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Investigating the Effects of Lactic-Citric Acid Blend and Sodium Lauryl Sulfate on the Inhibition of Shiga Toxin-Producing *Escherichia coli* in a Broth System

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Abstract

Food grade organic acids are used to control undesirable microbes in foods and are commonly diluted in water to facilitate application of desired concentrations of antimicrobial agents. However, water is a poor wetting agent for hydrophobic environments. In hydrophobic environments this problem can be circumvented by use of a food grade processing aid. Sodium lauryl sulfate (SLS) is a surfactant that is generally regarded as a safe food additive. The purpose of this study was to investigate the ability of a lactic-citric acid (LCA) blend and SLS to control the growth of Shiga toxin-producing *Escherichia coli* (STEC) in a broth system. *E. coli* O157:H7 and a cocktail of non-O157:H7 (O26, O45, O103, O11, O121, O145, O104:H4) strains were evaluated separately (8.0 log CFU/g). A blend solution of LCA at a 2.4% concentration and SLS at concentrations of 0.05%, 0.25%, and 0.5% were evaluated individually and in combination. Samples were plated onto Sorbitol MacConkey agar infused with rifampicin (100 µg/ml) and incubated at 37°C for 24 h. When used alone the treatments showed no individual effects ($p>0.05$) of the LCA blend or SLS on the control of O157:H7 and the non-O157:H7 cocktail in the BHI broth. Applying the combined SLS (0.05% and 0.5%) and LCA blend (2.4%) in the broth significantly ($p<0.01$) reduced the non-O157:H7 by 5.5 log CFU/g and 2.9 and 4.6 log CFU/g in O157:H7 stains. Increasing the SLS concentration (0.25%) in LCA blend (2.4%) was more effective ($p<0.01$) on O157:H7, showing 5 log CFU/g reduction. This work will assist with providing new information on enhancing the wettability and the exposure of pathogens to antimicrobial treatment.

Keywords: Surfactants; STEC; Organic acids; Lactic citric acid

Introduction

Despite the efforts that have been made by the scientific community to reduce the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) in food systems, this group of pathogens remains a serious public health concern. Recent outbreaks illustrate this [1] and therefore, efforts are being made to eliminate this risk from the food supply. A plethora of physical and chemical interventions have been examined to eliminate this pathogen in the food supply. Pulsed-electric field, high pressure processing, ultrasound, radiation, and biological and chemical interventions have been previously evaluated for their ability to reduce or control STEC populations in various foods [2]. Because no intervention system is 100% effective, "multiple-hurdle" systems have gained popularity in the food industry [3,4]. Although *E. coli* O157:H7 strains are the most studied of all STEC and has been evaluated against many intervention [5,6] data on the effectiveness of interventions on non-O157 STEC serotypes, such as O26, O45, O103, O111, O121, O145 and O104:H4 is lacking. Identification of the most effective interventions against STEC serotypes will aid in designing "multiple-hurdle" strategies to reduce pathogenic *E. coli* populations, and is important in protecting public health, especially since *E. coli* O157:H7 and strains of "The Big Six" serotypes are now both considered adulterants in ground beef products [7].

Coupled with the concern of having effective interventions to combat the prevalence of food pathogens in our food supply, another growing concern is that of biofilms. Biofilms are a mixture of bacteria and extracellular remnants that are secreted by bacterial cells and are difficult to eradicate from surfaces because they offer bacterial cells biological and physical as well as mechanical protection [8]. Biofilms continue to be a major problem in the meat industry [9]. Because of the frequent processing of meat parts and the continuous contact with various surfaces, this environment promotes the formation of biofilms, which increases the chances of a foodborne outbreaks [9,10]. Interactions between food pathogens and resident bacteria (microbiota) occur on production processing surfaces. These interactions make bacteria and the resulting biofilms immune to environmental stresses (pH, temperature) and can increase growth and maintain survival. Many interventions to control biofilm have been investigated [11,12]. An understanding of attachment and detachment of biofilms on various surfaces is key to finding the most effective treatment. A study conducted by Mendonca et al. [13] found that *Escherichia coli* O:157:H7 has the potential to adhere to surfaces commonly used in the food industry: stainless steel 304 (SS304), poly (vinyl chloride) film covered with thick cloth (PVC1) and poly(vinyl chloride) film covered with thin cloth (PVC2) at different contact times (0, 7, 24, 41 and 48 h) and temperatures (12, 17, 28, 39 and 44°C). In addition, Meira et al. [14] found that biofilms adhered over 4 log CFU/cm^2 on all surfaces at both temperatures and the cell detachment was 3 log CFU/cm^2. Park and Chen [15] performed a study to quantify biofilms formed by different STEC strains on polystyrene and stainless-steel surfaces to determine the effectiveness of 2% acetic or lactic acid and industry recommended concentrations of acidic or alkaline sanitizers, at 28°C. Results showed that the alkaline and acidic sanitizers were more effective than those with the organic acids for removing the biofilms.

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Food grade organic acids are used to control undesirable microbes in foods. Mitchell [16] evaluated the effects of lactic acid at different concentrations (0% (control), 1.5%, 2.0% and 2.5%) against STEC (O157:H7 and non-STEC O157 (O103, O111, O145 and O26) on spinach and soybean sprouts with and without heat at various times. The results showed that temperature did not enhance the antimicrobial ability of the lactic acid, but the lactic acid (1.5%, 2% and 2.5%) was effective against all organisms. Another study was conducted to determine the ability of eugenol and surfactant micelles to control STEC on beef trimmings. The treatments included: free micelle-encapsulated eugenol (free eugenol and 1% sodium dodecyl sulfate), 2% lactic acid (55°C) and distilled water (25°C) as a control. Results indicated that there was no significant difference among treatments. In fact, the log reduction was between 6.4 and 6.6 $\log_{10}$ CFU/g. Further results showed that after five days of storage, all treatments significantly reduced the pathogenic load by 0.2 and 0.3 $\log_{10}$ CFU/g (p=0.014) [17]. Tolen et al. [18] evaluated the synergistic blend of sodium dodecyl sulfate (SDS), lactic acid (LA) and chitosan acid (CA) against non-STEC O157 using cattle hide decontamination model. The treatments were (vol/vol): CA (1% chitosan in 1% acetic acid), SDS (1%), SDS (2%), LA (1%), CA-SDS combination (1% chitosan in 1% acetic acid mixed with 1% SDS), and LA-SDS combination in two different concentrations (1% LA mixed with 1% SDS, and 1% LA mixed with 2% SDS) and phosphate buffer water as a control. The 1% LA-2% SDS combinations significantly ($p<0.05$) reduced the *E. coli* O157:H7 concentration. There was no significant difference in the antibacterial effect of 1% LA and 2% SDS when used alone. All other antimicrobials blends reduced the pathogen by 1.8 log CFU/cm^2. The results showed that the antibacterial ability of 1% LA against *E. coli* O157:H7 was enhanced when blended with 1% SDS. Chemical treatments such as lactic and acetic acid are being used to destroy microorganisms on whole carcasses that contain fat in which microorganisms may be embedded [19]. Organic acids are commonly diluted in water to facilitate application of desired concentrations of these antimicrobial agents. However, water is a poor wetting agent for hydrophobic environments [20,21]. This problem can be circumvented by use of a processing aid such as a food grade surfactant, which increases the wettability and thus enhances the exposure of pathogens to the antimicrobial treatment.

Sodium lauryl sulfate (SLS) is a generally regarded as a safe (GRAS) food additive (10 to 5,000 ppm) that is used in animal fats, vegetable oils, fruit juices and beverages, gelatin, marshmallows and egg whites [22]. Sodium lauryl sulfate is also classified as an anionic surfactant [23]. Surfactants are amphiphilic, hydrophilic and have hydrophobic moiety. Its hydrophobic moiety consists of saturated and unsaturated fatty acids that have the ability to accumulate between interfaces. They also show great stability under extreme temperatures and pH [24]. Because the surfactant has the ability to interact with the membrane phospholipids, it is able to interfere with a pathogen's physiological functions, which changes the permeability of the pathogens membrane [25,26]. Sodium lauryl sulfate is believed to cause membrane damage and protein denaturation in microorganisms when its activity is enhanced at pH below 4.0 [27]. The action of LCA works by reducing the bacterial pH and interrupting the motive force of the transmembrane of the pathogens proton [28]. The addition of a surfactant increases the exposure of the pathogen to the antimicrobial because it can penetrate intercellular spaces like those of poultry [29].

More recently this approach has been applied to control *Listeria monocytogenes* on the surface of frankfurters [30]. Sewlikar et al. [31] demonstrated that 125 ppm of SLS in combination with 0.5% LA reduced initial counts of *Salmonella typhimurium* attached to broiler skins by 1.3 log CFU/g. Other surfactants have also been evaluated. Calcium hydroxide (1%) combined with Tween 80 (1%) enhanced inactivation of *Salmonella enterica* on alfalfa seeds by 1.3 log CFU/g compared to calcium hydroxide (1%) applied alone [32]. Although there are published studies that have investigated the effect of organic acid-surfactants on the inactivation of *E. coli* [33], studies are needed to evaluate the effects of lactic citric acid in combination with surfactants. Therefore, the objective of this study was to evaluate the most effective concentrations of a lactic citric acid blend with an added surfactant to aid in the inhibition of STEC using a broth system.

Materials and Methods

Preparation of bacterial strains

Seven strains of non-O157:H7 (O26, O45, O103, O11, O121, O145, O104:H4) and one O157:H7 strain of *E. coli* were used in the study. One single colony of each strain was individually inoculated in 10 ml of TSB and incubated 37°C for 24 h. Two consecutive transfers of each strain were performed. For this study, two cocktails were prepared: one containing the seven non-O157:H7 strains (prepared by mixing all 10 ml of each strain in one sterile container) and another containing only the O157:H7 strain. Bacterial suspensions were then centrifuged at 5,000 g for 10 min and the pellet was washed and re-suspended in 0.1% peptone water. Initial cell count was 8.0 CFU/ml for both cocktails [34].

Preparation of treatments

For this study, treatments were prepared using a combination of lactic-citric acid (LCA) blend (2.4% concentration) and Sodium lauryl sulfate was prepared by first making a 10% stock solution and then diluting into 0.05, 0.25, and 0.5% concentrations. Of each concentration, 10 mL were aseptically transferred into sterile glass bottles before combination with the organic acids. The LCA blend was then added and brought up to a volume of 100 ml using Brain Heart Infusion (BHI) broth and gently mixed. To maintain the 1/10 dilution effect, 0.1 mL was removed from each bottle and replaced with 0.1 mL of the prepared bacterial cocktail. Samples containing each concentration of SLS with no organic acid blend, the organic acid blend alone, and the BHI alone served as controls for the study. The pH of each treated sample was also observed and recorded [35].

Microbial analysis

To evaluate survival, 1.0 ml aliquots of inoculated broth were serially diluted (10-fold) in buffered peptone water (BPW; pH 7.2). Samples of appropriate dilutions were then surface plated onto tryptic soy agar with 0.6% yeast extract (TSAYE). Inoculated TSAYE plates were incubated at 35°C and survival cells were enumerated after 24 h [35].

Statistical analysis

The results of the statistical analysis are presented as means + the standard error. ANOVA is also included with the alpha error set at 0.05, using S.A.S. 9.4. Each experiment was replicated three times.

Results and Discussion

The survival of *E. coli* O157:H7 and the non-O157:H7 cocktail containing only SLS in a broth system showed that there was not a significant decrease of the pathogen. At concentrations of 2.4% LCA and 0.05% SLS, there was a 6 log CFU/g reduction in non-O157:H7 cocktail as opposed to the *E. coli* O157:H7, which showed a 3 log CFU/g

reduction at that same concentration. The 6 log CFU/g reduction remained the same with the remaining concentration amounts for the non-STEC O157:H7 cocktail. These findings suggest that the combination of LCA and SLS is very effective against the non-O157:H7 in broth (Figures 1 and 2).

Moreover, the most effective concentration against non-O157:H7 in a broth was 2.4% LCA and 0.25% SLS, which gave a 6 log CFU/g reduction. There was a slight decrease at a lower concentration, 2.4% LCA and 0.05% SLS against non-O157:H7 at a 5 log CFU/g reduction. These outcomes are consistent with published studies. Bjornsdottir et al. [36] found that non-O157:H7 in an aqueous antimicrobial solution with or without beef purge were more susceptible to antimicrobial compounds including lactic and citric acid providing a 3 log CFU/g reduction. A similar study conducted by Raybaudi-Massilia et al. [37] that used lactic and citric acid at 2% concentration with manual and automatic application on various serotypes (O157, O26, O103, O111, O145 and O121) of STEC showed that lactic acid at 2% with automatic application was the most effective treatments on pre-rigor beef carcasses.

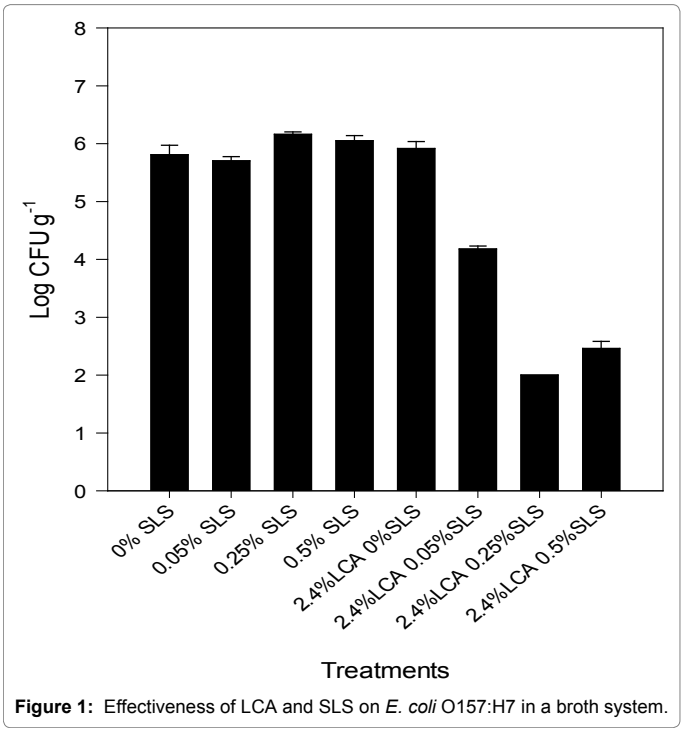

Figure 1: Effectiveness of LCA and SLS on *E. coli* O157:H7 in a broth system.

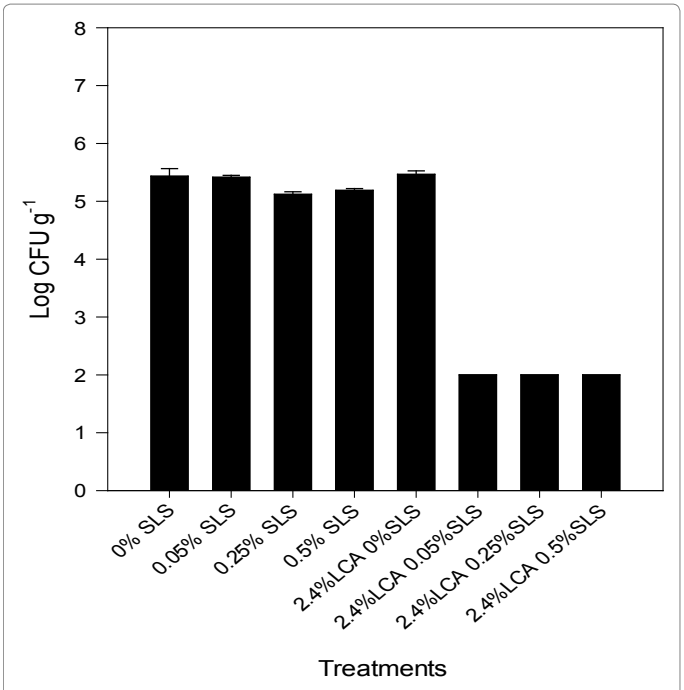

Figure 2: Effectiveness of LCA and SLS on cocktail of non-O157:H7 strains in a broth system.

Other studies have shown that non-O157:H7 is very sensitive to natural antimicrobials when compared to *E. coli* O157:H7. Studies on the effectiveness of *Quillaja saponaria* aqueous bark extracts showed that after 16 h incubation at room temperature, log reduction ranged from 6.81 to 4.55 log CFU against non-O157:H7. Once incubated at 37°C, the pathogen was reduced to undetectable levels within 1 h [38].

Antimicrobial solutions comprised of organic acids and those combined with plant extracts are more preferred given their GRAS status. The effectiveness of organic acids has been proven especially against *E. coli* O157:H7 on fresh produce [39]. One study investigated the effects of lactic acid against STEC and non-O157:H7 (O103, O111, O145 and O26) on soybean sprouts and spinach leaves. The concentrations of lactic acid (1.5%, 2% and 2.5%) was effective against all organisms evaluated in the study [40]. Oh et al. [41] investigated malic, acetic, lactic and citric acid (pH of 3.2) against *E. coli* strains. When these acids were added at greater concentrations, but remained at the same pH, it aided in the inhibition of the *E. coli* cells, resulting in a 6-log reduction. Further evidence showed that the synergistic blend of LCA and SLS achieved a 1.1 to 1.4 log CFU/g. Additionally, a study reported that adding surfactants, comparable to sodium lauryl sulfate (SLS), into organic acid solutions (LCA) increased the antimicrobial activity of sanitizers in produce wash water [42]. Other studies show that many surfactants are effective against food borne pathogens. An experiment was done evaluating the effectiveness of 1% citric acid and different concentrations of Tween 20 on Perilla leaves (an edible/medicinal plant) inoculated with *E. coli* O157:H7. The results showed that when these two antimicrobials were combined, *E. coli* O157:H7 was not detectable on the leaves [43].

The results of this study suggest that the five-strain cocktail of *E. coli* used in this study can effectively be reduced by natural antimicrobials. In addition, the use of a surfactant enhanced the effectiveness of the organic acid.

The influence of SLS/LCA on the pH of *E. coli* is shown in Figure 3. The pH for SLS alone was consistent at all concentrations (0%, 0.05%, 0.25% and 0.5%). However, when the LCA at 2.4% and SLS were combined, the pH remained at 4.5. The results indicate that a lower pH may have an influence on the effectiveness of the combination of antimicrobials on non-O157:H7 in broth at 6 log CFU/g. This has been demonstrated in many studies. Zaragoza et al. [44] conducted a study to evaluate the affects of a lactic acid and copper blend against *Salmonella* and *E. coli* O157:H7. The results showed that the blend was very effective against the pathogens. Further, the pH of the treatment solutions and the quality of the organic acid dissociation influenced the effectiveness of the antimicrobial. Also, the inhibitory action of lactic acid was mainly due to the compound crossing the plasma membrane in a state of dissociation. In a high acid environment, lactic acid remains undissociated and is in its most active antimicrobial

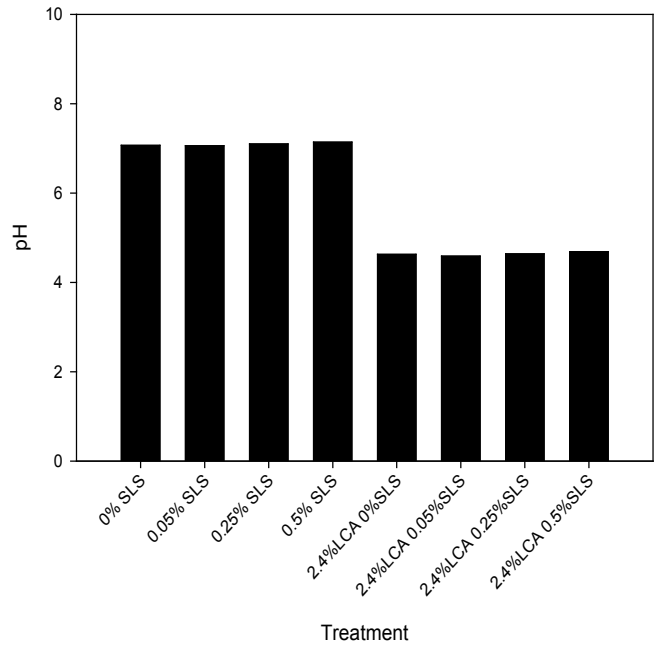

Figure 3: pH of treatments in a broth system.

form. Another study was conducted that investigated the effects of pH, lactic acid and NaCl in a cheddar cheese extract contaminated with *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes* and STEC. Results showed that all treatments did control the growth of all the pathogens. The results show that the pH had the greatest effect [45].

Although SLS was a vital component to the combination of antimicrobials, the pH of the organic acids played a role in its ability to reduce the log value of the pathogen by 3 to 6 log CFU/g. Therefore, the pH of this surfactant did not contribute to its ability to reduce both the pathogens. An experiment performed by Beier et al. [46] showed that the effect of the pH of the antimicrobial can be effective against non-O157:H7. The statistical analysis showed that there were no individual effects ($p>0.05$) of the LCA blend or SLS on the control of O157:H7 and the non-O157:H7 cocktail in the broth. When combined, the synergistic blend of SLS (0.05% and 0.5%)) and LCA blend (2.4%) in the broth significantly ($p<0.01$) reduced the non-O157:H7 by 6 log CFU/g and 2.9 and 4.6 log CFU/g in O157:H7 stains. When the SLS concentration was increased to 0.25% and the LCA blend was 2.4%, the synergistic blend was more effective ($p<0.01$) on O157:H7, showing 5 log CFU/g reduction. In this study, the application of SLS (with or without LCA) as antimicrobial treatments in a broth system effectively inactivated the *E. coli* O157:H7, with reduction that ranged from 3 to 6 log CFU/g. The results also indicated that the combination of SLS and LCA at the appropriate concentrations are potential alternatives to controlling *E. coli* O157:H7 and non-O157:H7 in a food system. The results of this study could be used to investigate the effects of the treatments in a food system and on the control of biofilms on food contact surfaces. Future studies are needed to determine whether such treatments will adversely affect the sensory attributes of the food product.

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