PAPER

INACTIVATION OF *LISTERIA MONOCYTOGENES* ATCC 7644 ON TOMATOES USING SODIUM DODECYL SULPHATE, LEVULINIC ACID AND SODIUM HYPOCHLORITE SOLUTION

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ABSTRACT

The human pathogen *Listeria monocytogenes* poses a serious threat to public health. A study was carried out to evaluate the effectiveness of four sanitizers, used individually or combined, against *L. monocytogenes* ATCC 7644. The contact times for bacteria and sanitizer were varied to 1, 3 and 5 minutes. Levulinic acid, sodium dodecyl sulphate (SDS), sodium hypochlorite solution (chlorine) and a combination of SDS and levulinic acid (mixture) were tested. Results revealed that 0.5% levulinic acid, when used individually, is capable of reducing the surviving colonies by 3.63 log CFU/mL, 4.05 log CFU/mL, 6.71 log CFU/mL after exposure for 1, 3 and 5 minutes respectively. SDS resulted in an 8 log CFU/mL reduction after 1, 3 and 5 minutes. A combination of 0.5% levulinic acid and 0.05% SDS caused a 3.69 log CFU /mL reduction, 4.4 log CFU/mL reduction, 7.97 log CFU/mL reduction for 1, 3 and 5 minutes respectively. Chlorine was the least effective with 2.93 log CFU/mL reduction, 3.16 log CFU/ mL reduction and 4.53 log CFU/ mL reduction respectively. When stored for up to 72 hours at 4°C, the surviving colonies remained viable and decreased in number significantly $P < 0.05 = 0.001$. The titratable acidity of samples treated with levulinic acid and samples treated with SDS/Lev mixture was lowered significantly compared to the control sample. No significant differences were noted in these same parameters for samples treated with chlorine or SDS. The application of SDS in the fresh produce industry as a sanitizing agent may be successful in eradicating or reducing the viability of *L. monocytogenes* on fresh produce, thereby replacing the routine chlorine washing.

- Keywords: food safety, Listeria monocytogenes, fresh produce, food borne illnesses, tomatoes, food borne pathogens -

INTRODUCTION

The increase in fresh produce consumption has caused a rapid evolution in the fresh produce industry (JOHNSTON *et al.*, 2005). This, coupled with recommendations to eat minimally processed foods, has led to an increase in the consumption of fresh fruits and vegetables among consumers (BERGER *et al.*, 2010). The consumption of minimally processed foods and fresh produce has also been encouraged among the immune compromised populations, such as those affected by HIV/AIDS, children and pregnant women (BERGER *et al.*, 2010; GANDHI and CHIKINDAS, 2007). Consumer demands and habits have also shifted, with many consumers in the busy world preferring to eat ready-to-eat foods and eating from salad bars (OMS-OLIU *et al.*, 2010; BERDEGUÉ *et al.*, 2005).

A variety of fresh produce such as lettuce cantaloupes, peppers, tomatoes, herbs and green leafy vegetables, among others, have been linked to food borne illnesses associated with either Salmonella, *Escherichia coli* O157:H7 or *Listeria monocytogenes* contamination (TAUXE *et al.*, 2010). Contamination of fresh produce by these pathogens occurs by various means. IJABADENIYI *et al.* (2011a) cited irrigation water as major pre-harvest source of contamination of fresh produce. Other factors as cited by JOHNSTON *et al.* (2005) include use of biocides as fertilizer, poor worker hygiene and poor sanitation.

L. monocytogenes among other food borne pathogens have been implicated as a public health threat (VELUSAMY *et al.*, 2010) and are estimated to cause about 1,600 incidents of illness, more than 1400 hospitalisations and about 250 deaths per year in United states (KYLE, 2012). These pathogens are responsible for food borne Listeriosis. They can grow in the soil, drains and on food preparation surfaces (GÁLVEZ *et al.*, 2010; PAN *et al.*, 2006; DJORDJEVIC *et al.*, 2002). They have been largely associated with dairy products, but recent research has also shown their increasing association with fresh produce (GANDHI and CHIKINDAS, 2007) including tomatoes.

Tomatoes are widely consumed and can be eaten raw, partially cooked or can be processed into other products. They are a very rich source of carotenoids, folate, vitamin C, mineral elements and phenolic compounds (FRUSCIANTE *et al.*, 2007). Of major importance are the antioxidants (carotenoids). Epidemiological research has shown that the antioxidants are capable of preventing chances of cancers and cardio vascular diseases (LEONARDI *et al.*, 2000). Tomatoes also provide a dietary source of soluble and insoluble fibres such as pectin, hemicellulose, and cellulose. Due to their nutritional value, they form an important part of the human diet. The elimination of food borne pathogens that can contaminate tomatoes is essential for preventing food borne illnesses that may be associated with the consumption of tomatoes.

Many methods are being used to try and eliminate the food borne pathogens. Use of phage or phage products in food production has been considered as a novel method for bio-control of pathogens in fresh and ready-to-eat food products (HAGENS and LOESSNER, 2010), but the cost associated with their use is very high. Other methods include bacteriocin-activated films high-hydrostatic pressure, high-pressure homogenization, in-package pasteurization, food irradiation, pulsed electric fields, or pulsed light and electrolyzed water (GÁLVEZ *et al.*, 2010). Sanitizers such as carvacrol, vanillin, peroxyacetic acid, hydrogen peroxide, N-acetyl-l-cysteine and citrox among others have also been tried (ABADIAS *et al.*, 2011). Sanitizers affect cell components, for example proteins, DNA, RNA and cell wall constituents through physicochemical interactions or chemical reactions. They cause irreversible damage to these structures and a loss of cell contents, thereby rendering the bacteria inactive or dead (CERF *et al.*, 2010).

The action of sanitizers is governed by contact time (exposure time), pH and temperature, among other factors. Some researchers conclude that sanitizers are not effective in eradicating food borne pathogens when used individually, although a combination of agents increases the sanitizer ability (SAGONG *et al.*, 2011; ZHAO *et al.*, 2009). Recent studies have also shown that if not used properly, sanitizers can be detrimental to the quality of fresh produce (SALGADO *et al.*, 2013; GUAN *et al.*, 2010). With regard to tomatoes, pH and acidity are the most important determinants of tomato quality (ANTHON *et al.*, 2011), hence the interaction of tomatoes with sanitizers during washing should be monitored. The study was performed to evaluate the effectiveness of SDS, chlorine and levulinic acid in reducing the viability of *L. monocytogenes* on tomatoes and the effect of these sanitizers on pH, titratable acidity and total soluble solids.

MATERIALS AND METHODS

Fresh produce

Tomatoes were purchased from a local supermarket on three separate occasions in Durban, South Africa. On the day of purchase the tomatoes were washed in running water. The tomatoes were then washed in 70% alcohol (IJABADENIYI *et al.*, 2011a). Prior to subjection to different sanitizer treatments, the tomatoes were tested for the presence of *L. monocytogenes*.

Bacterial strains

Listeria monocytogenes ATCC 7644 (Merck, South Africa) was used for this study. The

strain was cultured in Fraser broth for 24 hours at 37°C and stored at 4°C (IJABADENIYI *et al.*, 2011a). Prior to each experiment, a fresh culture was prepared from the stock culture by sub-culturing in Fraser broth for 24 hours at 37°C, an 8 log cfu/mL culture of *L. monocytogenes*, using McFarland Standards (JI *et al.*, 2010).

Chemicals and chemical treatments

Sodium dodecyl sulphate (SDS), levulinic acid, sodium hypochlorite solution, all purchased from Merck, South Africa, were tested, individually or combined with varying contact times (1, 3 and 5 minutes) for their effect on *L. monocytogenes* ATCC 7644 in tomatoes. The chemicals were used as follows;

1% SDS individually

0.5% Levulinic acid individually

200 ppm Sodium hypochlorite solution individually and 0.5% levulinic acid/0.05% SDS combined and termed mixture.

Inoculation of bacterial strains into tomatoes

The method of ZHAO *et al.* (2009) was followed. A 25 g sample of tomatoes was cut into approximately 5 cm long pieces in the lamina flow hood. The samples were submerged into bacterial suspension (108 cfu/mL, 50 mL of bacterial solution into 950 mL of distilled water) for 60 seconds and then air dried for 20 minutes in the lamina flow hood. The samples were then suspended into 500 mL test solution and agitated by a magnetic stirrer at 100 rpm for 1, 3 and 5 minutes. Following treatment, the individual samples were placed in double zipper bags containing 25 mL of phosphate buffered saline and pummelled for one minute. The suspension was serially diluted (1:10) in 0.1% buffered peptone water and enumerated for *L. monocytogenes* ATCC 7644.

Enumeration of *L. monocytogenes*

A method by Taormina and BEUCHAT (2001) was followed. Populations of *L. monocytogenes* ATCC 7644 were determined by surface plating serially diluted samples; 0.1 mL in duplicates on Listeria Selective Agar (Oxford formulation; Oxoid Ltd, Wade Road, Basingstoke, Hants UK). Plates were incubated for 24 hours at 37°C, after which colonies were counted.

Preparation of samples for Scanning Electron Microscopy

Untreated samples and samples subjected to chlorine, levulinic and SDS/Lev were used for SEM viewing. A method used by Ijabadeniyi *et al.* (2011b) was followed with a few modifications, according to the requirements of University of KwaZulu Natal microscopy unit. Pieces of tomatoes inoculated with *L. monocytogenes* ATCC 7644 and subjected to different treatments were cut into small pieces of 2 x 2 mm using a sterile blade. Primary fixation was carried out in 2.5% glutaraldehyde for 12 hours, followed by rinsing three times in phosphate buffer (0.1 M, pH 7.0). Post fixation was done using 0.5% Osmium tetroxide for one hour. Fixed samples were dehydrated in graded ethanol (30%, 50%, 75% and 100%) each for 5 minutes. The samples were then dried in a critical point dryer with carbon dioxide as a transition gas. The samples were mounted on specimen stubs and coated with gold palladium. The samples were then analysed using Desmond Clarence scanning electron microscopy.

Analysis of tomato physicochemical properties

Preparation of samples: The method of ZHAO *et al.* (2009) was followed for sample preparation, except that the tomato was further homogenised into slurry. A 25 g sample of tomatoes was cut into approximately 5 cm long pieces. The samples were then suspended into 500 mL test solutions as follows:

25 grams of tomatoes + 500 mL de-ionised water (control)

25 grams of tomatoes + 500 mL 1% SDS

25 grams of tomatoes + 500 mL of 0.5% levulinic acid

25 grams of tomatoes + 500 mL of 200 ppm sodium hypochlorite solution

25 grams of tomatoes + 500 mL of 0.5% levulinic acid/0.05% SDS (mixture)

The samples were agitated by a magnetic stirrer at 100 rpm for 1, 3 and 5 minutes (contact times). After each contact time was achieved, samples were immediately drained and the tomatoes were homogenized to form a slurry using Waring Commercial Laboratory blender. The slurry was used to test for pH, titratable acidity and total soluble solids immediately.

Determination of pH

The determination of pH was done on freshly made tomato paste using the Thermo Scientific Orion 2star pH meter. The electrodes were rinsed with distilled water in between samples.

Determination of Titratable Acidity

For estimating titratable acidity, the slurry was filtered using Whatman syringe filters. A 100 mL of the filtrate was titrated by adding 0.1N sodium hydroxide until a pH of 8.1 was attained. The volume of the sodium hydroxide added to the solution was multiplied by a correction factor of 0.064 to estimate titratable acidity as a

percentage of citric acid (CHEEMA *et al.*, 2014; TURHAN and SENIZ, 2009).

Determination of Soluble Solids Content

TSS is an index of soluble solids concentration in fruit. For an estimation of soluble solids content, 1.5 mL tomato slurry was centrifuged at 10,000 rpm (15 min, 25°C), and the supernatant was filtered through Whatman nonsterile syringe filters (0.45 μm). The filtered tomato serum (40 μL) was measured using a digital refractometer ATAGO (ATAGO, USA Inc. Kirkland, WA, USA). Measurements were taken once for each sample, and 70% ethanol was used to clean in between samples. The refraction index was expressed as percent soluble solids in°Brix (Wilkerson *et al.*, 2013; Javanmardi and Kubota, 2006).

Data analysis

Three trials were conducted for each experiment. Analysis of the data was performed using SPSS version 21 (IBM Statistics). Analysis of variance was conducted with repeated measures and Greenhouse Geisser correction to study the effect of contact time on the survival of *L. monocytogenes*, ATCC 7644 and the effect of each sanitizer on the survival of *L. monocytogenes* ATCC 7644 at varied time intervals (0, 24, 48 and 72 hours). The number of surviving colonies was plotted against contact time (1, 3 and 5 minutes) and also against time interval (0, 24, 48 and 72 hours). Log reductions for each contact time and sanitizer were also calculated and is presented in a table. Pair wise comparison with Bonferroni adjustment was used to determine any significant difference between subjects. To analyse results for physicochemical properties, ANOVA was used to assess if there was a significant difference in pH, total soluble solids and titratable acidity of treated and untreated tomato samples.

RESULTS

Effect of storage time, sanitizer treatments and contact time on the survival of *L. monocytogenes* ATCC 7644

The treatment of *L. monocytogenes* with sanitizers resulted in a decrease in the populations of bacteria. All the sanitizers tested had the ability to reduce the surviving colonies, with varying degree of effectiveness. Among the sanitizers tested, sodium hypochlorite solution was the least effective, with the highest counts of surviving colonies. The next in the list is levulinic acid, then a mixture of SDS and levulinic (termed mixture), with SDS the most effective of them all. The results of repeated measures (CASTRO *et al.*) with Greenhouse-Geisser correction showed that there was a significant difference at 5% level between effectiveness of sanitizers used, [$F(3, 9) = 63.00$; $P < 0.05 = 0.01$]. The surviving colonies were reduced progressively as storage time increased from 0 hours to 72 hours. The means of surviving colonies are shown in Table 1.

Marginal means for each sanitizer's contact time were also plotted in Fig. 1 for 1, 3 and 5 minutes. As shown in the figure, sodium hy-

Table 1 - Mean 1 count of *L. monocytogenes* ATCC 7644 after treatment with different sanitizers at different contact times and storage times.

Contact times		Time intervals			
		0 Hours	24 Hours	48 Hours	72 Hours
Chlorine	1 minute	[a] 5.36±0.02	[a] 5.14±0.03	[a] 5.02±0.03	[a] 4.75±0.04
	3 minutes	[a] 5.06±0.03	[a] 5.06±0.03	[a] 4.78±0.05	[a] 4.45±0.04
	5 minutes	[b] 4.17±0.09	[b] 3.77±0.09	[b] 3.33±0.10	[b] 2.60±0.09
SDS/Lev	1 minute	[c] 4.60±0.01	[c] 4.59 ±0.02	[c] 4.27±0.08	[c] 4.01±0.06
	3 minutes	[c] 4.35±0.05	[c] 4.24 ±0.06	[c] 3.39±0.36	[c] 2.53±0.08
	5 minutes	[d] 1.33±0.15	[d] 1.40±0.03	[d] 0.56±0.09	[d] 0.00
Levulinic	1 minute	[e] 4.68±0.03	[e] 4.60±0.02	[e] 4.15±0.14	[e] 4.06±0.11
	3 minutes	[e] 4.68±0.03	[e] 4.34±0.09	[e] 4.12±0.10	[e] 2.60±0.30
	5 minutes	[f] 3.17±0.07	[f] 2.06±0.04	[f] 1.50±0.10	[f] 0.43±0.20
SDS	1 minute	[g] 0.00	[g] 0.00	[g] 0.00	[g] 0.00
	3 minutes	[g] 0.00	[g] 0.00	[g] 0.00	[g] 0.00
	5 minutes	[g] 0.00	[g] 0.00	[g] 0.00	[g] 0.00

Mean counts ±Standard Deviation (Log 10 CFU /mL).
[1]Means followed by different letters in the same column are significantly different.

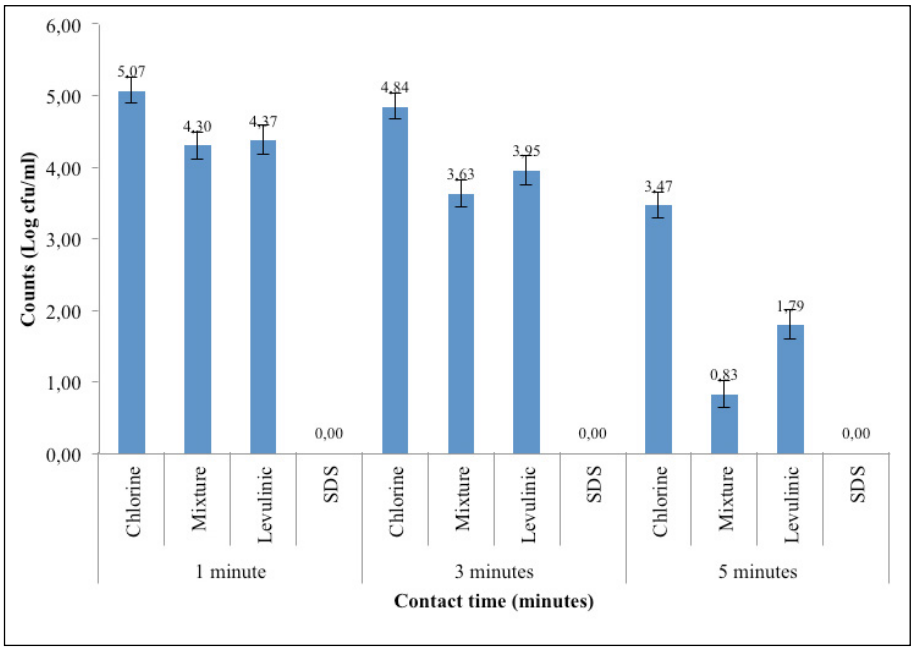

Fig. 1 - Means of surviving colonies of *L. monocytogenes* ATCC 7644; based on marginal means. The highest means associated with chlorine show that it was least effective.

pochlorite has the highest mean values, meaning that the highest number of surviving colonies was observed after exposure to this sanitizer compared to other solutions. Sodium chloride was thus not very efficient in reducing survival of the pathogen in this particular study.

Increasing the contact time (1, 3 and 5 minutes) significantly reduced the surviving colonies for all sanitizers tested at 5% level; [F (3, 180) = 30.70; P< 0.001]. However, the results of ANOVA with Green House Geisser correction showed that the reduction for 1 minute and 3 minutes of treatment were not significantly different (P = 0.16). This shows that increasing the contact time of each of the sanitizer to 3 minutes did not make much difference to the surviving colonies.

Overall log reductions

When exposed for 1 minute to 200 ppm chlorine, *L. monocytogenes* were inactivated by 2.93 log CFU/mL. A log reduction of 3.16 log CFU/mL and 4.53 log CFU/mL was achieved after increasing contact time to 3 minutes and 5 minutes respectively. A mixture of 0.5% levulinic acid and 0.05% SDS (mixture) reduced the surviving colonies to 3.69 log CFU/mL, 4.4 log CFU/mL and log 7.97 CFU/mL after exposure for 1 minute, 3 minutes and 5 minutes, respectively. Using 0.5% levulinic acid resulted in log reductions of 3.63 log CFU/mL, 4.05 log CFU/mL and 6.71 CFU/mL after exposure for 1 minute, 3 minutes and 5 minutes. The overall log reductions are presented in Table 2.

Observations of specimens using a scanning electron microscope

Samples of tomatoes treated with sodium hypochlorite solution, levulinic acid and mixture were viewed under SEM to verify the existence of colonies even after exposure to sanitizers. The results for this current work showed that there were surviving colonies after exposure to sodium chlorite solution, levulinic acid and a mixture. However, viewing samples treated with the above sanitizers did not clearly show the remains of surviving colonies. The SEM images did not show the presence of an abundance of bacteria on the surfaces. It is possible that the bacteria that were inoculated on the surfaces could have been washed out during the sample preparation procedure. The procedure used for sample preparation might not be suitable in this specific case. Bacteria might also have migrated into other hidden sections of the pictures due to the irregularities of the topography.

Table 2 - Log reductions (CFU/ mL) for chlorine, mixture, levulinic acid and SDS at 1, 3 and 5 minutes.

	Overall log reduction		
Sanitizer	**1 minute**	**3 minutes**	**5 minutes**
Chlorine	2.93	3.16	4.53
Mixture	3.69	4.40	7.17
Levulinic	3.63	4.05	6.71
SDS	8.00	8.00	8.00

Titratable acidity, pH and total soluble solids of tomato samples

Table 3 presents the results of the TA, pH and TSS. The TA of tomato samples treated with levulinic acid and SDS /Lev mixture was significantly different from the control ($P< 0.05$). The TA for levulinic acid treated tomatoes was 2.78%, 2.81%, and 2.81%; while the TA for mixture treated tomatoes was 3.81%, 3.73%, 3.74% for 1, 3 and 5 minutes respectively. The pH for levulinic acid and mixture treated tomato samples was relatively lower than the pH of the control sample, as shown in the table. There was no significant difference between the TA and pH of the mixture and levulinic acid treated samples. The TA for tomato samples treated with SDS was 0.16 and for samples treated with chlorine was 0.15%, 0.14% and 0.14%. These results did not vary significantly from the control. The pH for the SDS and chlorine treated samples were also slightly different from the control sample, as shown in the table. TSS for levulinic acid treated samples were reduced significantly to 3.20% brix for 1, 3 and 5 minutes, while the TSS for mixture treated samples was reduced to 3.24, 3.26, 3.24% brix respectively. Though the TSS for SDS treated and chlorine treated samples were reduced, the effect was not significant according to the findings of this study. Contact time was varied from 1 minute to 5 minutes; but there were no significant changes in theses parameters from 1 minute to 5 minutes.

DISCUSSION

The food manufacturing industry depends on the use of sanitizers for reducing the risk associated with food borne pathogens. Many sanitizers have been tried, but to date food borne pathogens are still a problem in the food and fresh produce industry. Some researchers have suggested that this is due to development of resistance by the bacteria with repeated exposure to sanitizers (MANI-LÓPEZ *et al.*, 2012; RIAZI and MATTHEWS, 2011).

Most fruits and vegetable units resort to chlorine based sanitizers because they are cheaper and have a long standing credibility with reducing surviving bacteria. However, this is proved not to be the case in this current research, as well as other previous research. Findings from this study show that though chlorine has been widely used for washing produce and sanitising food surfaces, it is not really capable of killing all food borne pathogens. This is shown by high mean counts associated with chlorine as presented in the results above. Chlorine washing has also been tried on *Escherichia coli* O157:H7 and Salmonella, but the reports on that work also shows that chlorine is not effective against food borne pathogens (KESKINEN *et al.*, 2009b). Other researchers also agree that chlorine cannot reduce food borne pathogens effectively (IJABADENIYI *et al.*, 2011b; ALLENDE *et al.*, 2009; MAHMOUD *et al.*, 2007).

Several research projects are under way to try

Table 3 - Effects of levulinic acid, chlorine, SDS/Lev mixture and SDS on physicochemical properties of tomatoes.

Tomato treatment	Contact times	pH of sample	Titratable acidity (% citric acid)	Total soluble solids (%Brix)
Distilled water	1 minute	4.77 a	0.16 a	4.90 a
	3 minutes	4.78 a	0.14 a	4.90 a
	5 minutes	4.78 a	0.16 a	4.90 a
Levulinic acid	1 minute	3.61 b	2.78 b	3.20 b
	3 minutes	3.67 b	2.81 b	3.20 b
	5 minutes	3.69 b	2.81 b	3.20 b
Mixture (SDS/Lev)	1 minute	3.81 b	2.76 b	3.24 b
	3 minutes	3.73 b	2.78 b	3.26 b
	5 minutes	3.74 b	2.78 b	3.24 b
Chlorine	1 minute	5.09 a	0.15 a	4.60 a
	3 minutes	5.17 a	0.14 a	4.63 a
	5 minutes	5.20 a	0.14 a	4.61 a
SDS	1 minute	4.68 a	0.16 b	4.65 b
	3 minutes	4.88 a	0.16 b	4.61 b
	5 minutes	4.87 a	0.16 b	4.63 b

Each value represents the mean of three trials. For each parameter, the values significantly different at $P \leq 0.05$ are indicated by different letters. Samples treated in distilled water were used as control. Chlorine = Sodium hypochlorite solution.

to find other alternative sanitizers because of the challenges that are associated with chlorine (KESKINEN *et al.*, 2009a). Some researchers point out that its pH sensitivity affects its effectiveness (ZHAO *et al.*, 2009). Another challenge is that it diminishes quickly upon contact with organic matter and hence leads to reduced effectiveness (NEAL *et al.*, 2012). Other concerns raised include the environmental and health risks associated with the formation of carcinogenic halogenated disinfection by-products such as trihalomethanes (GIL *et al.*, 2009; KIM *et al.*, 2009). For these reasons chlorine has not been gainfully useful in the fresh produce industry in recent years. Though it has been a long standing sanitizer in the food industry, other sanitizers that have been shown to be more effective than chlorine; through this research and previous research can be employed for the betterment of microbiologically quality of fresh produce.

Levulinic acid is applied in the food manufacturing industry as a food additive. It has been designated as a generally safe additive to food by the Food and Drug Administration (FDA)(ZHAO *et al.*, 2009). Levulinic acid disrupts the membrane structure of bacteria due to its polarity, thereby exposing cell constituencies and lethality (Thompson, 2007). Levulinic acid can be used over a wide pH and temperature range (SAGONG *et al.*, 2011). In this particular study, levulinic acid showed mean counts that were much lower than those of chlorine. With these findings, it can be concluded that levulinic acid at 0.5% can perform better than a 200ppm sodium hypochlorite solution against *L. monocytogenes* ATCC 7644. Other researchers also tried levulinic acid in their work with related findings. THOMPSON *et al.* (2008) concluded that it was effective in inhibiting outgrowth of *L. monocytogenes* in ready-to-eat meat products. Other studies using lactic acid, acetic acid and levulinic acid on meat revealed that though levulinic acid is effective, it does not provide as effective decontamination as lactic acid, nor as much residual protection as acetic acid (CARPENTER *et al.*, 2011). Levulinic acid shows potential in the fresh produce industry, and therefore further research can be pursued on the most usable concentrations and most applicable pathogens. Its detrimental effects on quality should be taken into consideration as well.

Sodium dodecyl sulphate is generally regarded as a safe (GRAS) food additive (LU and WU, 2012). In this study, using 1% SDS alone resulted in 8 log CFU/mL reduction of *L. monocytogenes.* SDS has amphilic properties (12 carbon chain attached to sulphate group) and its anti-microbial effectiveness increases when pH is decreased, it has the ability to denature cell proteins and damage cells membranes irreversibly (ZHAO *et al.*, 2009). The action of SDS was much better than that of levulinic acid in this particular study when they were used individually. This is because levulinic acid has a shorter carbon chain (5 carbons and a hydroxyl group), which makes it a weak acid, therefore its effectiveness is less than SDS. Extra care must be taken if SDS is employed in fresh produce as it was established during this study that very low concentrations of 1% can have a very large impact on survival of pathogens.

A combination of 0.05% SDS and 0.5% levulinic acid was also used in this study. Findings show that this mixture achieved better results as compared to levulinic acid alone. Many researchers have reported on the advantages of mixing SDS and levulinic acid. The findings of ZHAO *et al.* (2009) show an increased antimicrobial activity by the combination of SDS and levulinic acid against Salmonella and E. coli O157: H7. Gurtler and Jin (2012) found that a combination of 2% acetic acid, lactic acid and levulinic acid reduce Salmonella on tomatoes. ORTEGA *et al.* (2011) reported that a combination of levulinic acid and SDS was highly effective against E. coli when exposure times were increased to 30 and 60 minutes. On the contrary, GUAN *et al.* (2010) reported that a combination of these had no commercial value as they have detrimental effects on the quality of fresh produce. Combining sanitizers has shown to have a positive contribution in the food market. This has potential for implementation in the fresh produce industry. The implementation of a combination of sanitizers can be tried together with an assessment of their effects on sensory qualities.

Increasing exposure time significantly decreased the surviving colonies of *L. monocytogenes.* In this particular study, a greater fall in surviving colonies was achieved at 5 minutes exposure time. This is evidence that the longer the bacteria are exposed to chemicals, the greater the chances of reducing their survival. PARK *et al.* (2011) also reported that log reductions increase with increasing contact times. Other writers indicate that an exposure time of 3 minutes is effective against food pathogens (MATTSON *et al.*, 2011). DING *et al.* (2011) and Møretrø *et al.* (2012) also reported that the effectiveness of a sanitizer depends on treatment time. Other writers also note that a significant decrease in the bacterial counts occurs in the first minute and the subsequent decrease after one minute is not significant (STEBBINS *et al.*, 2011; TIRPANALAN *et al.*, 2011). From the reports written by other writers and from this research, it can be said that contact time is one of the factors that should be monitored when using sanitizers. Insufficient contact time will lead to high survival after treatment, while extended contact time may lead to damage in the sensory qualities of fresh produce.

The bacteria where further stored for a period of 72 hours at 4 0 C. During this storage period *L. monocytogenes* survived up to 72 hours after being treated with sanitizers, except in

SDS. Survival of pathogens after a storage period of 72 hours is also reported by IJABADENIYI *et al.* (2011b). Sufficient exposure of pathogens to sanitizers is paramount to reduce surviving colonies, as some have the ability to recover even after being treated with sanitizer.

For sanitizers to be effectively used on tomatoes, they should cause negligible changes to pH and titratable acidity of the tomatoes, the major determinants of tomato quality (ANTHON *et al.*, 2011). In this research it was revealed that sanitizers can alter the physicochemical attributes of fresh tomatoes if the sanitizers come into contact with the sub surfaces, thereby affecting the final sensory quality of tomatoes. Other recent studies also point out that sanitizers can affect sensory qualities of fresh produce to some extent (PÉREZ-GREGORIO *et al.*, 2011). In this study, major effects were noted on the pH, TA and TSS with levulinic acid and mixture. Changes effected by SDS and chlorine were not significant. These findings pronounce SDS as a better sanitizer to replace the routine chlorine washing as it causes minimal changes to quality.

In previous studies, SDS was tested together with organic acids and hydrogen peroxide on blue berries and no significant difference was detected in pH and total anthocyanin value between untreated and treated blueberries (LI and WU, 2013). In another study using Iceberg and Romane lettuce, chlorine had high quality scores for Romane lettuce, but caused quality deterioration on Iceberg lettuce. A combination of SDS and Tsunami did not show any effect on sensory attributes of Iceberg lettuce either (SALGADO *et al.*, 2013).

Though levulinic acid did not have favourable results in this particular study, previous studies report that using levulinic acid caused no sensory changes in turkey meat and pork sausages (VASAVADA *et al.*, 2003). A combination of SDS and levulinic did not give favourable results in this study. Other studies also report that SDS used in combination with other sanitizers such as levulinic acid are of low commercial value compared to chlorine, since they cause detrimental effects to sensory attributes (GUAN *et al.*, 2010).

Total soluble solids were reduced for all treatments with levulinic acid having the highest reductions followed by SDS/Lev mixture. This could have been attributed to leaching of contents into treatment solutions as a larger surface area of the subsurface area of tomatoes was exposed. Leaching of materials is also reported by ALEGRIA *et al.* (2009). Though previous studies also report that longer contact times result in deterioration of sensory characteristics (Rico *et al.*, 2007), there was no significant difference for all attributes tested in relation to contact time in this particular study. Contact of sanitizers with sub-surfaces of fresh produce should be minimised to prevent unnecessary damage to sensory quality attributes.

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CONCLUSION

This works confirms that use of sanitizers in food processing at shorter contact time of 1 minute may not eradicate food borne pathogens. SDS alone is capable of destroying *L. monocytogenes,* causing no detrimental effect to sensory attributes of tomatoes. It is also important to consider exposure time to increase the effectiveness of sanitizers. Sanitizers can have detrimental effects on the sensory attributes of fresh produce; hence careful consideration is required when selecting sanitizers for particular produce. Further studies are required to validate the application of levulinic acid and SDS as sanitizers in food processing as well as their efficacy.

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