MICROBIOLOGY AND FOOD SAFETY

Inhibitory effect of ethanol and thiamine dilaurylsulfate against loosely, intermediately, and tightly attached mesophilic aerobic bacteria, coliforms, and *Salmonella* Typhimurium in chicken skin

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ABSTRACT The effects of 3 ethanol levels (30, 50, and 70%) with and without thiamine dilaurylsulfate (**TDS**; 1,000 ppm) were evaluated for the reduction of natural mesophilic aerobic bacteria (**MAB**), coliforms, and inoculated *Salmonella* Typhimurium (*S.* Typhimurium) in chicken skin. The chicken skin was inoculated with a 7 log cfu/mL suspension of *S.* Typhimurium. Loosely, intermediately, and tightly attached cells were recovered from chicken skin through shaking at 200 rpm for 5 min, stomaching for 1 min, and blending for 1 min, respectively. Increasing the ethanol concentration reduced the number of MAB, coliforms, and *S.* Typhimurium on the chicken skin, whereas TDS treatment without ethanol was not effective. Intermediately and tightly attached microorganisms (total MAB, coliforms, and *S.* Typhimurium) were more resistant to chemical disinfectants than loosely attached microorganisms. The combination of 70% ethanol with TDS was most effective than the combination of TDS with lower concentrations of ethanol in reducing populations of loosely, intermediately, and tightly attached MAB (by 1.88 log cfu/g, 1.21 log cfu/g, and 0.84 log cfu/g, respectively), coliforms (by 1.14 log cfu/g, 1.04 log cfu/g, and 0.67 log cfu/g, respectively), and *S.* Typhimurium (by 1.62 log cfu/g, 1.72 log cfu/g, and 1.27 log cfu/g, respectively). However, the chicken skin treated with higher concentrations of ethanol was tougher ($P < 0.05$) and more yellow and less red ($P < 0.05$) than that treated with lower concentrations of ethanol or with water (control). On the other hand, a combination of 30% ethanol and TDS yielded the best results, showing the reduction greater than 0.5 log cfu/g in *S.* Typhimurium, with no negative effect on chicken skin color or texture. Thus, a combination of 30% ethanol and TDS appears to be the optimal treatment for reducing microbial contamination of skin-on chicken products to enhance poultry safety without decreasing food quality, and this treatment could be applied in the poultry industry.

Key words: *Salmonella* Typhimurium, chicken skin, thiamine dilaurylsulfate (TDS), ethanol, food quality

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INTRODUCTION

Salmonella is the most common foodborne pathogen associated with various foods (CDC, 2014) and a major cause of intestinal infectious diseases (CDC, 2005; 2014). In several regions, a large proportion of foodborne diseases can be attributed to hazardous salmonellosis caused by infectious *Salmonella* (Zhang et al., 2019). Kramer et al. (2000) reported that in the United Kingdom, 30–40% of chicken sold is contaminated by *Salmonella*. According to the Food Safety and Inspection Service (FSIS) (2014) survey from 1998 to 2013, among the 31,374 samples of raw meat and poultry investigated, positive rates of *Salmonella* were ground chicken (18.0%), ground turkey (15.0%), young chicken (3.9%), turkey (2.35%), and ground beef (1.6%). This suggests that poultry meat is widely associated with foodborne *Salmonella* infections (Bryan and Doyle, 1995; Newell et al., 2010). The FAO (Food and Agriculture Organization) and WHO (World Health Organization) (2002) also reported that 26% of foodborne diseases are related to eggs, poultry, and poultry products. Despite common *Salmonella* infections, the consumption of poultry meat has continually increased worldwide (FAO, 2010, 2013). Among more than 2,500 *Salmonella* serovars, the 5 predominant serotypes associated with human infections are *S.* Typhimurium

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(19%), *S.* Enteritidis (14%), *S.* Newport (9%), *S.* Heidelberg (6%), and *S.* Javiana (5%) (FAO, 2010, 2013). Among them, *S.* Typhimurium is the serotype most commonly associated with laboratory-confirmed diseases (Braden, 2006).

In the poultry industry, contamination by *Salmonella* spp. commonly occurs on the surface and inside of poultry skin during the various stages of processing, such as defeathering, scalding, evisceration, washing, and chilling (Buhr et al., 2005; McKee, 2012). In addition, *Salmonella* has known to persist on chicken skin during processing as it can possibly attach the skin and become entrapped in a deeper skin layer, crevice, or feather follicles which could provide suitable environment for bacteria to lodge (McMeekin et al., 1979; Chantarapanont et al., 2004). Furthermore, the bacteria lodged deeper in skin are hardly recovered by rinsing or stomaching. Although poultry meat undergoes chemical treatment before packaging, microorganisms are not completely eliminated during processing (Ko et al., 2005; Zhang et al., 2011). Hinton and Cason (2008) reported that microorganisms in poultry skin cannot be eliminated by soft washing because they can be attached to the feather follicles, folds, and microcracks in the skin. In addition, according to Thomas and McMeekin (1980), a larger variety of microorganisms can be found in poultry skin that has been immersed in water than in untreated poultry skin. Contamination can occur through tearing in the poultry skin, which can occur during defeathering. Natural microorganisms are more often intermediately and tightly attached to the skin rather than loosely attached (Lee et al., 2014). Because poultry skin is generally consumed with the meat, various efforts have been made to reduce microorganism contamination on poultry.

Disinfectants are chemical agents such as acidified sodium chlorite, chlorine dioxide, bromine, organic acid, trisodium phosphate, peracetic acid, and monochloramine, and they have been applied as antimicrobial agents (Møretrø, et al., 2012). Ethanol is another compound that has also been widely used for centuries as a disinfectant in food (Barker and Park, 2001; Kalathenos and Russell, 2003). Various studies have found the inhibitory effect of ethanol on *Saccharomyces cerevisiae* and *Bacillus cereus* (Thomas and Rose, 1979; Ingram, 1989), and 30% or higher concentrations of ethanol have been effective in the reduction of *B. cereus* (Jang et al., 2003). In addition, ethanol treatments have been used to extend the shelf life by inhibiting growth of microorganisms in fresh noodles in many Asian countries (Fu, 2008; Kim et al., 2011a). Thiamine dilaurylsulfate (**TDS**), a precursor of vitamin B1, is a nutrient that has been used as a food additive and a preservation enhancer for noodles in Korea (KFDA, 2009). The antimicrobial effects of TDS are known to be due to its structure and components, such as sodium lauryl sulfate (**SLS**), the thiazole ring, and thiamine (Choi et al., 2015). Several studies have reported its bactericidal activity and synergistic effect of TDS with various chemicals against foodborne pathogens (Kim et al, 2005, 2011a; Lee et al., 2010). TDS has synergistic effects with disinfectants such as malic acid and chlorine in the sterilization of alfalfa seeds (Fransisca et al., 2012), oysters (Kim et al., 2011b), rice (Lee and Ha, 2008), and lettuce (Ha et al., 2012). Kim et al. (2005) reported that TDS has antimicrobial effects on gram-positive bacteria than on gram-negative bacteria, and the combination of TDS and ethanol treatment can be even more effective than either alone, as TDS dissolves well in organic solvents such as ethanol and acetic acid.

However, a combination of ethanol and TDS has not yet been tested against natural indigenous mesophilic aerobic bacteria (**MAB**), coliforms, and inoculated *S.* Typhimurium on chicken skin. Thus, the purpose of this study was to investigate the efficacy of 30, 50, and 70% ethanol and TDS for the reduction of loosely, intermediately, and tightly attached MAB, coliforms, and *S.* Typhimurium on chicken skin.

MATERIALS AND METHODS

Bacterial Strains and Inoculum Preparation

S. Typhimurium with resistance to novobiocin (**NO**; Sigma-Aldrich Co., St. Louis, MO) and nalidixic acid (**NA**; Sigma-Aldrich Co.), which was previously isolated from poultry, was used in this study. The strain was transferred from a stock culture and stored at −80°C in tryptic soy broth (Difco Laboratories, Detroit, MI) containing 50% glycerol (Fisher Scientific, Itasca, IL). The strain was subcultured twice at 37°C for 24 h in 10 mL of tryptic soy broth for activation, and then the cells were centrifuged at 12,000 × *g* for 10 min at 4°C. The cell pellet was washed twice and suspended in phosphate-buffered saline (**PBS**; Oxoid, Basingstoke, UK). The cell suspension was diluted in PBS to obtain a final cell concentration of 7 log cfu/mL for inoculation. *S.* Typhimurium were counted after plating on xylose lysine deoxycholate agar (Difco Laboratories) supplemented with 25 μg/mL of NA and 25 μg/mL of NO and incubating at 37°C for 24 h.

Sample Preparation and Inoculation

Chicken breast skin was purchased from a local market (Anseong, Korea) and stored at 4°C before the experiment. The chicken skin was cut into uniform 5-g pieces (5 × 5 cm) using sterile stainless steel scissors and used immediately. To remove background flora from the chicken skin in the inoculated *S.* Typhimurium experiment, samples were treated with UV light (Sankyo UV Co. Ltd., Seoul, Korea) at 1,000 μW s/cm^2 for 5 min and then rinsed once with sterile distilled water for 2 min. However, the UV treatment was not conducted for samples used in the experiments with natural indigenous MAB and coliforms. Samples were dried on a clean bench for 10 min, and the surfaces of the chicken skin were spot inoculated with 0.5 mL of *S.* Typhimurium suspension for 10 min. The samples of inoculated *S.*

Typhimurium were stored at 4°C for 1 h for attachment and then rinsed with sterile distilled water for 20 s to eliminate nonattached cells. Uninoculated skin samples were used to evaluate the effectiveness of treatment for coliforms and MAB.

Disinfection Treatments

Ethanol concentrations of 30, 50, and 70% (Biosesang, Seongnam, Korea) were used as chemical treatments to eliminate *S.* Typhimurium, MAB, and coliforms on chicken skin. TDS of 1,000 ppm was prepared in 3% ethanol, dissolved by sonication, and immediately added to the ethanol treatment. Sterile distilled water was used as a control. All disinfectant solutions were prepared before use and applied at room temperature (24°C). The *S.* Typhimurium, MAB, and coliforms were counted, and experiments were repeated 3 times.

Enumeration of Microorganisms

Microbial analysis was performed as described by Zhang et al. (2013). Samples (10 g) treated with chemical disinfectants (ethanol and TDS) were placed in 90 mL of 0.1% peptone water (**PW**, Oxoid) in sterile glass beakers and shaken at 200 rpm for 5 min in a shaking incubator (VS-101Si, Vision Science, Daejeon, Korea) at room temperature. The recovered microorganisms were classified as loosely attached cells. Rinsed chicken skin samples were transferred to stomacher bags (Nasco, Fort Atkinson, WI) containing 90 mL of 0.1% PW and stomached for 1 min in a stomacher (SH-IIM, Elmex, Tokyo, Japan). The recovered microorganisms were classified as intermediately attached cells. Finally, stomached chicken skin samples were transferred to sterile bottles containing 90 mL of PW and ground using a blender (SMX 760J, Shinil, Seoul, Korea) for 1 min. The recovered microorganisms were classified as tightly attached cells. From this point on, the 3 levels of loosely, intermediately, and tightly attached MAB, coliforms, and *S.* Typhimurium refer to the microorganisms detected from the rinsed, stomached, and blended skins, respectively. *S.* Typhimurium, MAB, and coliforms were counted in 10-fold serial dilutions of the rinsed, stomached, or blended samples, after incubation on xylose lysine deoxycholate agar (Difco Laboratories) containing 25 μg/mL of NA and 25 μg/mL of NO, tryptic soy agar (Difco Laboratories), and violet red bile agar (Difco Laboratories), respectively.

Color and Texture Measurement

The color and texture of all treated chicken skin were measured to assess the changes in chicken skin with single and combined treatments. The color of chicken skin was measured using a color difference meter (UltraScan PRO, HunterLab Co., Reston, VA). Measurements were taken from 5 different spots for each sample, expressed as lightness (L*), redness (a*), and yellowness (b*). The sample texture was measured by stretching the chicken skin using a texture analyzer (TAHDi/500, TAHD Co., London, UK) at a speed of 0.4903 N (Salim et al., 2012).

Field Emission Scanning Electron Microscopy

Field emission scanning electron microscopy (**FE-SEM**; Sigma, Carl Zeiss, Germany) was performed as described by Lee et al. (2014) to observe changes in the number of *S.* Typhimurium on the surface of chicken skin after the 70% ethanol, TDS, and combined 70% ethanol and TDS treatments. The results were compared with those of controls treated with sterile distilled water. The samples were dipped into a suspension of *S.* Typhimurium (8 log cfu/mL) for 10 min and dried for 1 h on a clean bench. The chicken skin samples were then gently washed with PBS and fixed overnight with 2% glutaraldehyde (Sigma-Aldrich Co.). After fixing, the skin samples were washed with PBS for 10 min and treated in 2% osmium tetroxide (OsO_4, Sigma-Aldrich Co.) for 1 h for after fixation. The samples were washed in PBS for 15 min twice to eliminate the fixation solution and subsequently dehydrated using a gradual series of ethanol (50, 60, 70, 80, 90, and 100%). Each ethanol treatment was conducted for 15 min, and the final 100% ethanol treatment was conducted 3 times. The samples were then further dehydrated with 25, 50, 75, and 100% hexamethyldisilazane (Sigma-Aldrich Co.) in ethanol for 15 min. Finally, samples were dried in a freeze dryer for 3 D before being coated with gold palladium for observation by FE-SEM. The FE-SEM was performed at an acceleration voltage of 3 kV at a 5-mm working distance.

Statistical Analysis

All experiments were repeated 3 times with duplicate samples. Experimental data were analyzed by the analysis of variance (ANOVA) procedure using the Statistical Analysis System software, version 9.2 (2008, SAS Institute, Cary, NC). The differences between the chemical treatments were determined using Duncan's multiple range tests, and significant differences were reported at $P < 0.05$.

RESULTS AND DISCUSSION

Autochthonous Flora on Chicken Skin

Poultry meat (including chicken breasts, wings, legs, and skin) is widely consumed around the world, including Korea. In addition, chicken meat in the traditional markets of Korea is often sold without plastic or vinyl containers. Therefore, chicken products can be severely microbially contaminated. In particular, contamination on the surface of chicken skin can be a significant problem. Thus, studying microbial reduction methods in skin-on poultry meat is crucial. This study found contamination of loosely (5.87 log cfu/g),

Table 1. Populations (log cfu/g) of loosely, intermediately, and tightly attached mesophilic aerobic bacteria and coliforms in chicken skin.

Item	Loosely	Intermediately	Tightly
Mesophilic aerobic bacteria	5.87 ± 0.15^{y}	6.84 ± 0.20^{x}	6.72 ± 0.32^{x}
Coliform	3.51 ± 0.27^{x}	3.77 ± 0.26^{x}	3.36 ± 0.41^{x}

$^{x–y}$Means value within the same row with no common superscripts were different ($P < 0.05$).

intermediately (6.84 log cfu/g), and tightly (6.72 log cfu/g) attached MAB on chicken skin (Table 1). This study also found contamination of loosely (3.51 log cfu/g), intermediately (3.77 log cfu/g), and tightly (3.36 log cfu/g) attached coliforms on chicken skin (Table 1). The bacterial counts of naturally existing MAB and coliforms were highest for the intermediately attached cells. In another study, naturally existing MAB and coliform populations in loosely, intermediately, and tightly attached cells were 5.84–4.61 log cfu/g, 6.69–5.06 log cfu/g, and 6.59–5.59 log cfu/g, respectively (Lee et al., 2014). It can be assumed that microorganisms can proliferate on chicken skin that has been damaged during defeathering, plucking, chilling, and subsequent stages of the commercial process (Thomas and McMeekin, 1984; Zhang et al., 2011).

Effect of Treatment on Reduction of Autochthonous Flora on Chicken Skin

The effects of ethanol and TDS treatment on loosely, intermediately, and tightly attached MAB and coliforms present on chicken skin have been shown in Tables 2 and 3. The populations of loosely, intermediately, and tightly attached MAB and coliforms on water-treated (0% ethanol) chicken skin samples were 4.48 and 2.58 log cfu/g, 5.04 and 3.52 log cfu/g, and 5.22 and 3.31 log cfu/g, respectively (data not shown). The single 30% ethanol treatment did not significantly ($P > 0.05$) reduce any of the 3 types of attached MAB and coliforms. However, the 50% and 70% ethanol treatments significantly ($P < 0.05$) reduced loosely (1.02 and 1.59 log cfu/g, respectively), intermediately (0.81 and 0.97 log cfu/g, respectively), and tightly (0.59 and 0.67 log cfu/g, respectively) attached MAB on chicken skin. The 50% and 70% ethanol treatments also significantly ($P < 0.05$) reduced loosely (0.96 and 1.05 log cfu/g, respectively), intermediately (0.73 and 0.93 log cfu/g, respectively), and tightly (0.53 and 0.64 log cfu/g, respectively) attached coliforms on chicken skin. Another study showed that a 10% ethanol treatment alone reduced the number of total coliforms and mesophilic bacteria in cabbage to 2.37 log cfu/g (from 6.60 log cfu/g to 4.23 log cfu/g) and 2.43 log cfu/g (from 9.21 log cfu/g to 6.78 log cfu/g) respectively (Cho et al., 2004). Furthermore, Piernas and Guiraud (1998) observed that a 70% ethanol treatment alone for 10 min rapidly reduced the number of total mesophilic bacteria to about 3.50 log cfu/g in rice sprouts. These studies also demonstrated that the reduction of MAB and coliforms increased as the ethanol concentration increased, which was also observed in this study. TDS treatment alone did not significantly affect ($P > 0.05$) the populations of MAB (0.02–0.10 log cfu/g) or coliforms (−0.02–0.00 log cfu/g) in any of the 3 attachment categories (data not shown). Another study reported that treatment with TDS (100–2,000 mg/L) alone for 5 min was not effective in reducing total aerobic bacteria (0.09–0.88 log cfu/g) or *Escherichia coli* (0.08–0.31 log cfu/g) in head lettuce (Ha et al., 2012). These studies showed that TDS treatment is ineffective for microbial reduction, which was also observed in this study. The combined treatments of 30, 50, or 70% ethanol with TDS stepwise increased ($P < 0.05$) the reduction of MAB in the loosely (0.61, 1.39, and 1.88 log cfu/g), intermediately (0.59, 0.94, and 1.21 log cfu/g), and tightly (0.47, 0.79, and 0.84 log cfu/g) attached cells. Similarly, the combined treatments of 30, 50, or 70% ethanol with TDS resulted in the reduction of coliforms in the loosely (0.41, 1.02, and 1.14 log cfu/g), intermediately (0.38, 0.73, and 1.04 log cfu/g), and tightly (0.34, 0.55, and 0.67 log cfu/g) attached cells. Compared with the ethanol treatment alone, the combination of ethanol and TDS was more effective in reducing loosely attached MAB and coliforms ($P < 0.05$). The combined treatment of 70% ethanol and TDS was effective in reducing intermediately attached MAB. In addition, the ethanol and TDS treatments were more effective in reducing the 3 types of attached MAB than in reducing coliforms (Tables 2 and 3). Ha et al. (2012) reported that the combined treatment of NaOCl (200 ppm) and TDS (1,000 mg/kg) for 5 min reduced MAB and coliforms in head lettuce to 0.86 and 0.74 log cfu/g, respectively. Lee et al. (2014) also reported that the combined treatment of NaOCl (200 ppm) and TDS was more effective in reducing MAB (0.04–0.58 log cfu/g) and coliforms (0.15–0.31 log cfu/g) than a treatment using NaOCl (200 ppm) alone. These results are consistent with the results of this study, which shows that the addition of TDS in the treatment method results in a slightly more reduction of MAB than coliforms.

Effect of Treatment on Reduction of S. Typhimurium on Chicken Skin

Table 4 shows the effects of treatments with ethanol alone, TDS alone, and both ethanol and TDS on loosely, intermediately, and tightly attached *S.* Typhimurium on chicken skin. The average bacterial counts of *S.* Typhimurium on chicken skin treated with water (0% ethanol)

Table 2. Reduction efficacy (log cfu/g) of ethanol alone and ethanol and TDS against loosely, intermediately, and tightly attached MAB in chicken skin.

Treatments	Ethanol (%)	Loosely	Intermediately	Tightly
Without TDS[1]	30	$0.69 \pm 0.18^{c,x}$	$0.60 \pm 0.06^{c,x}$	$0.43 \pm 0.04^{b,x}$
	50	$1.02 \pm 0.02^{b,c,x}$	$0.81 \pm 0.01^{b,c,y}$	$0.59 \pm 0.11^{a,z}$
	70	$1.59 \pm 0.17^{a,b,x}$	$0.97 \pm 0.15^{a,b,y}$	$0.67 \pm 0.04^{a,z}$
with TDS	0	$0.10 \pm 0.12^{d,x}$	$0.10 \pm 0.12^{d,x}$	$0.02 \pm 0.11^{c,x}$
	30	$0.61 \pm 0.10^{c,d,x}$	$0.59 \pm 0.40^{c,x}$	$0.47 \pm 0.09^{b,x}$
	50	$1.39 \pm 0.02^{a,b,x}$	$0.94 \pm 0.17^{a,b,y}$	$0.79 \pm 0.25^{a,y}$
	70	$1.88 \pm 0.19^{a,x}$	$1.21 \pm 0.06^{a,x,y}$	$0.84 \pm 0.15^{a,y}$

[a–f]Means value within the same column with no common superscripts were different ($P < 0.05$).
[x–z]Means value within the same row with no common superscripts were different ($P < 0.05$).
Abbreviation: MAB, mesophilic aerobic bacteria.
[1]TDS: thiamine dilaurylsulfate (1,000 ppm).

were 5.93 log cfu/g, 5.45 log cfu/g, and 4.40 log cfu/g for loosely, intermediately, and tightly attached cells, respectively (data not shown). The 30, 50, and 70% ethanol treatments significantly ($P < 0.05$) reduced the number of loosely (0.64, 1.21, and 1.49 log cfu/g, respectively), intermediately (0.55, 1.25, and 1.59 log cfu/g, respectively), and tightly (0.38, 0.79, and 1.17 log cfu/g, respectively) attached *S.* Typhimurium on chicken skin. The results clearly demonstrate higher reductions of microorganisms with increasing ethanol concentrations. Another study reported that the treatment of a *S.* Typhimurium suspension with ethanol (0–50%) for 5 min reduced the microbial content from 9 log cfu/g to 3 log cfu/g with the increase in the ethanol concentration (Jang et al., 2003). Ethanol is present naturally in a variety of fermented foods and beverages and has been approved as a food additive to enhance flavor and colors in various foods (Seiler and Russell, 1991). Strong antibacterial effect of ethanol which can solubilize lipids and denature proteins causing membrane destruction has been widely studied (Shapiro et al., 1978; Basu and Poddar, 1994; Barker and Park, 2001; Phongphakdee and Nitisinprasert, 2015). TDS treatment (1,000 ppm), on its own, was not effective for the reduction (0.17–0.22 log cfu/g) of loosely, intermediately, or tightly attached *S.* Typhimurium on chicken skin. Similarly, Koo et al. (2018) also demonstrated that 2% TDS–treated tofu reduced *S.* Typhimurium by 0.36 log cfu/g. However, Choi et al. (2015) reported relatively high reduction (2.37 log cfu/g) compared with the present study. The study found that addition of 2% TDS (2%) significantly reduced the population of *S.* Typhimurium inoculated on custard cream by 2.37 log cfu/g during storage at 25°C. The authors also reported that TDS treatment was more effective at 25°C than 4°C, indicating cell membrane fluidity was dependent on temperature. The combined effects of temperature and disinfectant treatment on inactivation of bacteria have been demonstrated (Beuchat and Scouten, 2002; Rahman et al., 2010; Choi et al., 2015). Generally, the TDS was more effective at room temperature (25°C) than at refrigeration temperature (4°C) (Kim et al., 2005; Choi et al., 2015; Koo et al., 2018). Therefore, the low reduction of TDS treatment in this study could be caused by the temperature of chicken skin which were stored in refrigerators before the experiment. In addition, gram-negative bacteria (S. Typhimurium and coliforms) are more susceptible to TDS than gram-positive bacteria, explaining relatively low antibacterial effect of TDS in the present study. The mechanism is due to the effect of antimicrobial peptides which are first attracted to the target bacterial surface by electrostatic interaction between positive charge on the peptides and negative charge on the surface of the bacteria (Brogden, 2005). Zhang et al. (2016) reported that the difference of antimicrobial activity of TDS against gram-positive and

Table 3. Reduction efficacy (log cfu/g) of ethanol alone and ethanol and TDS against loosely, intermediately, and tightly attached coliforms in chicken skin.

Treatments	Ethanol (%)	Loosely	Intermediately	Tightly
without TDS[1]	30	$0.53 \pm 0.17^{bc,x}$	$0.41 \pm 0.05^{c,x}$	$0.33 \pm 0.43^{ab,x}$
	50	$0.96 \pm 0.24^{ab,x}$	$0.73 \pm 0.01^{b,y}$	$0.53 \pm 0.18^{ab,z}$
	70	$1.05 \pm 0.44^{a,x}$	$0.93 \pm 0.14^{ab,x}$	$0.64 \pm 0.11^{a,y}$
with TDS	0	$0.00 \pm 0.11^{d,x}$	$-0.02 \pm 0.03^{d,x}$	$0.00 \pm 0.13^{c,y}$
	30	$0.41 \pm 0.15^{c,x}$	$0.38 \pm 0.01^{c,x}$	$0.34 \pm 0.11^{b,x}$
	50	$1.02 \pm 0.17^{ab,x}$	$0.73 \pm 0.10^{b,y}$	$0.55 \pm 0.11^{ab,z}$
	70	$1.14 \pm 0.10^{a,x}$	$1.04 \pm 0.14^{a,x}$	$0.67 \pm 0.01^{a,y}$

[a–d]Means value within the same column with no common superscripts were different ($P < 0.05$).
[x–z]Means value within the same row with no common superscripts were different ($P < 0.05$).
[1]TDS: thiamine dilaurylsulfate (1,000 ppm).

Table 4. Reduction efficacy (log cfu/g) of ethanol alone and ethanol and TDS against loosely, intermediately, and tightly attached *Salmonella* Typhimurium in chicken skin.

Treatments	Ethanol (%)	Loosely	Intermediately	Tightly
without TDS[1]	30	$0.64 \pm 0.01^{d,x}$	$0.55 \pm 0.04^{cd,y}$	$0.38 \pm 0.01^{e,z}$
	50	$1.21 \pm 0.56^{c,x}$	$1.25 \pm 0.48^{ab,x}$	$0.79 \pm 0.02^{c,y}$
	70	$1.49 \pm 0.01^{b,x}$	$1.59 \pm 0.03^{a,y}$	$1.17 \pm 0.23^{b,z}$
with TDS	0	$0.17 \pm 0.02^{e,x}$	$0.21 \pm 0.05^{d,x}$	$0.22 \pm 0.01^{f,x}$
	30	$0.64 \pm 0.05^{d,xy}$	$0.79 \pm 0.06^{bc,x}$	$0.59 \pm 0.01^{d,y}$
	50	$1.29 \pm 0.06^{c,x}$	$1.38 \pm 0.02^{a,x}$	$1.08 \pm 0.06^{b,y}$
	70	$1.62 \pm 0.23^{a,x}$	$1.72 \pm 0.06^{a,x}$	$1.27 \pm 0.02^{a,y}$

[a–f]Means value within the same column with no common superscripts were different ($P < 0.05$).
[x–z]Means value within the same row with no common superscripts were different ($P < 0.05$).
[1]TDS: thiamine dilaurylsulfate (1,000 ppm).

gram-negative bacteria may have caused the structure of their respective cell wall. While the cell of gram-negative bacteria is formed of a thin peptidoglycan layer, the cell wall of gram-positive bacteria is composed of 1 layer of membrane, which can interact electrostatically with the positively charged area of the bacterial cell membrane (Fransisca et al., 2012). As these different mechanisms of TDS depending on the type of bacteria and correspondence of antimicrobial peptide, appropriate inactivation technique should be applied.

The treatment with TDS alone did not reduce the number of MAB, coliforms, or *S.* Typhimurium in the present study. The combined treatment of 30, 50, or 70% ethanol with TDS did not result in significant reductions ($P > 0.05$) of loosely (0.64–1.62 log cfu/g) or intermediately (0.79–1.72 log cfu/g) attached cells, but it did result in a significant reduction of tightly (0.59–1.27 log cfu/g) attached cells. The best treatment for *S.* Typhimurium was the combination of 70% ethanol and TDS. The reduction of microorganisms was also more effective when 70% ethanol was combined with TDS than by the treatment with only 70% ethanol, which indicates that TDS is effective for microbial reduction in combination with ethanol. Furthermore, the advantages of TDS include not only its antimicrobial properties but also its nutritional function, as it provides vitamin B. Choi et al. (2015) found that the antimicrobial effects of TDS are due to its components and structures, such as SLS and the thiazole ring. SLS damages cell membranes and interferes with the proliferation of microorganisms by disrupting protein functions (Rykke et al., 1990). In addition, the thiazole ring of TDS can disrupt the lipid bilayer of the cytoplasmic membrane (Thorsteinsson et al., 2003). A study previously reported that a combined treatment with NaOCl (200 ppm) and TDS did not reduce the number of *S.* Typhimurium (0.01–0.27 log cfu/g) significantly as compared with treatment with only NaOCl (200 ppm) (Lee et al., 2014). Because TDS dissolves better in organic solvents such as ethanol and acetic acid (Kim et al., 2005), TDS may have been effective in the present study because TDS dissolves better in ethanol than in NaOCl. Thus, the combination of ethanol and TDS can be used as an antimicrobial treatment to prevent *S.* Typhimurium. However, further studies are required on the synergistic effect of TDS in combination with other chemicals to broaden antibacterial effect of TDS. In addition, various food handling conditions such as storage temperature should be considered for commercial sanitizers and its impact on product quality and shelf life.

Field Emission Scanning Electron Microscopy

FE-SEM micrographs of loosely, intermediately, and tightly attached *S.* Typhimurium on chicken skin are

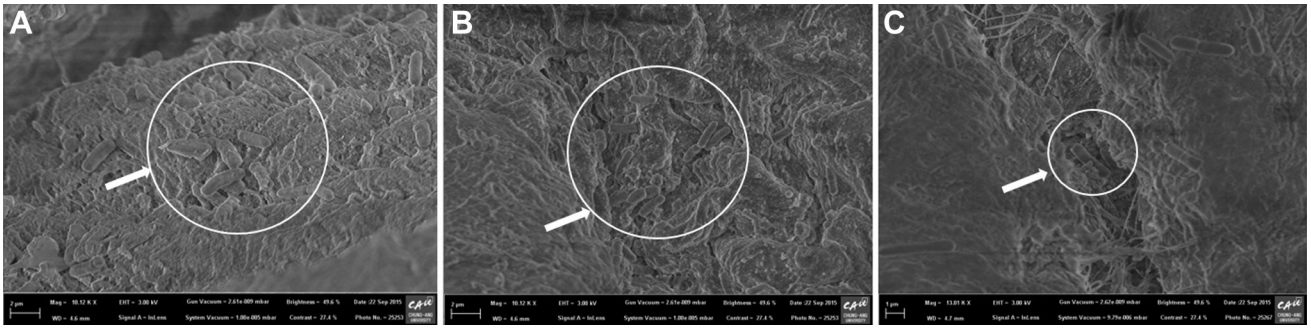

Figure 1. Field emission scanning electron microscopy (FE-SEM) images of *S.* Typhimurium attached to chicken skin (arrows; 10,000 × magnification). (a) Loosely attached *S.* Typhimurium on chicken skin. (b) Intermediately attached *S.* Typhimurium on chicken skin. (c) Tightly attached *S.* Typhimurium on chicken skin.

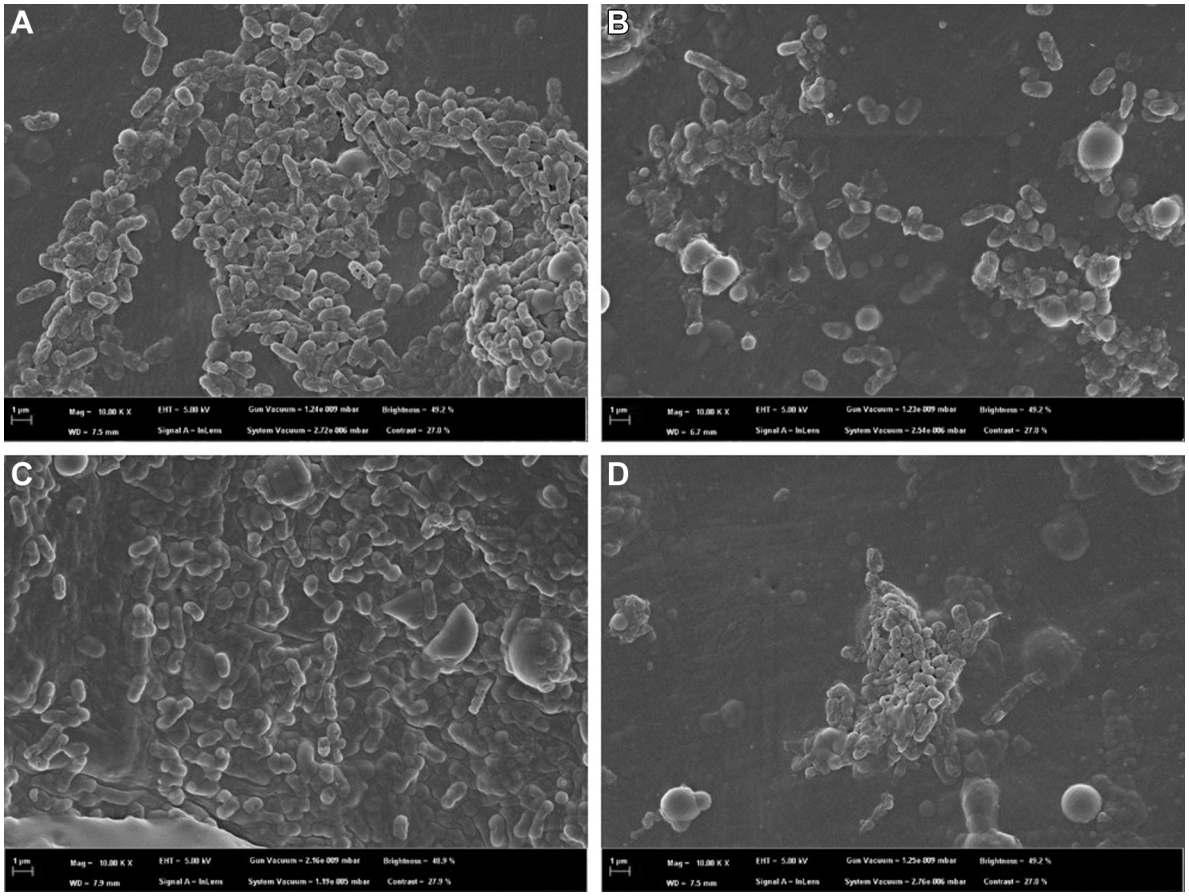

Figure 2. FE-SEM images of *S.* Typhimurium attached to chicken skin after 70% ethanol and TDS treatment (arrows; 20,000 × magnification). (a) Effect of sterile distilled water (control) on *S.* Typhimurium on chicken skin. (b) Effect of ethanol treatment on *S.* Typhimurium on chicken skin. (c) Effect of TDS treatment on *S.* Typhimurium on chicken skin. (d) Effect of combined ethanol and TDS treatment on *S.* Typhimurium on chicken skin. FE-SEM, field emission scanning electron microscopy; TDS, thiamine dilaurylsulfate.

shown in Figure 1. The loosely attached *S.* Typhimurium on the flat surfaces of chicken skin (Figure 1a) and the intermediately (Figure 1b) and tightly (Figure 1c) attached *S.* Typhimurium on the ridges and crevices of chicken skin were visualized. The intermediately attached *S.* Typhimurium was visually much more prominent than loosely attached cells. The results show that a high number of *S.* Typhimurium remained attached to the ridges and crevices of the chicken skin after treatment with sterile water. The FE-SEM micrographs in Figure 2 show the effect of water (control) (Figure 2a) which has no effect on numbers of S. Typhimurium on chicken skin, 70% ethanol (Figure 2b), TDS treatment (Figure 2c), and combined treatment with 30% ethanol and TDS (Figure 2d) on *S.* Typhimurium. The results show that the cell membranes of *S.* Typhimurium might be

Table 5. Color parameters (L*, a*, and b*)[1] and shear force values (kg/cm^2) for chicken skin treated with ethanol alone and ethanol and TDS.

Treatments					
Ethanol (%)	TDS	L*	a*	b*	Shear force (kg/cm^2)
Control	0	84.75 ± 0.27^{NS}	4.30 ± 0.32^{b}	13.37 ± 0.90^{e}	0.33 ± 2.52^{c}
without TDS[2]	30	84.92 ± 0.65	3.96 ± 0.210^{d}	14.87 ± 1.23^{b}	0.33 ± 4.54^{bc}
	50	84.40 ± 0.85	2.29 ± 0.70^{d}	16.70 ± 0.31^{c}	0.39 ± 4.82^{ab}
	70	84.88 ± 0.51	2.75 ± 0.08^{cd}	16.35 ± 0.46^{c}	0.43 ± 1.74^{a}
with TDS	0	84.72 ± 0.72	3.69 ± 0.38^{bc}	13.80 ± 0.47^{de}	0.31 ± 1.44^{c}
	30	84.08 ± 0.57	3.83 ± 0.09^{a}	14.38 ± 0.23^{cd}	0.34 ± 0.88^{bc}
	50	84.85 ± 0.33	2.51 ± 0.15^{d}	18.46 ± 0.33^{b}	0.39 ± 1.43^{a}
	70	84.29 ± 1.84	2.73 ± 1.00^{bc}	18.87 ± 0.49^{a}	0.41 ± 3.60^{a}

^{NS}No significance; means value within a same column are no different ($P > 0.05$).

$^{a–c}$Means value within the same column with no common superscripts were different ($P < 0.05$).

The means and standard deviations were calculated based on 10 replicates (color) and 10 replicates (texture).

[1]Color are L* (lightness), a* (redness), b* (yellowness).

[2]TDS: thiamine dilaurylsulfate (1,000 ppm).

destroyed by 70% ethanol treatment (Figure 2b), which is consistent with the finding that ethanol can pass freely through the membranes of microorganisms because of its small molecular size (Ingram, 1989). Furthermore, ethanol can inhibit the cross-linking of peptidoglycans through ethanol decomposition. These results demonstrate that ethanol is highly effective in microbial reduction. *S.* Typhimurium became inflated when surrounded by TDS molecules (Figure 2c); however, TDS alone minimally eliminated the number of cells in this study. A previous study reported that the peptidoglycans of *S.* Typhimurium were severely bloated by TDS treatment and that the growth of the microorganisms was decreased through the enzyme-inhibiting enzymatic activities of TDS molecules that were absorbed into the cell membrane (Kim et al., 2005). Addition of other chemical disinfectants that interact with their outer membrane could improve the inactivation of TDS against gram-negative bacteria. In addition, as a large number of *Salmonella* were entrapped in the ridges and crevices after a water treatment, the bacteria could be difficult to remove. Noriega et al. (2011) reported the difficulty of removing pathogens from crevices or folded sections with a single treatment. Treatment with 30% ethanol and TDS (Figure 2d) showed a lower density of bacteria in the crevices of chicken skin and remarkably reduced the cells.

Color and Texture

Table 5 shows changes in the quality (color and texture) of chicken skin treated with ethanol and TDS. Significant differences ($P > 0.05$, Table 5) were not observed in Hunter color L* values. However, as the ethanol concentration increased, color a* values decreased and color b* values increased ($P < 0.05$, Table 5). Thus, chicken skin samples treated with higher ethanol concentrations were slightly more yellow or slightly less red than chicken skin samples treated with lower ethanol concentrations or water (control). In addition, as the ethanol concentration increased, the shear force, or the hardness of chicken skin, increased ($P < 0.05$, Table 5). Treatments with ethanol at concentrations higher than 50% changed the color and texture of chicken skin, which are qualities important to consumers (Sharma, Ates, Joseph, Nannapanei and Kiess, 2013). Weak chicken skin can be easily ruptured during mechanical operation, which can reduce the shelf life of the meat (Salim et al., 2012). A study by Lee et al. (2014) suggested that TDS treatment does not change the color of chicken skin. However, chicken skin treated with TDS was weaker than the control sample. The present study found that the color and texture of chicken skin were significantly changed by treatment with 50% or higher concentrations of ethanol. Previously, various studies have confirmed with sensorial and instrumental analysis (Basu and Poddar, 1994; Lachenmeier, 2008; Phongphakdee and Nitisinprasert, 2015) that chicken skin samples become yellower and harder with increasing concentrations of ethanol. Hall and Spencer (1964) reported that a 70% ethanol treatment increased the shelf life of packaged chicken, although the water-treated chicken (control) was reported to have better flavor than the ethanol-treated chicken. Further study is required to determine an ethanol concentration that does not affect the quality of chicken meat.

CONCLUSION

This study demonstrated that the intermediately and tightly attached bacteria on chicken skin were more resistant to the combination of ethanol and TDS than the loosely attached bacteria. The reduction of the loosely attached cells was enhanced in all treatment conditions compared with the intermediately and tightly attached cells. Increasing concentrations of ethanol improved the reduction of loosely, intermediately, and tightly attached MAB, coliforms, and *S.* Typhimurium on chicken skin. The reductions of microorganisms achieved with 30, 50, and 70% ethanol treatments were comparable ($P < 0.05$) with those achieved with combined treatments of 30, 50, and 70% ethanol with TDS. Changes in the color and texture of chicken skin were observed in all treatments with 50% ethanol or higher. The combination of 30% ethanol and TDS was considered the best treatment because it did not change the quality of the chicken skin. In conclusion, the results of the present study suggest that the combination of 30% ethanol and TDS may be the optimal treatment for the reduction of contamination in skin-on chicken products, and it may enhance poultry safety without decreasing food quality.

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SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

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