons (5 cm × 2 cm). Stainless steel and PVC coupons were immersed in 70 % ethanol for 20 min and rinsed with sterilized distilled water. After washing, coupons were dried in a laminar flow biosafety hood (22 ± 2 °C) for 3 h. The method used for biofilm formation was similar to that as described by Kim et al. [29]. Each prepared stainless steel and PVC coupon was immersed in a sterile 50-ml conical centrifuge tube (SPL Lifesciences, Pocheon, Korea) containing 30 ml of each cell suspension of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in PBS (ca. 10^7–10^8 CFU/ml). Conical centrifuge tubes were incubated at 4 °C for 24 h to facilitate attachment of cells. After incubation, coupons were removed from conical centrifuge tubes with a sterile forceps and washed in 500 ml of sterile distilled water for 10 s (22 ± 2 °C). Washed coupons were transferred to 50-ml conical centrifuge tubes containing 30 ml of TSB and incubated at 25 °C for 6 days.

Steam pasteurization

A steam pasteurization apparatus developed and constructed at Seoul National University (Seoul, Korea) and Young Jin Engineering (Daegu, Korea) was used in this study. Coupons were removed from TSB and washed in 500 ml of distilled water for 10 s (22 ± 2 °C), and coupons were steam pasteurized at 75 and 85 °C for 5, 10, 20, 30, 40, and 50 s. All experiments were performed at room temperature (22 ± 2 °C).

Bacterial enumeration

After steam treatment, stainless steel and PVC coupons were transferred to sterile 50-ml conical centrifuge tubes containing 30 ml of PBS and 3 g of glass beads (425–600 μm; Sigma-Aldrich, St. Louis, MO, USA), and then vortexed for 1 min. Cell suspension was tenfold serially diluted in 9 ml of BPW, and 0.1 ml of undiluted cell suspension or diluents was spread-plated onto the appropriate medium. Sorbitol MacConkey agar (SMAC; Difco), Xylose Lysine Desoxycholate agar (XLD; Difco), and Modified Oxford Medium (MOX; Difco) were used as selective media for the enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. The plates were incubated at 37 °C for 24–48 h. For the enumeration of injured *E. coli* O157:H7, phenol red agar base with 1 % sorbitol (SPRAB; Difco) was used. One hundred microliters of undiluted cell suspension or diluents was spread-plated and incubated at 37 °C for 24 h. Injured cells of *S.* Typhimurium and *L. monocytogenes* were enumerated using the overlay (OV) method proposed by Kang and Fung [30, 31]. One hundred microliters of undiluted cell suspension or diluents was spread-plated onto TSA and incubated at 37 °C for 2 h to allow injured cells to resuscitate before overlaying with 7 ml of XLD (OV–XLD) or MOX (OV-MOX) for *S.* Typhimurium and *L. monocytogenes*, respectively. The plates were incubated at 37 °C for 22 h after the overlay solidified. When low bacterial numbers were anticipated, 250 μl of undiluted cell suspension was plated onto four plates of each respective medium.

Statistical analysis

All experiments were repeated three times. Data were analyzed by ANOVA using Statistical Analysis System (SAS

Institute, Cary, NC, USA) and separation of means by Duncan's multiple range test at a probability level of $P < 0.05$.

Results

Inactivation of biofilm cells formed on stainless steel

The effects of steam treatment against the biofilm cells of three tested pathogens on stainless steel are shown in Figs. 1, 2, 3. The initial biofilm cells of *E. coli* O157:H7,

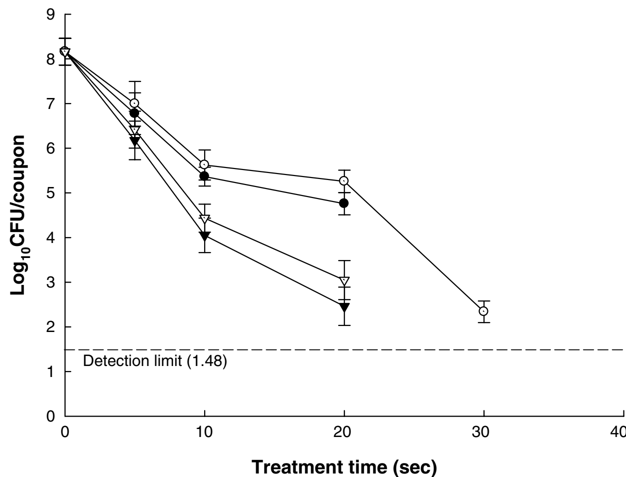

Fig. 1 Survival (log CFU/coupon) of biofilm cells of *Escherichia coli* O157:H7 formed on stainless steel coupons after steam pasteurization. *open circle*, SPRAB (75 °C); *filled circle*, SMAC (75 °C); *open inverted triangle*, SPRAB (85 °C); *filled inverted triangle*, SMAC (85 °C)

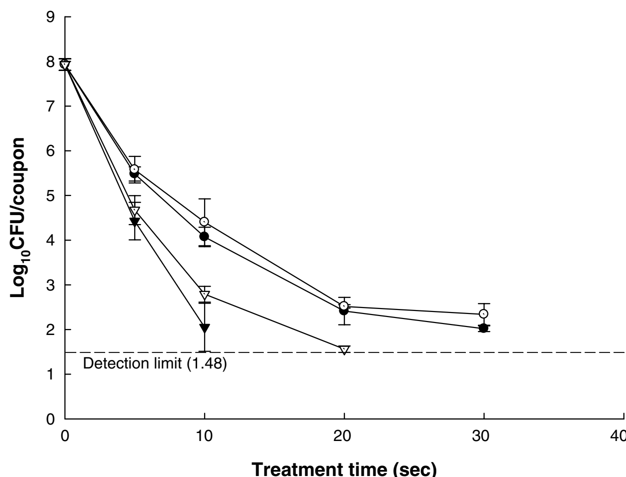

Fig. 2 Survival (log CFU/coupon) of biofilm cells of *Salmonella* Typhimurium formed on stainless steel coupons after steam pasteurization. *open circle*, OV-XLD (75 °C); *filled circle*, XLD (75 °C); *open inverted triangle*, OV-XLD (85 °C); *filled inverted triangle*, XLD (85 °C)

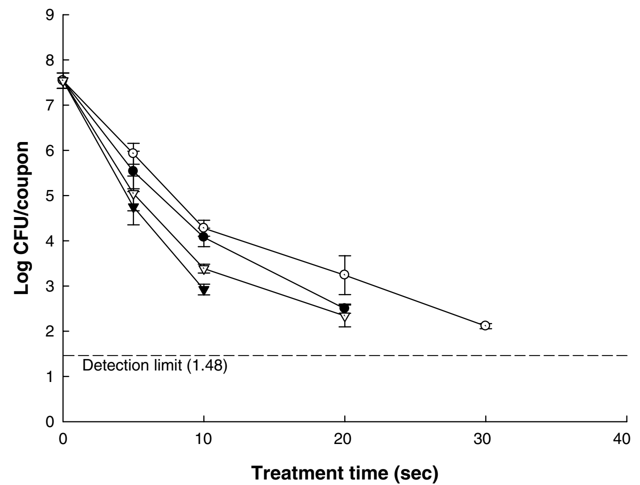

Fig. 3 Survival (log CFU/coupon) of biofilm cells of *Listeria monocytogenes* formed on stainless steel coupons after steam pasteurization. *open circle*, OV-MOX (75 °C); *filled circle*, MOX (75 °C); *open inverted triangle*, OV-MOX (85 °C); *filled inverted triangle*, MOX (85 °C)

S. Typhimurium, and *L. monocytogenes* were 8.16, 7.93, and 7.54 log CFU/coupon, respectively. Steam treatment effectively reduced biofilm cells, and high reductions were achieved as steam treatment time and temperature increased. The number of biofilm cells of *E. coli* O157:H7 and *L. monocytogenes* was reduced to below the detection limit (1.48 log CFU/coupon) within 30 s when treated with steam at 75 °C. Levels of biofilm cells of *S*. Typhimurium were reduced to below the detection limit (1.48 log CFU/coupon) after 40 s of steam treatment at 75 °C. Significant ($P < 0.05$) further reductions were observed with steam treatment at 85 °C. Treatment with steam at 85 °C for 20 s reduced the number of biofilm cells of *S*. Typhimurium and *L. monocytogenes* to below the detection limit (1.48 log CFU/coupon). Overall, the levels of injured cells enumerated on SPRAB, OV-XLD, and OV-OAB were not different from the levels of healthy cells enumerated on SMAC, XLD, and OAB. However, significant ($P < 0.05$) differences between the levels of injured cells and healthy cells were observed as steam treatment time increased.

Inactivation of biofilm cells formed on PVC

The initial biofilm cell counts of *E. coli* O157:H7, *S*. Typhimurium, and *L. monocytogenes* on PVC were 7.68, 7.30, and 7.33 log CFU/coupon, respectively (Figs. 4, 5, 6). Steam treatment led to less reduction in biofilm cells on PVC compared to those on stainless steel. Levels of biofilm cells of *E. coli* O157:H7, *S*. Typhimurium, and *L. monocytogenes* were reduced by steam treatment at 75 °C for 50 s, showing 2.47, 2.57, and 2.04 log CFU/coupon reductions, respectively. Significant ($P < 0.05$) further reductions were

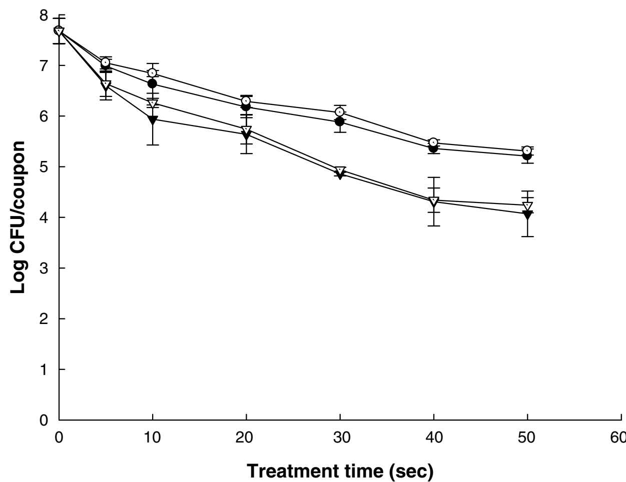

Fig. 4 Survival (log CFU/coupon) of biofilm cells of *Escherichia coli* O157:H7 formed on PVC coupons after steam pasteurization. *open circle*, SPRAB (75 °C); *filled circle*, SMAC (75 °C); *open inverted triangle*, SPRAB (85 °C); *filled inverted triangle*, SPRAB (85 °C)

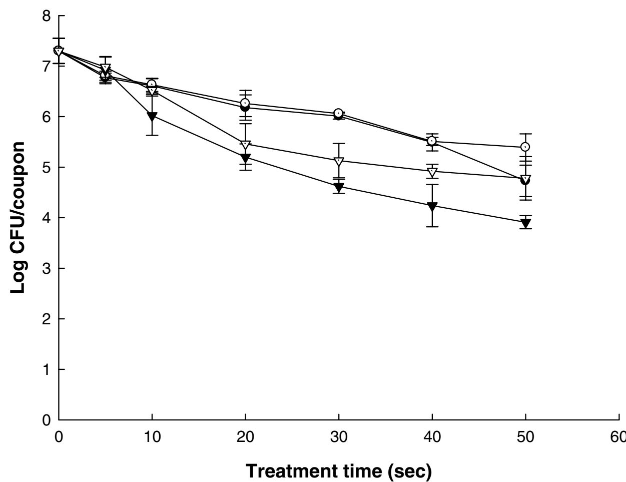

Fig. 5 Survival (log CFU/coupon) of biofilm cells of *Salmonella* Typhimurium formed on PVC coupons after steam pasteurization. *open circle*, OV-XLD (75 °C); *filled circle*, XLD (75 °C); *open inverted triangle*, OV-XLD (85 °C); *filled inverted triangle*, XLD (85 °C)

observed with steam treatment at 85 °C. Steam treatment at 85 °C for 50 s caused 3.61, 3.39, and 2.52 log CFU/coupon reductions, respectively. Similar to the results of stainless steel, significant ($P < 0.05$) differences between the levels of injured cells and healthy cells were observed as steam treatment time increased.

Discussion

Steam pasteurization for the decontamination of meat has been used extensively in the USA, Canada, Australia, and

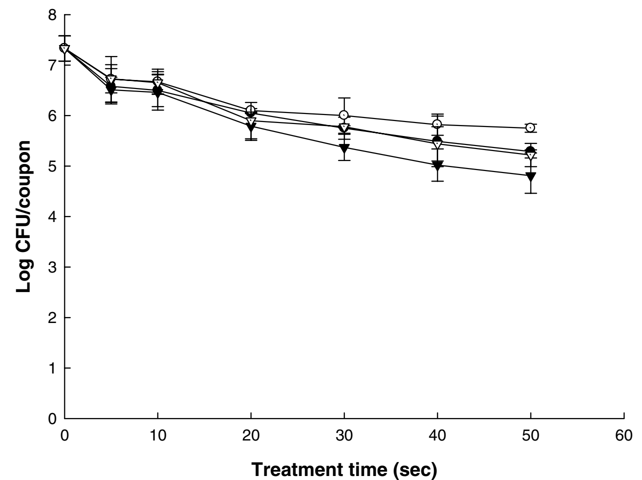

Fig. 6 Survival (log CFU/coupon) of biofilm cells of *Listeria monocytogenes* formed on PVC coupons after steam pasteurization. *open circle*, OV-MOX (75 °C); *filled circle*, MOX (75 °C); *open inverted triangle*, OV-MOX (85 °C); *filled inverted triangle*, MOX (85 °C)

the European Union (EU) [25]. The antimicrobial effect of steam on meat products has been evaluated, and the data suggest that steam pasteurization may be effective in controlling meat contaminated with foodborne pathogens [28]. Significant ($P < 0.05$) reductions in the counts of *Campylobacter* were observed on broiler carcasses exposed to steam at 90 °C for 12 s [27]. Murphy et al. [32] reported that about 3 log CFU/cm^2 reduction in *L. monocytogenes* was achieved when frankfurters were treated with steam at 114 °C for 1.5 s. Steam treatment at 99–101 °C for 15 s reduced *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* populations on surfaces of freshly slaughtered beef by 3.53, 3.74, and 3.44 log CFU/cm^2, respectively [26].

In the present study, steam pasteurization showed significant antimicrobial effect against biofilm cells of foodborne pathogens on stainless steel and PVC. Biofilm cells of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on stainless steel coupons were reduced to below the detection limit (1.48 log CFU/coupon) after steam treatment at 75 °C for 30–40 s, and at 85 °C for 20–30 s. Steam treatment caused less reduction in biofilm cells of three foodborne pathogens on PVC coupons. Levels of biofilm cells of three foodborne pathogens were reduced by 2.04–2.57 log CFU/coupon and 2.52–3.61 log CFU/coupon after 50 s of steam treatment at 75 and 85 °C, respectively. Differences in microbial reduction patterns between stainless steel and PVC may be due to different thermal conductivity. When stainless steel and PVC coupons were treated with steam at 75 °C, the temperature of stainless steel coupons reached 75 °C within 5 s, while PVC coupons reached this temperature within 15 s (data not shown). In this study, significant ($P < 0.05$) differences between the levels of injured cells and healthy cells were observed as steam treatment time increased. Because injured

cells of foodborne pathogens are potentially as dangerous as their uninjured counterparts [33, 34], sub-lethally injured foodborne pathogens could assume added significance following steam treatment. To completely inactivate biofilm cells of foodborne pathogens, a higher steam temperature than that evaluated in this study should be applied.

Other physical control methods have been evaluated to remove biofilms on food processing surfaces. Baumann et al. [21] reported that ultrasound treatment (20 kHz, 120 W) for 60 s at a distance of 2.54 cm from biofilm chips reduced levels of *L. monocytogenes* biofilms by 3.8 log CFU/ml. Nanostructured TiO_2 thin films on stainless steel and glass are an alternative means of disinfecting contaminated surfaces [35]. The biofilm cells of *L. monocytogenes* on glass were reduced by 3 log CFU/cm^2 when TiO_2 was activated by ultraviolet A light for 90 min. Gamma irradiation (3 kGy) decreased the biofilm cells of *E. coli* formed on polypropylene and polyester to below the detection limit ($<10^1$ CFU/ml) [36]. However, these methods cannot be applied to control biofilms in inaccessible areas in the food processing line, while steam pasteurization can be used for cleaning food processing surfaces.

Conclusions

Control of biofilms is an important issue for the food processing industry. This study showed that steam pasteurization was able to effectively inactivate biofilm cells of foodborne pathogens on stainless steel and PVC, which are common food processing surfaces. It may provide the food industry with a cleaning procedure for controlling biofilms of foodborne pathogens in food processing facilities. However, it is suggested that a post-removal procedure is needed to completely remove biofilm residues. Also, further study is required to increase the effectiveness of steam treatment to achieve desired safety limits on PVC surfaces.

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Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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