Viability of *Salmonella* Typhimurium biofilms on major food-contact surfaces and eggshell treated during 35 days with and without water storage at room temperature

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ABSTRACT *Salmonella* is one of the main foodborne pathogens that affect humans and farm animals. The *Salmonella* genus comprises a group of food-transmitted pathogens that cause highly prevalent foodborne diseases throughout the world. The aim of this study was to appraise the viability of *Salmonella* Typhimurium biofilm under water treatment at room temperature on different surfaces, specifically stainless steel **(SS)**, plastic **(PLA)**, rubber **(RB)**, and eggshell **(ES)**. After 35 D, the reduction of biofilm on SS, PLA, RB, and ES was 3.35, 3.57, 3.22, and 2.55 log CFU/coupon without water treatment and 4.31, 4.49, 3.50, and 1.49 log CFU/coupon with water treatment, respectively. The d_R value (time required to reduce bacterial biofilm by 99% via Weibull modeling) of *S.* Typhimurium without and with water treatment was the lowest on PLA (176.86 and 112.17 h, respectively) and the highest on ES (485.37 and 2,436.52 h, respectively). The viability of the *S.* Typhimurium on ES and the 3 food-contact surfaces was monitored for 5 wk (35 D). The results of this study provide valuable information for the control of *S.* Typhimurium on different surfaces in the food industry, which could reduce the risk to consumers.

Key words: biofilm, eggshell, *S.* Typhimurium, survival ability, Weibull model

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INTRODUCTION

The bacteria on surfaces in food-processing environments are a potential source of cross-contamination and can lead to food spoilage or transmission of disease, through scratched or unclean food-contact surfaces in processing lines (Nidaullah et al., 2017; Coradini et al., 2019). Owing to condensation, these surfaces are favorable sites for bacteria to grow in static biofilms (Petridis et al., 2019). Biofilm do not possess a uniform structure, and bacterial species as well as several extrinsic factors (temperature, flow conditions, pH, presence of salts, nutrients, and and so on) play a major role in influencing biofilm formation and the degree of attachment (Jain and Chen, 2007; Hannig et al., 2018). Biofilm formation is strongly influenced by the food-contact surfaces (Jeon et al., 2018; Mizan et al., 2018).

In a biofilm, cells can initiate attachment on food products and food-contact surfaces (Jeon et al., 2018; Mizan et al., 2018), which provide a potential transmission route for foodborne pathogens (Hald et al., 2016). In the food industry, water cleaning is vital to control and remove biofilms (Liu et al., 2016; Esbelin et al., 2018). Nevertheless, poor or ineffective cleaning processes can increase the risk of foodborne outbreaks leading to public health concerns. In addition, the food industry may incur economic losses due to product recalls as well as legal and customer claims (Davey et al., 2013). Biofilms are involved in over 80% of all microbial diseases according to the US National Health Institute and the Centers for Disease Control and Prevention (Khatoon et al., 2018).

It has been inferred that biofilms formed on surfaces at various food-processing locations are a crucial source of *Salmonella* contamination of food (Khieu et al., 2013; Lamas et al., 2018). One of the most extensively used materials in machinery and food-contact surfaces in the food industry is stainless steel **(SS)**. Schlisselberg

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and Yaron (2013) described how various treatments of SS could affect the biofilms formation of *S.* Typhimurium. The crucial problem of biofilms on food industry surfaces is their transfer to food and resulting contamination of foodstuffs. In this point of view, Wang et al. (2015) evaluated the transfer of *Salmonella* biofilms formed on the food-contact surface of SS to foodstuff (meat). *Salmonella* spp. can contaminate fresh produce during any stage, from farm to table, through cross-contamination by washing with water, handling by workers, and contact with food surfaces (Kroupitski et al., 2009). *Salmonella* biofilm on eggshell **(ES)** can cause cross-contamination of *Salmonella* (Carrasco et al., 2012; Pande et al., 2016) onto other food-contact surfaces on egg-processing lines and can finally lead to food contamination (Carrasco et al., 2012).

More than 70% of human salmonellosis cases in the United States have been attributed to the consumption of contaminated chicken, turkey, and eggs with 175, 133, and 45 illness outbreaks involving 1,003, 358, and 11 people, respectively (CDC, 2019). Egg products associated with *S.* Typhimurium outbreaks have been frequently reported in Australia (Group, 2012; Kirk et al., 2014). Numerous egg-related human *S.* Typhimurium outbreaks have garnered significant interest from the general public, public health authorities, and egg industry (Chousalkar et al., 2017).

The major materials used for food-contact surfaces are known to be Teflon and nitrile butyl rubber, SS, glass, rubber **(RB)**, and polyurethane (Chia et al., 2009; Fink et al., 2017). In the present study, the viability of *S.* Typhimurium biofilm was evaluated when stored under room temperature for a long period (35 D) on ES and 3 different surfaces (SS, plastic **[PLA]**, and RB) treated with and without water. Indeed, the ability of pathogenic bacteria, including *S.* Typhimurium, to form biofilms on various food-contact surfaces and under different conditions for different periods has been investigated in several research studies (Lamas et al., 2016). In a review, Mizan et al. (2015) stated that *Salmonella* spp. biofilms self-gather and form flat or mushroom-shaped 3D structures on SS. Other studies have examined the viability of *Salmonella* spp. on polypropylene surfaces (Iibuchi et al., 2010), *Salmonella* spp. on ES surfaces at different temperatures (Park et al., 2015b), and specifically, *S.* Typhimurium on ES (McAuley et al., 2015).

In spite of the boundless appliance for forecasting mathematical models of survival and growth and the significant amount of scientific literature on the modeling of biofilm survival ability (Giertsen et al., 2011; Dimakopoulou-Papazoglou et al., 2016), no attempt has been made to study the effects of environmental factors during biofilm formation to establish and apply mathematical models. Therefore, the aim of this study was to establish and appraise predictive mathematical models for the effect of water treatment on the viability of *S.* Typhimurium biofilm on SS, PLA, RB, and ES.

The fitting and performance of linear and modified Weibull models were assessed to estimate the survival behavior of *Salmonella* spp. on SS, PLA, RB, and ES. Survival modeling of *S.* Typhimurium on table egg during storage at different temperatures (Pasquali et al., 2016) as well as the modeling of biofilm formation by *Salmonella enterica* ser. Newport as a function of pH and water activity (Dimakopoulou-Papazoglou et al., 2016) have been reported.

Many investigations have been carried out on food-contact surfaces concerning viability and biofilm attachment ability. However, there is still a need to explore the survival time and mathematical modeling of *S.* Typhimurium biofilm (the major cause of human bacterial gastroenteritis) on the 3 types of food-contact surface (SS, PLA, and RB), especially focusing on egg processing line materials and cookware utensils. Thus, the purpose of this study was to appraise the viability of *S.* Typhimurium on SS, PLA, RB, and ES surfaces.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

S. Typhimurium ATCC14028 was used in this study to evaluate the viability of *S.* Typhimurium on food-contact surfaces (SS, PLA, RB) and ES with and without water treatment. Bacterial stock culture was maintained at −70°C in Tryptic Soy Broth (**TSB**; Difco Laboratories Detroit, MI) supplemented with 15% (vol/vol) glycerol (Fisher Scientific, Itasca, IL). The strain was consecutively subcultured twice aerobically at 37°C for 24 h in TSB. Cultured cells were centrifuged at 11,000 × *g* at 4°C for 10 min and washed twice with sterile phosphate buffered saline (PBS; pH 7.2). The pellets were resuspended in peptone water (PW; Oxoid, Basingstoke, Hampshire, England). The bacterial cell suspension was diluted in 0.1% PW to yield the final cell concentration (10^5–10^6 CFU/mL) for inoculation to make biofilm on coupons. By plating on xylose lysine deoxycholate agar (Difco Laboratories) plates and incubating at 37°C for 24 h, microbial numbers were determined.

Preparation of SS/PLA/RB/ES Coupons, Inoculation, and Biofilm Formation

SS (SUS 304 2B; Posco Co., Ltd., Pohang, Korea), PLA (egg packaging; Join Co., Ltd., Eumseong, Korea), and RB (Komax Industrial Co., Ltd., Goyang-ro, Korea) were selected as delegate surfaces used in the food industry. In this study, we used SS, PLA, and RB coupons (2 cm × 2 cm × 0.1 cm) that were processed as earlier addressed (Sadekuzzaman et al. 2018; Hossain et al., 2020). ES coupons were processed as previously described by Park et al. (2018). Briefly, eggs were collected from a local grocery store in Anseong-Si, South Korea (Eggs with a remaining shelf life of at least 40 D were selected and stored at 4°C until use.). Each egg was gently broken, and the ES was cut into 2 cm × 2 cm × 0.1 cm coupons using a sterilized knife. Immediately after, the ES was soaked into 70% alcohol for 10 min, washed 3 times with sterilized deionized water, and then treated with

UV in a laminar flow biosafety hood for 15 min on each side to remove background flora before inoculation. The bacterial cell suspension was diluted at 1:50 into 50-mL Falcon tubes with each coupon completely submerged in 10-mL TSB. The 50-mL Falcon tubes were incubated without shaking to form biofilms on the coupons at 37°C for 24 h.

With or Without Water Treatment, Storage and Biofilm Detachment

After incubation of biofilm formation, half of the *S.* Typhimurium biofilms on SS, PLA, RB, and ES coupons were washed under running sterile water with swirling of 10 s (3 times) and stored at room temperature in a humidity chamber (relative humidity 50%; V8111H-150; Vision Scientific Co., Ltd., Gyeonggi-do, Korea). The remaining half of each sample was stored at room temperature in a humidity chamber (relative humidity 50%) without water treatment except for an initial wash process for removing planktonic cells. The viability of the *S.* Typhimurium on ES and the 3 food-contact surfaces was monitored for 5 wk (35 D). Biofilm cells detachment was done according to the study by Jahid et al. (2014) with minor modifications. After incubation, each coupon expect ES was shifted in a small petri dish (55 mm × 12 mm) containing 2 mL of 0.1% PW and agitated by holding the SS, PLA, and RB coupons on the petri dish using sterile tweezers at the same time to rotate clockwise and anticlockwise. Agitation was always performed by the same person to ensure the same amount of pressure was applied to all the coupons. The suspension was then transferred to a test tube and ultrasonicated for 2 min in a sonicator (380 W, 37 kHz, Elmasonic P; Elma Schmidbauer, GmbH, Singen, Germany) to disperse the biofilm population. Each sample of the ES coupon was vortexed with 10 glass beads and 10-mL PW in a 50-mL Falcon tube for 1 min. In PW, the dispersed biofilm cells were serially diluted for cells counting by plating on xylose lysine deoxycholate agar and incubated for 24 h at 37°C.

The Modified Weibull Model

The modified Weibull model (a two-parameter nonlinear model) can be expressed as

$$\mathrm{Log}\,(N_t\,/\,N_0) = N_0 - 1\,/\,2.303 * (t/b)^{\mathrm{n}} \qquad (1)$$

where N_t is the concentration of biofilm (CFU/coupon) after exposure time t, N_0 is the initial concentration of biofilm (CFU/coupon), t is the exposure time, and b and n are the scale (characteristic time) and shape parameters as a behavior index, respectively (van Boekel, 2002). To reduce the first log cycle, the value of b represents the required time to reduce the bacterial biofilm population, while that of n indicates the shape of the survival curve ($n = 1$ corresponds to a linear survival curve, while $n > 1$ and $n < 1$ correspond to downward and upward concavity, respectively). For the calculation of $\mathrm{d_R}$ (analogous to the traditional D value) from the Weibull parameters, the following equation is used (Buzrul and Alpas, 2007):

$$\mathrm{d_R}(t) = b * 4.606^{(1/n)} \qquad (2)$$

where $\mathrm{d_R}$ is the time required to reduce the bacterial biofilm by 99%. GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA) was used to appraise the inactivation kinetics for nonlinear regression which was fitted by the modified Weibull method.

Field Emission Scanning Electron Microscopy

Field emission scanning electron microscopy images of the *S.* Typhimurium biofilms on SS, PLA, RB, and ES coupons were obtained according to previously reported procedures (Mizan et al., 2018; Hossain et al., 2020) with some modifications. The adhered cells on coupons were fixed for 24 h with 2% glutaraldehyde (Sigma Aldrich, St. Louis, MO) in PBS and then washed 3 times with PBS. The fixed cells were serially treated with ethanol (50, 60, 70, 80, and 90% for 15 min, respectively, and 100% 2 times for 15 min each) and with 33, 50, 66, and 100% hexamethyldisilazane (Sigma Aldrich) in ethanol and successively dehydrated for 15 min each to observe the dehydrated samples which were coated with platinum and visualized on field emission scanning electron microscopy (**FE-SEM**; Carl Zeiss, Oberkochen, Germany) with an accelerating voltage of 5 kV and 5 mm working distance.

Statistical Analysis

Data were analyzed via one-way analysis of variance (Duncan's test) using SAS version 9.2 (SAS Institute Inc., Cary, NC). Three independent trials were used in all experiments repeated 3 times. Data were considered to be statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Viability of S. Typhimurium biofilm on Food-Contact Surfaces and ES With and Without Water Treatment Stored Under Room Temperature

The viability of *S.* Typhimurium biofilm was measured at predetermined times (0, 1, 2, 3, 5, 7, 14, 21, 28, and 35 D), after inoculation on SS, PLA, RB, and ES surfaces. The amounts of *S.* Typhimurium biofilm detected from the surfaces with and without water treatment significantly decreased over time ($P < 0.05$; Figure 1).

The viability of *S.* Typhimurium biofilm on food-contact surfaces without and with water treatment at room temperature is shown in Figures 1A and 1B, respectively. The reduction of *S.* Typhimurium biofilm after 35 D on food-contact surfaces without water treatment (Figure 1A) was the highest on PLA (3.57

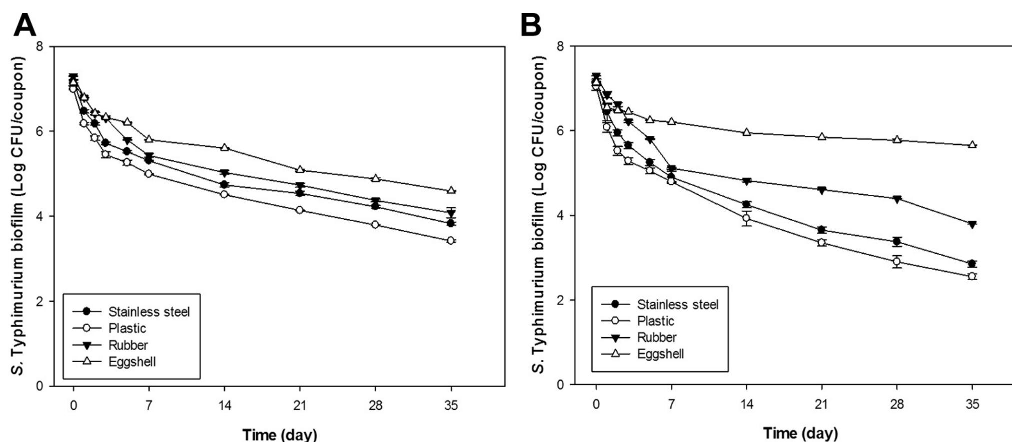

Figure 1. The viability of *Salmonella* Typhimurium biofilm formation on different food-contact surfaces on different time intervals without (A) and with (B) water treatment at room temperature.

$\log_{10}$ CFU/coupon) and the lowest on ES (2.55 $\log_{10}$ CFU/coupon). For SS and RB, the corresponding values were 3.35 and 3.22 $\log_{10}$ CFU/coupon, respectively. The amounts of *S.* Typhimurium biofilm detected on the surfaces of ES and the 3 food-contact surfaces significantly decreased over time ($P < 0.05$). From these data, *S.* Typhimurium biofilm survived for the shortest period on PLA among the 3 food contact surfaces, and the viability was maintained for the longest period on ES among all the surfaces. The characteristics of the food-contact surfaces could be important for bacteria attachment and viability (Stepanović et al., 2004). The bacterial biofilm viability on different food-contact surfaces depends on various factors, including temperature, humidity, and nutrient availability for bacteria (Lamas et al., 2018). According to Brankatschk et al. (2014), *Salmonella* uses its genetic marker to effectively attach to surfaces and finally colonize.

The viability of the *S.* Typhimurium biofilms is rated from the highest to the lowest as ES > RB > SS > PLA (Figure 1A), due to the properties of the surfaces. According to our FE-SEM study, the surface roughness is rated from highest to lowest as RB > ES > SS > PLA. FE-SEM images of the *S.* Typhimurium biofilm formation on the study surfaces are depicted in Figure 2. FE-SEM images reveal that *S.* Typhimurium colonized and consequently formed compact or dense biofilms on the surfaces. The adhesion forces for *Streptococcus* spp. on composite resins with different surface roughness values were evaluated by Mei et al. (2011), who confirmed that they increased with increasing surface roughness. It was also reported that the surface roughness of a polyester urethane conveyor belt had a significant influence on the biofilm-forming ability of *Listeria monocytogenes* (Stepanović et al., 2004). For this reason, *S.* Typhimurium could make a stronger biofilm on RB than other surfaces (Ronner and Wong, 1993). However, the viability was higher on ES than on RB (Figures 1A, 1B). ES has a surface roughness and porosity similar to those of wood, which allow the gas and water exchange necessary for the developing chick embryo but also microbial ingress and contamination of the egg contents. Thus, *S.* Typhimurium (1 μm) can enter through an ES pore (15–65 μm) and make a strong biofilm (Ghaneian et al., 2011; Abramian and El-Rassy, 2012). According to our FE-SEM study, we observed pore on ES (Figures 2E, 2F).

After 35 D, the reduction of the *S.* Typhimurium biofilms on food-contact surfaces with water treatment was the highest on PLA (4.49 $\log_{10}$ CFU/coupon) and the lowest on ES (1.49 $\log_{10}$ CFU/coupon) (Figure 1B). The reduction values for SS and RB were 4.31 and 3.50 $\log_{10}$ CFU/coupon, respectively. The amounts of *S.* Typhimurium biofilm detected on the 3 surfaces (SS, PLA, and RB) decreased significantly over time ($P < 0.05$), but they decreased significantly less ($P < 0.05$) on the surface of ES than on the other surfaces. The order of viability of the biofilms on the washed surfaces was the same as that on an unwashed surface, with the *S.* Typhimurium biofilm on ES being the highest (Kim et al., 2016; Jung et al., 2018). This difference could have been due to ES absorbing water through its pore during the 3 times washing step. Although the ES was dried and stored for 24 h in a humidity chamber before washing, its interior region could have remained wet and thus absorbed most of the biofilm. This may explain the high observed viability of the *S.* Typhimurium biofilm on the ES surface (Kim et al., 2016). Similarly, it has been suggested that wood encourages biofilm formation because of its porosity and absorbency, which can entrap organic material and bacteria (Adetunji and Isola, 2011; Al-kafaween et al., 2019). Moreover, moisture retention on the ES could also account for the greater viability of *S.* Typhimurium at low temperatures because the eggs were stored while still wet (Rizk et al., 1966).

Overall, it was found that the viability of *S.* Typhimurium biofilm (rated from highest to lowest: ES > RB > SS > PLA) showed several sharp decreases after 1, 2, 3, 5, and 7 D, but after 7 D, the decrease in the rate of viability was slight (Figure 1). It was also determined that the biofilms on the 3 food-contact surfaces (except for ES) were more viable when not washed with water, although the difference in the amount of *S.* Typhimurium biofilm was not significant ($P > 0.05$). The viability of *S.* Typhimurium biofilm on washed ES

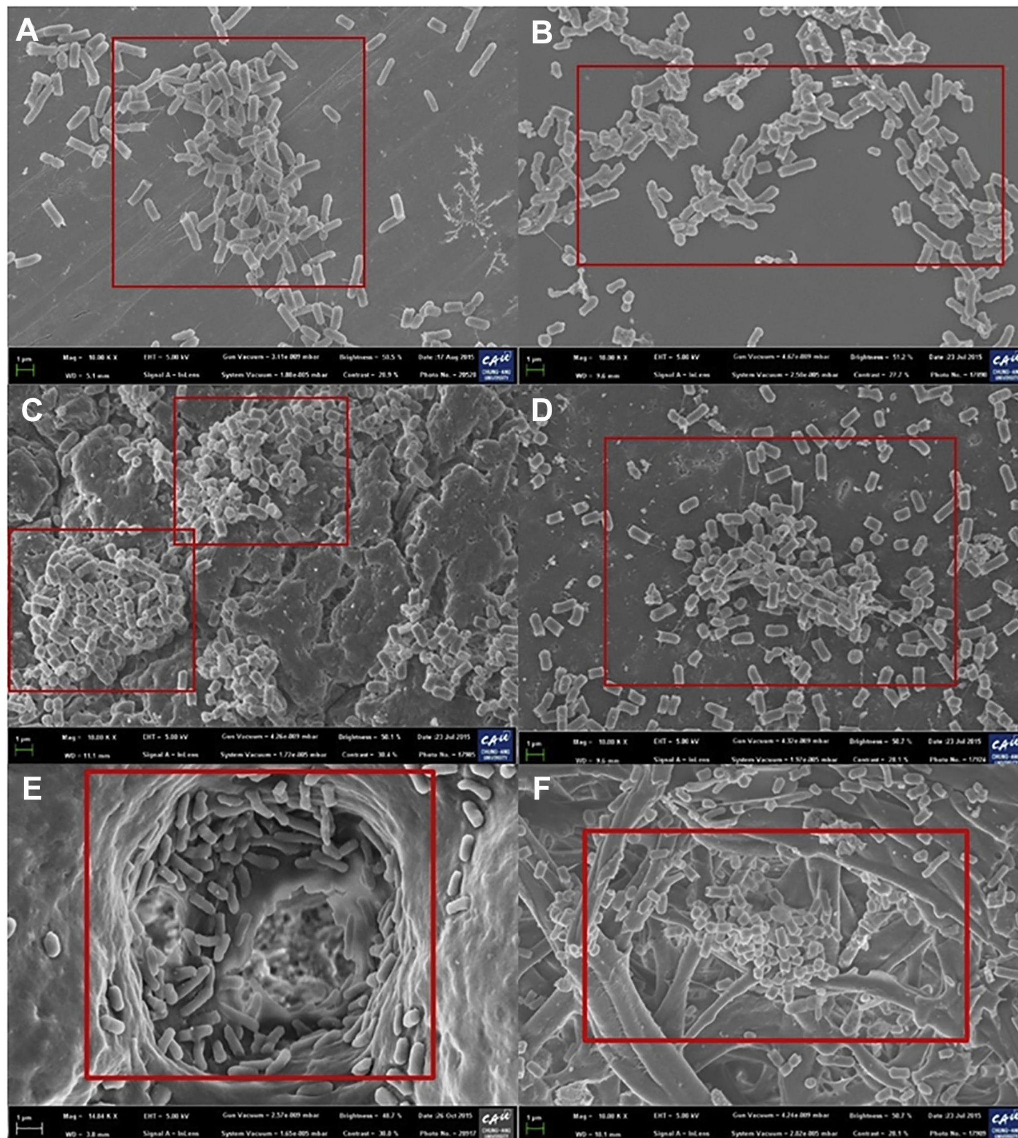

Figure 2. FESEM images of *S.* Typhimurium biofilm formation without water treatment on different food-contact surfaces. SS (A); PLA (B); RB (C, D); and ES (E, F). Red box indicates the biofilm formation ability on different surfaces.

was significantly higher in value than that on unwashed ES (Wolf-Hall and Nganje, 2017), and the amount of *S.* Typhimurium biofilm was significantly different ($P < 0.05$). This could have been due to ES absorbing water via its pores during the washing step (Pande et al., 2016).

After 35 D with and without water treatment on all food-contact surfaces, the results of the present study show that the viability of *S.* Typhimurium biofilms was maintained. McAuley et al. (2015) found that *S.* Typhimurium biofilm was attached early to the ES surface after exposure for only 20 min but was not maintained after 2 wk. It was also recently reported that the viability of *S. enterica* on ES surfaces was maintained after 3 wk (Park et al., 2015a). The results of these 2 studies are contradictory because *S.* Typhimurium cells attached to an ES surface had weaker adhesive power than *S. enterica* biofilm (exposure for 24 h). The results of the study by Park et al. (2015a) showed that the validity curve for *S. enterica* on ES was similar to that for *S.* Typhimurium biofilm in the present study. Consequently, the viability of *S.* Typhimurium on food-contact surfaces is variable according to the biofilm state and biofilm formation ability.

Weibull Modeling to Obtain Survival d_R Value Against S. Typhimurium Biofilms on the Food-Contact Surfaces

In food-contact surfaces, the viability data of *S.* Typhimurium biofilm were fitted with the Weibull model for nonlinear microbial survival. The values of the Weibull model parameters (b, n, d_R, and R^2) are reported in Table 1. The survival curves were a good fit for this model by using R^2 to estimate the goodness of fit of the model, whereas the values were over 0.96. Microbial inactivation has commonly been modeled using a first-order kinetics process (Whiting et al., 1996; Pankaj et al., 2013) fitted for comparison with the Weibull model. Moreover,

Table 1. Weibull modeling parameters for *Salmonella* Typhimurium survivability on the food-contact surfaces.

Water treatment	Parameters	Surface types			
		Stainless steel	Plastic	Rubber	Egg shell
Without	b ± SE	3.36 ± 1.10	2.88 ± 0.67	6.44 ± 2.24	18.6 ± 4.72
	n ± SE	0.37 ± 0.02	0.37 ± 0.01	0.42 ± 0.03	0.47 ± 0.03
	d_R ± SE	204.04 ± 23.90^{a,B,C}	176.86 ± 14.50^{a,C}	241.33 ± 30.23^{a,B}	485.37 ± 31.14^{b,A}
	R^2	0.99	0.99	0.98	0.98
With	b ± SE	3.54 ± 0.72	2.67 ± 0.65	5.71 ± 3.43	9.62 ± 2.28
	n ± SE	0.42 ± 0.01	0.41 ± 0.02	0.42 ± 0.05	0.28 ± 0.01
	d_R ± SE	133.28 ± 10.94^{b,B,C}	112.17 ± 11.44^{b,C}	216.75 ± 55.05^{a,B}	2,436.52 ± 35.81^{a,A}
	R^2	0.99	0.99	0.96	0.99

Values are mean ± SE.

b = scale parameter; n = shape parameter (concave upward survival curve if n < 1, concave downward if n > 1, and linear if n = 1); d_R = time (h) required to reduce the bacterial biofilm by 99%; R^2 = correlation coefficient (a higher R^2 value indicates a better fit to the data). Means in the same column with superscript lowercase letters are significantly different via Duncan's multiple range test ($P < 0.05$). Means in the same row with superscript uppercase letters are significantly different via Duncan's multiple range test ($P < 0.05$).

many researchers have revealed that the Weibull model might have a better fit than first-order models for kinetic viability of bacteria and viruses, such as mixed-culture biofilms of *S.* Typhimurium (Jahid et al., 2015), reduction of *Cladosporium cladosporioides* and *Penicillium citrinum* (Park and Ha, 2015), and survival of norovirus (Kim et al., 2014) and the hepatitis A virus (Bae et al., 2014). Thus, for its simplicity and flexibility, the Weibull model is used extensively (Chen and Hoover, 2004; Chen, 2007; Muñoz-Cuevas et al., 2013).

The Weibull model, which has 2 parameters (b and n), can be affected by external conditions (temperature, pH, the presence of a preservative, and so on) (Peleg and Cole, 2000; Mattick et al., 2001). The Weibull model (without water treatment) was used to predict survival curves and calculate the d_R values. In this study period, the R^2 values from the linear model and the Weibull model were 0.85 to 0.90 (data not shown) and 0.98 to 0.99, respectively, whereas the Weibull model was a better fit to the data than the linear model. The Weibull parameters (b and n) represent the required time to reduce the bacterial biofilm by 1 $\log_{10}$ by calculating the d_R value. The calculated d_R values were 204.04, 176.86, 241.33, and 485.37 h for SS, PLA, RB, and ES, respectively. The d_R values for *S.* Typhimurium biofilm without water treatment rated from significantly highest to lowest ($P < 0.05$) were in the order of ES > RB > SS > PLA (Table 1). The Weibull model (with water treatment) was used to predict survival curves and calculate the d_R values. The R^2 values were 0.75 to 0.89 (data not shown) and 0.96 to 0.99, respectively, from the linear model and the Weibull model, indicating the better fit of the data to the Weibull model than to the linear model. The d_R value which was calculated from the Weibull parameters (b and n) represents the required time to reduce the bacterial biofilm by 1 $\log_{10}$. The calculated d_R values were 133.28, 112.17, 216.75, and 2,436.52 h for SS, PLA, RB, and ES, respectively. The d_R values for *S.* Typhimurium biofilm with water treatment rated from significantly highest to lowest ($P < 0.05$) were in the order of ES > RB > SS > PLA (Table 1).

Overall, it was found that the d_R values for *S.* Typhimurium biofilm rated from highest to lowest were in the order of ES > RB > SS > PLA. The d_R values of *S.* Typhimurium biofilm on unwashed surfaces (RB, SS, and PLA) except for ES were significantly higher than those on washed surfaces ($P < 0.05$). However, the d_R values of *S.* Typhimurium biofilm on washed ES were higher than those on unwashed ES. The amounts of *S.* Typhimurium biofilm on the unwashed and washed surfaces of ES were significantly different ($P < 0.05$). From this result, it can be determined that *S.* Typhimurium biofilm inactivation on the surface of PLA requires less time than on the other surfaces. The *S.* Typhimurium biofilm inactivation on the surface of ES was estimated to take a longer time than on the other surfaces. Consequently, the results indicate that the Weibull modeling arrived at the same result as the experiments.

In summary, the viability of *S.* Typhimurium biofilm on food-contact surfaces without and with water treatment was investigated. The reduction of *S.* Typhimurium biofilm without and with water treatment was highest on PLA (3.57 and 4.49 $\log_{10}$ CFU/coupon, respectively) and lowest on ES (2.55 and 1.49 $\log_{10}$ CFU/coupon, respectively). The order of reduction value (from highest to lowest) was PLA > SS > RB > ES. After 35 D treated with and without water, the viability of *S.* Typhimurium biofilm was maintained on all food-contact surfaces.

Weibull modeling was conducted to obtain survival d_R values for *S.* Typhimurium biofilms on the food-contact surfaces without and with water treatment. The d_R values of *S.* Typhimurium biofilm without and with water treatment were lowest on PLA (176.86 and 112.17 h, respectively) and highest on ES (485.37 and 2,436.52 h, respectively). Overall, the d_R values of *S.* Typhimurium biofilm from highest to lowest were in the order of ES > RB > SS > PLA (Hingston et al., 2013). It was also found that the d_R values of *S.* Typhimurium biofilm on the unwashed RB, SS, and PLA (not ES) were less than those on the washed surfaces. The result of this study suggests that washing ES every day does not reduce *S.* Typhimurium biofilm viability significantly.

CONCLUSION

The viability of *S.* Typhimurium on food-contact surfaces varies according to the biofilm state and formation ability. The viability of *S.* Typhimurium biofilm over

time depends on the surface roughness and porosity. The results of this study show that *S.* Typhimurium biofilm formed during food formulation and processing can survive persistently in factories and cookware; thus, ensuring a safe environment is very crucial during these procedures. Reducing *S.* Typhimurium biofilm contamination during food processing will reduce the occurrence of associated diseases. The findings in this study provide valuable information for the control of *S.* Typhimurium on different food-contact surfaces in the food industry to prevent foodborne disease. However, the experimental scope of the study is limited to only one strain of *Salmonella.* Further studies are urged to extend the application of this study in food quality and safety regulations.

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REFERENCES

Abramian, L., and H. El-Rassy. 2012. Adsorption kinetics and thermodynamics of azo-dye orange II onto highly porous titania aerogel. Chem. Eng. J. 209:682.

Adetunji, V. O., and T. O. Isola. 2011. Crystal violet binding assay for assessment of biofilm formation by *Listeria monocytogenes* and *Listeria* spp on wood, steel and glass surfaces. Glob. Vet. 6:6–10.

Al-kafaween, M. A., A. B. M. Hilmi, N. Jaffar, H. A. N. Al-Jamal, and M. K. Zahri. 2019. Determination of optimum incubation time for formation of *Pseudomonas aeruginosa* and *Streptococcus pyogenes* biofilms in microtiter plate. Bull. Natl. Res. Cent. 43:100.

Bae, S. C., S. Y. Park, A. N. Kim, M. H. Oh, and S. D. Ha. 2014. Survival of hepatitis A virus on various food-contact surfaces during 28 days of storage at room temperature. Food Res. Int. 64:849–854.

Brankatschk, K., T. Kamber, B. Duffy, J. Pothier, and T. H. M. Smits. 2014. Transcriptional profile of *Salmonella enterica* subsp. enterica serovar Weltevreden during alfalfa sprout colonization. Microb. Biotechnol. 7:528–544.

Buzrul, S., and H. Alpas. 2007. Modeling inactivation kinetics of food borne pathogens at a constant temperature. Lwt-food Sci. Technol. 40:632–637.

Carrasco, E., A. Morales-Rueda, and R. M. García-Gimeno. 2012. Cross-contamination and recontamination by *Salmonella* in foods: a review. Food Res. Int. 45:545–556.

Centers for Disease Control and Prevention (CDC). 2019. *Salmonella*, outbreaks of *Salmonella* infections linked to backyard poultry. Accessed Oct. 2019. www.cdc.gov/*Salmonella*/backyardpoultry-05-19/index.html.

Chen, H. 2007. Use of linear, Weibull, and log-logistic functions to model pressure inactivation of seven foodborne pathogens in milk. Food Microbiol. 24:197–204.

Chen, H., and D. G. Hoover. 2004. Use of Weibull model to describe and predict pressure inactivation of *Listeria monocytogenes* Scott A in whole milk. Innov. Food Sci. Emerg. 5:269–276.

Chia, T., R. Goulter, T. McMeekin, G. Dykes, and N. Fegan. 2009. Attachment of different *Salmonella* serovars to materials commonly used in a poultry processing plant. Food Microbiol. 26:853–859.

Chousalkar, K. K., M. Sexton, A. McWhorter, K. Hewson, G. Martin, C. Shadbolt, and P. Goldsmith. 2017. *Salmonella typhimurium* in the Australian egg industry: Multidisciplinary approach to addressing the public health challenge and future directions. Crit. Rev. Food Sci. Nutr. 57:2706–2711.

Coradini, M. G. L., D. S. V. Maia, M. A. Iglesias, L. Haubert, G. V. Lopes, D. A. L. da Silva, L. A. Nero, and W. P. da Silva. 2019. Occurrence and characterization of *Listeria monocytogenes* from beef jerky processing line. J. Food Sci. Technol. 56:436–442.

Davey, K., S. Chandrakash, and B. O'Neill. 2013. A new risk analysis of Clean-In-Place milk processing. Food Control 29:248–253.

Dimakopoulou-Papazoglou, D., A. Lianou, and K. P. Koutsoumanis. 2016. Modelling biofilm formation of *Salmonella enterica* ser. Newport as a function of pH and water activity. Food Microbiol. 53:76–81.

Esbelin, J., T. Santos, and M. Hebraud. 2018. Desiccation: an environmental and food industry stress that bacteria commonly face. Food Microbiol. 69:82–88.

Fink, R., D. Okanovič, G. Dražič, A. Abram, M. Oder, M. Jevšnik, and K. Bohinc. 2017. Bacterial adhesion capacity on food service contact surfaces. Int. J. Environ. Heal Res. 27:169–178.

Ghaneian, M. T., M. H. Ehrampoush, G. H. Ghanizadeh, and M. Momtaz. 2011. Study of eggshell performance as a natural sorbent for the removal of reactive red 198 dye from aqueous solution. Spring 10:70–81.

Giertsen, E., R. Arthur, and B. Guggenheim. 2011. Effects of xylitol on survival of mutans streptococci in mixed-six-species *in vitro* biofilms modelling supragingival plaque. Caries Res. 45:31–39.

Group, O. W. 2012. Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: Annual report of the OzFoodNet network, 2010. Commun. Dis. Intell. Q. Rep. 36:E213.

Hald, T., W. Aspinall, B. Devleesschauwer, R. Cooke, T. Corrigan, A. H. Havelaar, H. J. Gibb, P. R. Torgerson, M. D. Kirk, F. J. Angulo, R. J. Lake, N. Speybroeck, and S. Hoffmann. 2016. World health Organization estimates of the relative Contributions of food to the Burden of disease due to selected foodborne Hazards: a structured Expert Elicitation. PLoS One 11:e0145839.

Hannig, C., R. Helbig, J. Hilsenbeck, C. Werner, and M. Hannig. 2018. Impact of the springtail's cuticle nanotopography on bioadhesion and biofilm formation *in vitro* and in the oral cavity. Roy. Soc. Open Sci. 5:171742.

Hingston, P. A., E. C. Stea, S. Knøchel, and T. Hansen. 2013. Role of initial contamination levels, biofilm maturity and presence of salt and fat on desiccation survival of *Listeria monocytogenes* on stainless steel surfaces. Food Microbiol. 36:46–56.

Hossain, M. I., M. F. R. Mizan, M. Ashrafudoulla, S. Nahar, H. J. Joo, I. K. Jahid, S. H. Park, K.-S. Kim, and S. D. Ha. 2020. Inhibitory effects of probiotic potential lactic acid bacteria isolated from kimchi against *Listeria monocytogenes* biofilm on lettuce, stainless-steel surfaces, and MBEC™ biofilm device. LWT 118:108864.

Iibuchi, R., Y. Hara-Kudo, A. Hasegawa, and S. Kumagai. 2010. Survival of *Salmonella* on a polypropylene surface under dry conditions in relation to biofilm-formation capability. J. Food Prot. 73:1506–1510.

Jahid, I. K., N. R. Han, S. Srey, and S. D. Ha. 2014. Competitive interactions inside mixed-culture biofilms of Salmonella Typhimurium and cultivable indigenous microorganisms on lettuce enhance microbial resistance of their sessile cells to ultraviolet C (UV-C) irradiation. Food Res. Int. 55:445–454.

Jahid, I. K., N. Han, C.-Y. Zhang, and S.-D. Ha. 2015. Mixed culture biofilms of *Salmonella* Typhimurium and cultivable indigenous microorganisms on lettuce show enhanced resistance of their sessile cells to cold oxygen plasma. Food Microbiol. 46:383–394.

Jain, S., and J. Chen. 2007. Attachment and biofilm formation by various serotypes of *Salmonella* as influenced by cellulose production and thin aggregative fimbriae biosynthesis. J. Food Prot. 70:2473–2479.

Jeon, H. R., M. J. Kwon, and K. S. Yoon. 2018. Control of *Listeria innocua* biofilms on food contact surfaces with slightly acidic electrolyzed water and the risk of biofilm cells transfer to duck meat. J. Food Prot. 81:582–592.

Jung, S.-J., S. Y. Park, and S. D. Ha. 2018. Synergistic effect of X-ray irradiation and sodium hypochlorite against *Salmonella enterica* serovar Typhimurium biofilms on quail eggshells. Food Res. Int. 107:496–502.

Khatoon, Z., C. D. McTiernan, E. J. Suuronen, T. F. Mah, and E. I. Alarcon. 2018. Bacterial biofilm formation on implantable

devices and approaches to its treatment and prevention. Heliyon 4:e01067.

Khieu, V., S. Srey, F. Schar, S. Muth, H. Marti, and P. Odermatt. 2013. Strongyloides stercoralis is a cause of abdominal pain, diarrhea and urticaria in rural Cambodia. BMC Res. Notes 6:200.

Kim, A.-N., S. Y. Park, S.-C. Bae, M.-H. Oh, and S. D. Ha. 2014. Survival of norovirus surrogate on various food-contact surfaces. Food Environ. Virol. 6:182–188.

Kim, M., S. Y. Park, and S. D. Ha. 2016. Synergistic effect of a combination of ultraviolet–C irradiation and sodium hypochlorite to reduce *Listeria monocytogenes* biofilms on stainless steel and eggshell surfaces. Food Control 70:103–109.

Kirk, M., L. Ford, K. Glass, and G. Hall. 2014. Foodborne illness, Australia, circa 2000 and circa 2010. Emerg. Inf. Dis. 20:1857.

Kroupitski, Y., R. Pinto, M. T. Brandl, E. Belausov, and S. Sela. 2009. Interactions of *Salmonella enterica* with lettuce leaves. J. Appl. Microbiol. 106:1876–1885.

Lamas, A., I. C. Fernandez-No, J. M. Miranda, B. Vázquez, A. Cepeda, and C. M. Franco. 2016. Biofilm formation and morphotypes of *Salmonella enterica* subsp. arizonae differs from those of other *Salmonella enterica* subspecies in isolates from poultry houses. J. Food Prot. 79:1127–1134.

Lamas, A., P. Regal, B. Vázquez, J. M. Miranda, A. Cepeda, and C. M. Franco. 2018. *Salmonella* and *Campylobacter* biofilm formation: a comparative assessment from farm to fork. J. Sci. Food Agric. 98:4014–4032.

Liu, S., C. Gunawan, N. Barraud, S. A. Rice, E. J. Harry, and R. Amal. 2016. Understanding, monitoring, and controlling biofilm growth in drinking water distribution systems. Environ. Sci. Technol. 50:8954–8976.

Mattick, K., J. Legan, T. Humphrey, and M. Peleg. 2001. Calculating *Salmonella* survival during non-isothermal heat inactivation. J. Food Prot. 64:606–613.

McAuley, C. M., L. L. Duffy, N. Subasinghe, G. Hogg, J. Coventry, and N. Fegan. 2015. *Salmonella* Typhimurium and *Salmonella* Sofia: growth in and persistence on eggs under production and retail conditions. Biomed. Res. Int. 2015:914987.

Mei, L., H. J. Busscher, H. C. van der Mei, and Y. Ren. 2011. Influence of surface roughness on streptococcal adhesion forces to composite resins. Dent. Mater. 27:770–778.

Mizan, M. F. R., M. Ashrafudoulla, M. Sadekuzzaman, I. Kang, and S. D. Ha. 2018. Effects of NaCl, glucose, and their combinations on biofilm formation on black tiger shrimp (*Penaeus monodon*) surfaces by *Vibrio parahaemolyticus*. Food Control 89:203–209.

Mizan, M. F. R., I. K. Jahid, and S. D. Ha. 2015. Microbial biofilms in seafood: a food-hygiene challenge. Food Microbiol. 49:41–55.

Muñoz-Cuevas, M., L. Guevara, A. Aznar, A. Martínez, P. M. Periago, and P. S. Fernández. 2013. Characterisation of the resistance and the growth variability of *Listeria monocytogenes* after high hydrostatic pressure treatments. Food Control 29:409–415.

Nidaullah, H., N. Abirami, A. K. Shamila-Syuhada, L. O. Chuah, H. Nurul, T. P. Tan, F. W. Z. Abidin, and G. Rusul. 2017. Prevalence of *Salmonella* in poultry processing environments in wet markets in Penang and Perlis, Malaysia. Vet. World 10:286–292.

Pande, V. V., A. R. McWhorter, and K. K. Chousalkar. 2016. *Salmonella enterica* isolates from layer farm environments are able to form biofilm on eggshell surfaces. Biofouling 32:699–710.

Pankaj, S., N. Misra, and P. Cullen. 2013. Kinetics of tomato peroxidase inactivation by atmospheric pressure cold plasma based on dielectric barrier discharge. Innov. Food Sci. Emerg. 19:153–157.

Park, S., S. Choi, H. Kim, Y. Kim, B. S. Kim, L. R. Beuchat, and J. H. Ryu. 2015a. Fate of mesophilic aerobic bacteria and *Salmonella enterica* on the surface of eggs as affected by chicken feces, storage temperature, and relative humidity. Food Microbiol. 48:200–205.

Park, S., Y. Oh, B. Jo, J. Lee, S. Lee, and J. Jeong. 2015b. Study of formation factor of biofilm on aluminum surface and removal efficiency of biofilm by antimicrobials. Korean Chem. Eng. Res. 53:730–739.

Park, S. Y., and S. D. Ha. 2015. Application of cold oxygen plasma for the reduction of *Cladosporium cladosporioides* and *Penicillium citrinum* on the surface of dried filefish (*Stephanolepis cirrhifer*) fillets. Int. J. Food Sci. Technol. 50:966–973.

Park, S. Y., S. J. Jung, and S. D. Ha. 2018. Synergistic effects of combined X-ray and aqueous chlorine dioxide treatments against *Salmonella* Typhimurium biofilm on quail egg shells. LWT 92:54–60.

Pasquali, F., G. Klein, F. Reich, G. Manfreda, and A. Valero. 2016. Modelling survival behaviour of *Salmonella enterica* ser. Enteritidis, Typhimurium and Tennessee on table eggs during storage at different temperatures. Food Control 59:314–319.

Peleg, M., and M. Cole. 2000. Estimating the survival of *Clostridium botulinum* spores during heat treatments. J. Food Prot. 63:190–195.

Petridis, X., F. H. Busanello, M. V. R. So, R. J. B. Dijkstra, P. K. Sharma, and L. W. M. van der Sluis. 2019. Chemical efficacy of several NaOCl concentrations on biofilms of different architecture: new insights on NaOCl working mechanisms. Int. Endod. J. 52:1773–1788.

Rizk, S., J. Ayres, and A. Kraft. 1966. Effect of holding condition on the development of *Salmonellae* in artificially inoculated hens' eggs. Poult. Sci. 45:825–829.

Ronner, A. B., and A. C. L. Wong. 1993. Biofilm development and sanitizer inactivation of *Listeria monocytogenes* and *Salmonella* Typhimurium on stainless-steel and Buna-n rubber. J. Food Prot. 56:750–758.

Sadekuzzaman, M., M. F. R. Mizan, H. S. Kim, S. Yang, and S. D. Ha. 2018. Activity of thyme and tea tree essential oils against selected foodborne pathogens in biofilms on abiotic surfaces. LWT 89:134–139.

Schlisselberg, D. B., and S. Yaron. 2013. The effects of stainless steel finish on *Salmonella* Typhimurium attachment, biofilm formation and sensitivity to chlorine. Food Microbiol. 35:65–72.

Stepanović, S., I. Ćirković, L. Ranin, and M. S. vabić-Vlahović. 2004. Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. Lett. Appl. Microbiol. 38:428–432.

van Boekel, M. A. 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. Int. J. Food Microbiol. 74:139–159.

Wang, H., X. Zhang, Q. Zhang, K. Ye, X. Xu, and G. Zhou. 2015. Comparison of microbial transfer rates from *Salmonella* spp. biofilm growth on stainless steel to selected processed and raw meat. Food Control 50:574–580.

Whiting, R., S. Sackitey, S. Calderone, K. Morely, and J. Phillips. 1996. Model for the survival of *Staphylococcus aureus* in nongrowth environments. Int. J. Food Microbiol. 31:231–243.

Wolf-Hall, C., and W. Nganje. 2017. Microbial Food Safety: A Food Systems Approach. CABI.