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Effect of thiamine dilaurylsulfate against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Listeria monocytogenes* and *Bacillus cereus* spores in custard cream

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ABSTRACT

This study was conducted to investigate the antimicrobial effect of thiamine dilaurylsulfate (TDS), known as a vitamin B_1 derivative, on custard cream. The inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Bacillus cereus* spores in custard cream over a 5 day storage period and the influence of storage temperature at 4 and 25 °C were investigated. Samples were inoculated with a cocktail of these pathogens and subjected to addition of TDS at concentrations ranging from 0 to 2%. As the concentration of TDS and storage time increased, the inactivation effect increased among all tested pathogens except for *B. cereus*. The addition of 2% TDS achieved 2.43-, 2.37-, and 0.93-log reductions in inoculated *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, during 5 day storage at 25 °C. Gram-negative bacteria were more susceptible to TDS than Gram-positive bacteria, and TDS was more effective at 25 °C than 4 °C. However, regardless of TDS concentration during storage at 4 and 25 °C, there were no significant ($P > 0.05$) differences in populations of *B. cereus* spores between untreated control and TDS-treated samples. These results suggest that TDS has potential for preventing spoilage of dairy products by improving microbiological safety.

1. Introduction

Custard cream is traditionally used to make cream puffs, pastries and cakes in a variety of culinary preparations. It is mainly made from milk and eggs (Arakawa et al., 2008). Although it sometimes contains sugar, colorants, flavors, and modified starch, custard cream is considered a dairy product due to its main ingredients (de Wijk, van Gemert, Terpstra, & Wilkinson, 2003). The 2010 Dietary Guidelines for Americans emphasize that most Americans should focus on consuming nutrient-dense foods like dairy products. However, foodborne outbreaks from contaminated dairy products such as milk, yogurt, cheese, and dairy desserts including custard cream, continue to be reported to the Center for Disease Control and Prevention (CDC) (Langer et al., 2012). This is because these can be sources of a variety of microorganisms and can readily harbor foodborne pathogens during re-processing, preservation, transportation, and sale (Oliver, Jayarao, & Almeida, 2005).

Custard cream has a shelf life which is close to or shorter than that of milk (Dieu & CuQ, 1989). Foodborne pathogens of high public health concern, including *Escherichia coli* O157:H7, *Salmonella* Typhmurium, *Listeria monocytogenes*, and *Bacillus cereus*, are able to grow in custard cream (Bennett, Walsh, & Gould, 2013; Oliver et al., 2005). *E. coli* O157:H7 causes hemolytic uremic syndrome and diarrhea, and has a long history of outbreaks involving raw foods and dairy products (CDC, 2011). *S.* Typhmurium is the most common pathogen among *Salmonella* serotypes. An outbreak of *Salmonella* infections due to consumption of contaminated custard cream occurred in Australia in January 2011. During this outbreak, a reported 74 people became ill (South Australian Department for Health and Ageing, 2012). In Massachusetts in

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November 2007, *L. monocytogenes* caused an outbreak of listeriosis associated with pasteurized milk (CDC, 2008). *B. cereus* has not been frequently linked to foodborne outbreaks associated with dairy products (Bennett et al., 2013). Nonetheless, *B. cereus* has a high incidence in dairy products because *B. cereus* spores survive the pasteurization process. *B. cereus* spoils the quality of dairy products as well as producing diarrheal and emetic toxins (Kim, Lee, & Ha, 2011; Wong, Chang, & Fan, 1988).

For this reason, custard cream should receive an additional antimicrobial intervention before packaging, even though the milk component was pasteurized previously. To reduce foodborne pathogens and prolong the shelf life of custard, thermal processing and chemical preservatives have been evaluated (Arakawa et al., 2008). Many combinations of time and temperature such as low temperature long time (LTLT), high temperature short time (HTST), and ultra high temperature (UHT) pasteurization are widely used in industry. However, these thermal processes can cause the Maillard reaction and destroy heat sensitive compounds, thus resulting in loss of nutrients and flavor (Newton, Fairbanks, Golding, Andrews, & Gerrard, 2012). Although chemical preservatives can alleviate the problem of heat treatment, there is the limitation of poor consumer acceptance due to their perceived harmful effects on health. Biopreservatives like bacteriocins produced by lactic acid bacteria have been proposed to prevent bacterial growth in custard cream (Arakawa et al., 2009), but constraints regarding their narrow antimicrobial activity spectrum still limit their widespread use (Mills, Stanton, Hill, & Ross, 2011).

There has been an increasing interest in food preservatives that not only have a broad spectrum of antimicrobial effects but are also beneficial to human health. One of these preservatives is thiamine dilaurylsulfate (TDS) which is known as a vitamin B_1 derivative. TDS is nontoxic and bland tasting, and is an amphiphilic compound that forms a dispersed emulsion in water. The antibiotic effects of TDS are due to its structure and components such as sodium lauryl sulfate (SLS) and a thiazole ring. SLS damages biological membranes and inhibits proliferation of microorganisms resulting from disrupting the conformation of protein molecules (Rykke, Rolla, & Sonju, 1990). It is also reported that the quaternary amine group in the thiazole ring of TDS can perturb lipid bilayer membranes that constitute the cytoplasm membrane (Thorsteinsson et al., 2003). Thiamine, another component of TDS, exhibits nutritional functions by involvement in a variety of metabolic processes such as carbohydrate metabolism (Kaneda et al., 1997). The advantages of TDS include that it maintains its antimicrobial properties and improves the membrane transport ability of thiamine in cells (Wei et al., 2014).

TDS has been approved for use as a food additive in Korea and Japan. However there are few research studies on its antimicrobial effects and application. Thus, the overall objective of this study was to investigate the effect of TDS for inactivating *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes* and *B. cereus* spores in custard cream.

2. Materials and methods

2.1. Bacterial strains

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889 and ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43174 and DT 104), *L. monocytogenes* (ATCC 7644, ATCC 19114 and ATCC 19115), and *B. cereus* (ATCC 10876, ATCC 13061, W-1) were obtained from the Department of Food and Animal Biotechnology culture collection at Seoul National University (Seoul, South Korea). Stock cultures in 0.7 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD, USA) and 0.3 ml of 50% glycerol were stored at −80 °C. Working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37 °C for 24 h, and stored at 4 °C.

2.2. Preparation of pathogen inocula

Each strain of *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes* was cultured in 5 ml TSB at 37 °C for 24 h, harvested by centrifugation at 4000 × g for 20 min at 4 °C and washed three times with buffered peptone water (BPW; Difco). The final pellets were resuspended in BPW, corresponding to approximately 10^9 CFU/ml. To produce *B. cereus* spores, 0.1 ml of cell suspensions grown in TSB for 24 h at 37 °C were spread-plated onto TSA, and incubated at 37 °C for 7 days until at least 80% sporulation was reached as determined by microscopic examination. Spores of each strain were individually harvested by depositing 1 ml of sterile water onto the surface of TSA culture plates. Spores were dislodged by gently rubbing with a sterile swab. Pooled spore suspensions of 10 plates of each strain were centrifuged at 4000 × g for 20 min at 4 °C, and washed 3 times with BPW. The final spore concentration in BPW was approximately 10^8 spores/ml. Subsequently, resuspended pellets of each strain of all pathogen species were combined to construct a 4-pathogen mixed culture cocktail.

2.3. Sample preparation and inoculation

Custard cream was obtained from Samlip Inc., (Seoul, Republic of Korea) and stored at 4 °C prior to the experiment. In this study, five concentrations of TDS (0.1%, 0.5%, 1%, and 2%) were selected. One hundred μl of the mixed culture cocktail (*E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes* and *B. cereus* spores) and predetermined amounts of TDS (Sanigen Co., Ltd., Gyeonggi, Republic of Korea) were added to 25 g of each sample of custard cream in 250 ml sterile containers at room temperature (22 ± 2 °C), and then mixed for 2 min by stirring with a sterile spatula. The final cell concentration of pathogens and spores was 10^6–10^7 CFU/g and 10^5–10^6 CFU/g, respectively. In order to identify the effect of TDS on inactivation of *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes*, and *B. cereus* spores in custard cream during storage, samples were packed into a sterile container and stored at 4 °C for 5 days. Inoculated and conditioned custard cream samples were used in subsequent experiments.

2.4. Bacterial enumeration

For enumeration of pathogens, each conditioned 25 g sample was transferred into a sterile stomacher bag (Labphas, Inc., Sainte-Juilie, Quebec, Canada) containing 50 ml BPW and homogenized with a stomacher (Easy Mix; AES Chemunex, Rennes, France) for 2 min. One ml aliquots of stomached samples were serially diluted in 9 ml of BPW, and 0.1 ml of sample or diluent was spread-plated onto each selective medium. Sorbitol MacConkey agar (SMAC; Difco), xylose lysine desoxycholate agar (XLD; Difco), and Oxford agar base (OAB; Difco) with antimicrobic supplement (Bacto Oxford antimicrobic supplement, Difco) were used as selective media for enumeration of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*, respectively. For *B. cereus* spores, samples were subjected to heat-shocking at 90 °C for 10 min to stimulate spore germination and inactivate vegetative cells, including those of the other pathogen species. Subsequently, samples were serially diluted and spread-plated onto mannitol egg yolk polymyxin agar (MYP, Difco). All plates were incubated at 37 °C for 24–48 h, and then typical colonies were counted.

Table 1
Survival of *Escherichia coli* O157:H7 in custard cream containing TDS stored at 4 and 25 °C for 5 days.[a]

TDS conc. (%)[b]	Population (log_{10} CFU/g) by storage time						
	0 Day	1 Day		3 Day		5 Day	
		4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
0	7.40 ± 0.21 A	7.20 ± 0.01 Aa	6.77 ± 0.50 Aa	6.94 ± 0.08 Aa	6.45 ± 0.40 Aa	6.60 ± 0.27 Aa	6.10 ± 0.17 Aa
0.1	7.28 ± 0.29 A	7.24 ± 0.25 Aa	6.34 ± 0.30 ABb	6.83 ± 0.23 Aa	6.10 ± 0.35 ABb	6.34 ± 0.38 ABa	5.64 ± 0.56 ABa
0.5	7.22 ± 0.24 A	7.11 ± 0.32 Aa	6.20 ± 0.36 ABb	6.40 ± 0.26 BCa	5.69 ± 0.21 BCb	6.09 ± 0.09 ABa	5.26 ± 0.45 Bb
1	7.36 ± 0.11 A	7.12 ± 0.16 Aa	6.35 ± 0.52 ABa	6.69 ± 0.27 ABa	5.64 ± 0.46 BCb	6.17 ± 0.48 ABa	5.20 ± 0.43 Ba
2	7.29 ± 0.29 A	6.78 ± 0.42 Aa	5.93 ± 0.20 Bb	6.26 ± 0.22 Ca	5.14 ± 0.29 Cb	5.83 ± 0.42 Ba	4.97 ± 0.31 Bb

[a] Means ± standard deviations from three replications. Means with the same uppercase letter in the same column are not significantly different ($P > 0.05$). Means with the same lowercase letter in the same row per storage time are not significantly different ($P > 0.05$).
[b] TDS conc., TDS concentration.

2.5. Statistical analysis

All experiments were repeated three times with duplicate samples. Data were analyzed by the analysis of variance procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Means values were separated using Duncan's multiple-range test, and $P < 0.05$ was used to determine significant differences.

3. Results and discussion

3.1. Inactivation of foodborne pathogens in custard cream with TDS

The effectiveness of TDS for reduction of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in custard cream at 4 or 25 °C for up to 5 days storage was investigated, and results are shown in Tables 1, 2, and 3, respectively. There were no significant ($P > 0.05$) differences in microbial populations between untreated control and TDS-treated samples on day 0. However, during storage, surviving populations of those pathogens decreased with increasing TDS concentration. The addition of 2% TDS significantly ($P < 0.05$) reduced levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* by 2.43, 2.37, and 0.93 log CFU/g, respectively, during 5 day storage at 25 °C. Although the number of pathogen cells in the control reduced slightly in accordance with storage due to depletion of nutrients necessary for survival, significant ($P < 0.05$) reductions were observed in custard cream with TDS compared to the control. Other researchers also reported the antimicrobial effect of TDS. Fransisca and Feng (2012) found that 1% TDS reduced *E. coli* O157:H7 87-23 by 2.17, 0.99, and 0.38 log CFU/g on alfalfa, broccoli, and radish seed surfaces, respectively. Wei et al. (2014) reported that TDS has an antimicrobial effect as a preservative in apple juice and pasteurized milk.

The average bacterial concentrations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* inoculated on custard cream were ca. 6 log CFU/g. A high-inoculum concentration was used to enumerate surviving bacteria easily. These inoculation levels were much higher than would occur in commercial dairy products, since the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) has established a zero tolerance of *L. monocytogenes* in ready-to-eat foods including dairy products (USDA, 1989). Even in raw milk, usually less than 2 log CFU/ml of pathogens are detected (Van Kessel, Karns, Gorski, McCluskey, & Perdue, 2004). Therefore, keeping in mind the low numbers of foodborne pathogens present custard cream products, the application of TDS capable of achieving a 1- to 2-log reduction of pathogens would be sufficient to render the TDS-treated product pathogen free.

The effect of TDS on product quality and sensorial properties were not investigated thoroughly, since this study was focused on its antimicrobial effect. After the maximum treatment applied for inactivation of foodborne pathogens, in appearance, color and texture of samples were not different those of the control. It has been proposed that TDS did not affect the product quality change. Lee, Park, Kang, and Ha (2014) found that the TDS treated chicken skin maintained color and texture. Fransisca, Park, and Feng (2012) concluded that the combination treatment of TDS and malic acid produced no loss in germination rate and caused little change in sensorial properties on alfalfa seeds. As a potential food preservative, more work needs to be published on the effectiveness of TDS for promoting microbiological safety and its impact on product quality.

3.2. Effect of cell wall structure of foodborne pathogens and storage temperature

In the present study, regardless of TDS concentration during storage at 4 and 25 °C, Gram-negative bacteria (*E. coli* O157:H7 and *S.* Typhimurium) were more susceptible to TDS than Gram-positive bacteria (*L. monocytogenes*). This is in agreement with the effect of antimicrobial peptides on inactivation of pathogens. The antimicrobial peptides are firstly attracted to the target bacterial surface

Table 2
Survival of *Salmonella* Typhimurium in custard cream containing TDS stored at 4 and 25 °C for 5 days.[a]

TDS conc. (%)[b]	Population (log_{10} CFU/g) by storage time						
	0 Day	1 Day		3 Day		5 Day	
		4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
0	7.26 ± 0.12 A	6.97 ± 0.25 Aa	6.72 ± 0.25 Aa	6.66 ± 0.15 Aa	6.46 ± 0.45 Aa	6.28 ± 0.15 Aa	5.81 ± 0.29 Aa
0.1	7.40 ± 0.40 A	7.14 ± 0.40 Aa	6.45 ± 0.17 ABa	6.71 ± 0.18 Aa	5.97 ± 0.21 ABb	6.11 ± 0.13 ABa	5.26 ± 0.31 Bb
0.5	7.01 ± 0.35 A	7.03 ± 0.40 Aa	6.37 ± 0.17 ABa	6.60 ± 0.19 Aa	5.77 ± 0.43 Bb	6.22 ± 0.19 Aa	5.16 ± 0.23 Bb
1	7.12 ± 0.18 A	7.02 ± 0.32 Aa	6.33 ± 0.35 ABa	6.66 ± 0.15 Aa	5.94 ± 0.21 Bb	6.06 ± 0.06 ABa	5.03 ± 0.23 Bb
2	7.15 ± 0.19 A	6.97 ± 0.27 Aa	6.23 ± 0.20 Bb	6.48 ± 0.10 Aa	5.74 ± 0.23 Bb	5.92 ± 0.06 Ba	4.89 ± 0.11 Bb

[a] Means ± standard deviations from three replications. Means with the same uppercase letter in the same column are not significantly different ($P > 0.05$). Means with the same lowercase letter in the same row per storage time are not significantly different ($P > 0.05$).
[b] TDS conc., TDS concentration.

Table 3
Survival of *Listeria monocytogenes* in custard cream containing TDS stored at 4 and 25 °C for 5 days.[a]

TDS conc. (%)[b]	Population (log_{10} CFU/g) by storage time						
	0 Day	1 Day		3 Day		5 Day	
		4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
0	6.78 ± 0.27 A	6.75 ± 0.05 Aa	6.56 ± 0.25 Aa	6.51 ± 0.05 Aa	6.61 ± 0.21 Aa	6.42 ± 0.11 Aa	6.20 ± 0.17 ABa
0.1	6.67 ± 0.17 A	6.59 ± 0.18 Aa	6.55 ± 0.13 Aa	6.46 ± 0.23 ABa	6.46 ± 0.11 Aa	6.24 ± 0.27 Aa	6.16 ± 0.32 ABa
0.5	6.63 ± 0.13 A	6.64 ± 0.20 Aa	6.52 ± 0.19 Aa	6.40 ± 0.14 ABa	6.48 ± 0.07 Aa	6.20 ± 0.03 Aa	6.21 ± 0.04 ABa
1	6.80 ± 0.12 A	6.69 ± 0.17 Aa	6.64 ± 0.18 Aa	6.36 ± 0.23 ABa	6.47 ± 0.27 Aa	6.19 ± 0.26 Aa	6.42 ± 0.21 Aa
2	6.64 ± 0.44 A	6.21 ± 0.62 Aa	6.49 ± 0.36 Aa	6.01 ± 0.44 Ba	6.03 ± 0.13 Ba	5.76 ± 0.24 Ba	5.85 ± 0.05 Ba

[a] Means ± standard deviations from three replications. Means with the same uppercase letter in the same column are not significantly different ($P > 0.05$). Means with the same lowercase letter in the same row per storage time are not significantly different ($P > 0.05$).
[b] TDS conc., TDS concentration.

by electrostatic interaction between the positive charge on the peptides and the negative charge on the surface of the bacterium. After attraction to the target surface, these peptides are able to disturb the cytoplasmic membrane by forming ion channels, transmembrane pores and eventually causing cell lysis (Brogden, 2005). The net negative charge on the surface of Gram-negative bacteria is attributed to the anionic phospholipids and phosphate groups of lipopolysaccharides on the outer envelope. While teichoic acids and lipoteichoic acids on the surface of Gram-positive bacteria contribute to its net negative charge (Murray, Rosenthal, & Pfaller, 2009). According to Chung et al. (2004), Gram-negative bacteria demonstrate a higher negatively charged density distribution than that of Gram-positive bacteria. This mechanism can apply to the different inactivation effect of TDS depending on the type of bacteria, because the structure of TDS corresponds with that of antimicrobial peptides (Brogden, 2005).

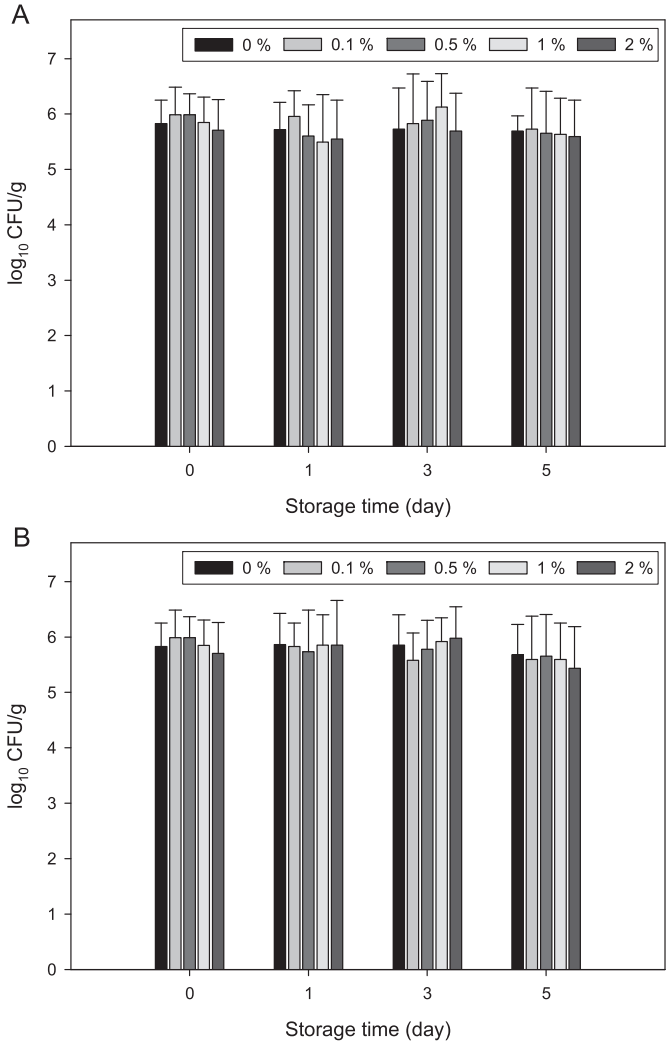

Fig. 1. Survival of *Bacillus cereus* spores in custard cream containing TDS stored at 4 (A) and 25 °C (B) for 5 days. The results are means from three experiments, and error bars indicate standard deviations.

There have been some research efforts to clarify the combined effect of temperature and chemical treatments on inactivation of foodborne pathogens (Bang, Kim, Kim, Beuchat, & Ryu, 2011; Beuchat & Scouten, 2002; Rahman, Ding, & Oh, 2010; Venkitanarayanan, Zhao, & Doyle, 1999). However, there has been no research on the inactivation of foodborne pathogens in dairy products by TDS treatment relative to storage temperature. In this study, we determined that storing custard cream with TDS at 25 °C produced significantly ($P < 0.05$) grater population decreases of *E. coli* O157:H7 compared to 4 °C, resulting in additional reductions of 0.85, 1.12, and 0.86 log for 1, 3, and 5 days, respectively. Although there was no significant ($P > 0.05$) difference between 4 and 25 °C for *L. monocytogenes* due to the slight effect of TDS on inactivation, patterns of inactivation for *S.* Typhimurium were similar to those of *E. coli* O157:H7. These results could be explained by the relationship between temperature and cell membrane fluidity. Membrane fluidity is dependent on temperature (Murray et al., 2009). Bigelow, Wiessner, Kleinman, and Mandel (1997) reasoned that increases in membrane fluidity enables increased molecular lateral motion within the membrane, resulting in easier formation of complementary bonding array on the cell membrane surface. This rearrangement could be supportive of bonding interactions between TDS and the cell membrane.

3.3. Inactivation of *B. cereus* spores in custard cream with TDS

Fig. 1 shows surviving populations of *B. cereus* spores in custard cream with TDS during 5 day storage at 4 and 25 °C. All the selected concentrations of TDS resulted in a less than 0.5-log reduction of spore numbers at 4 and 25 °C. These results are similar to those of another study reporting the survival of *B. cereus* spores in rice with 1000 ppm added TDS; there were no significant decreases in microbial populations (Lee, Ha, & Ha, 2010). Shin et al. (2008) concluded that 2.5% TDS demonstrated a slightly greater than 1 log CFU/ml reduction of *B. cereus* spores in distilled water 24 h after application. This low sporicidal activity was attributed to distinct structural characteristics of *B. cereus* spores. These spores have a multilayer structure to protect DNA and essential proteins from harmful environmental stresses. Synthesis of α/β type small acid soluble proteins and saturation to DNA during spore forming

contributes to the resistance of spores to adverse environmental interference (Montville & Matthews, 2008).

Many researchers have investigated the synergistic effects of TDS with other control methods for reduction of *B. cereus* spores. Lee et al. (2010) reported that sanitizer-treated rice with added TDS exhibited a greater reduction of *B.* cereus spores than sanitizer-treated rice alone without degrading sensory properties. Similar results were observed in distilled water treated with 60 °C heat and 2.5% TDS (Shin et al., 2008). Besides *B. cereus* spores, other pathogens have been shown to be inactivated by the synergistic effect of TDS in combination with commercial sanitizers such as hydrogen peroxide, chlorine, quaternary ammonium compounds, ethanol, and calcium oxide (Lee & Ha, 2008). Therefore, in order to increase the antimicrobial effect of TDS, further studies involving combined treatments are required.

4. Conclusions

The antimicrobial effect of TDS as a food preservative on custard cream was investigated. The addition of TDS significantly ($P < 0.05$) reduced levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* during 5 day storage. Gram-positive bacteria were more resistant to TDS than Gram-negative bacteria. As the concentration of TDS, storage time, and storage temperature increased, the inactivation effect increased among those pathogens. However, there were no significant ($P > 0.05$) differences in populations of *B. cereus* spores when untreated control and TDS-treated samples were compared. In order to broaden the TDS antimicrobial spectrum without affecting product quality, further studies are required on the synergistic effect of TDS in combination with commercial sanitizers and its impact on product quality and shelf life.

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