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Trisodium phosphate enhanced phage lysis of *Listeria monocytogenes* growth on fresh-cut produce

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ABSTRACT

Combination of bacteriophage and trisodium phosphate (TSP) to inactivate *Listeria monocytogenes* inoculated on fresh-cut produce was investigated. Fresh-cut tomato and watermelon inoculated with *L. monocytogenes* cocktail at log 10^7 CFU/g were treated with Listex P100 bacteriophage (10^8 PFU/g) alone, and in combination with TSP at 10, 30 and 60 mg/ml using chlorine at 200 mg/L as control. Treated samples were stored at 4 and 10 °C for 6 days. Effect of treatment on pH and colour of treated samples was evaluated. Phage treatment alone significantly ($p < 0.05$) reduced *L. monocytogenes* on fresh-cut tomato by approximately 0.5 and 0.6 log CFU/ml and on fresh-cut melon by 1.30 and 1.49 log CFU/ml at 4 and 10 °C respectively. Addition of phage-TSP at 10 mg/ml was only effective on fresh-cut melon, but phage-TSP at 30 and 60 mg/ml approximately reduced inoculated tomato by 1 and 2 logs, and melon by 2 and 5 logs at 4 and 10 °C respectively. Chlorine treatment showed 1–2 log reduction. Phage titer declined rapidly on tomato unlike melon, pH and colour parameters slightly increased with treatment combinations with no impairment on both samples. Phage-TSP combination could serve as effective tool to control listeriosis outbreak in fresh produce.

1. Introduction

Recent increase in production and consumption of fresh or slightly processed produce has been well documented (Abadias, Usall, Anguera, Solsona, & Viñas, 2008; FAO/WHO, 2008; Olaimat & Holley, 2012). Daily intake of fresh produce rich in phytonutrients at $\geq$ 400 g has been identified to improve body resistance against certain degenerative diseases such as coronary heart disease, diabetes and cancer (Allende, McEvoy, Luo, Artes, & Wang, 2006; Callejón et al. 2015; Goodburn & Wallace, 2013; WHO., 2003).

Despite these associated benefits, food safety challenge of disease outbreaks resulting from pathogenic contamination of fresh produce along the food chain is constantly increasing, with reported cases of hospitalization and death (Lynch, Tauxe, & Hedberg, 2009; Warriner, Huber, Namvar, Fan, & Dunfield, 2009). Growth of pathogen with high mortality rate such as *Listeria monocytogenes* has been reported in wide variety of fresh produce including tomato and watermelon (Beuchat, 2002; Botticella et al. 2013; Penteado & Leitão, 2004; Scallan et al. 2011). Meanwhile, alternative methods to traditional use of chlorine decontamination in fresh produce have been suggested, due to its associated toxicity concerns. This suggestion involves the use of safe, eco-friendly and non-toxic antimicrobial agent such as bacteriophage (Gopal, Coventry, Wan, Roginski, & Ajlouni, 2010; Meireles, Giaouris, & Simões, 2016).

Bacteriophages or phages are bacteria-killing viruses operating through a process of lysis (Hagens & Loessner, 2015; Simões, Simões, & Vieira, 2010). Phages are highly host-specific, very available hence can easily be isolated, self-replicative in host genome and possess effective means of causing lysis to their bacterial host with little or no deleterious organoleptic impact on the food substrate (Hughes, Sutherland, Clark, & Jones, 1998; Sharma, Ryu, & Beuchat, 2005; Sillankorva, Oliveira, & Azeredo, 2012; Spricigo, Bardina, Cortés, & Llagostera, 2013). Phage application for significant reduction of *L. monocytogenes* on various fresh produce has been reported (Leverentz et al. 2003; Oliveira et al. 2014; Perera, Abuladze, Li, Woolston, & Sulakvelidze, 2015). However, due to reported development of resistance by this pathogen, the need to combine phage with other antimicrobials becomes more imminent (Kim & Kathariou, 2009; Kim et al. 2012; Strydom & Witthuhn, 2015).

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Salts of inorganic acid such as trisodium phosphate (TSP) has been reported to exhibit inhibitory effect on common food-borne bacterial pathogens such as *Escherichia coli*, *Salmonella* species and *L. monocytogenes* (Capita, Alonso-Calleja, Prieto, García-Fernández, & Moreno, 2003; del Río, Capita, Prieto, & Alonso-Calleja, 2006). TSP as antimicrobial agent has been granted a "generally recognized as safe" (GRAS) status by the US Food and Drug Administration at 80–120 mg/ml (Federal-Register, 1994; USDA-FSIS 2012). Its mode of action is believed to be linked with surface-active action on microbial cell wall and denaturation of cytoplasmic protein in response to its alkaline nature (Cai et al. 2015).

Incidentally, Hoelzer, Pouillot, Van Doren, and Dennis (2014) reported 2.5–5.0 log CFU/g reduction of *L. monocytogenes* on fresh apple and lettuce when treated with TSP. Due to increased interest in phage biocontrol on pathogens of health importance in the fresh produce industry, there is an imminent need to enhance its potential limitation via combination with other antimicrobials. To the best of author's knowledge, no investigation on the combination of bacteriophage with TSP to inhibit *L. monocytogenes* growth on fresh-cut produce has been reported. Therefore, the objective of this study is to evaluate the inactivation of *L. monocytogenes* on fresh-cut tomato and water-melon when treated with bacteriophage and trisodium phosphate.

2. Materials and method

2.1. Fresh produce preparation

Matured, ripe and organically grown tomato (*Lycopersicon esculentum*) and water-melon (*Citrullus lanatus*) were purchased from woolworth grocery market in Durban, South Africa. Samples were rinsed with de-ionized water (pH 7.0 ± 0.2) at room temperature and surface cotton-disinfected with 70% ethanol for 1 min, rinsed again with de-ionized water and allowed to air-dry at ambient temperature for 5 min in a Class II biosafety cabinet (BSC-1500II B2-X Labotech. Midrand 1685, South Africa). Tomatoes were cut transversely into 10 mm thickness and 50 mm diameter, while watermelons were cut longitudinally into trapezoidal shapes of 50 mm height, 15 × 50 mm upper surface area and 15 × 80 mm base area. The seeds were carefully removed and rind sliced off on a cleaned cutting board using a pre-sterilized stainless steel knife.

2.2. Bacteria preparation

Three different L. *monocytogenes* strains: ATCC 35152 (1/2a), ATCC 7644 (1/2c) and LQC 15257 (4b) from (Thermofisher scientific. 200 Smit St., Fairland 2195, Johannesburg. South Africa) were used in the present study. Following a modified method reported by Singh, Mnyandu, and Ijabadeniyi (2014), individual working cultures obtained from stock culture (Thermofisher scientific) kept in glycerol (−80 °C) were thawed in water bath (WB 1024: Foss tecator technology Hoganas, Sweden) at 25 °C for 3 min. Each thawed culture was streaked on *Listeria* oxford media (LOM 75805, Sigma-Aldrich Inc., St Louis MO 63103 USA) containing oxford *Listeria* selective supplement (Fluka 75806 Sigma Aldrich Inc., Buch, Switzerland) and grown at 37 °C for 20–24 h.

Each bacterial colony was transferred into a 50-ml Frazer broth base (F6672 FB, Sigma Aldrich Buch Switzerland) containing *Listeria* selective supplement (F18038 FSS, Sigma-Aldrich Buch Switzerland), harvested by centrifugation (5810R; Eppendorf Hamburg, Germany) at 5000 × *g* for 10 min at 10 °C and re-suspended in a sterile saline peptone solution (SP; 8.5 g/L Nacl and 1 g/L peptone. Conda Lab. Madrid, Spain). Equal volume of each strain was combined to make cocktail of *L. monocytogenes*, and a final working culture suspension at approximately 7 $\log_{10}$ CFU/g was prepared before inoculation using McFarland standard solution.

2.3. Sample inoculation

Sample inoculation was done by a modified method reported by Chen and Zhu (2011). About 6 mm diameter well of approximately 5 mm depth was made on three different spots on each fresh-cut surface to contain the inoculum using a manual fruit corer (FCS 0020 Prestige Pty Ltd, Somerset west 7130. South Africa). 0.15 ml of *L. monocytogenes* bacterial cocktail containing 1×10^7 CFU/g was spot-inoculated into the sample well, placed on sterile petri-dishes and allowed to attach for 5 min under aeration at room temperature in a biosafety cabinet.

2.4. Antimicrobial treatment procedure

Antimicrobial treatment consisting 0.15 ml of Listex P100 phage (Micreos Food safety, Netherlands) at 1×10^8 PFU/g was applied into each spotted well by the method reported by Oliveira et al. (2014). Simultaneously, 0.15 ml of trisodium phosphate solution (TSP 7601, Sigma Aldrich, Pty Ltd, Aston Manor. 1630 South Africa) at 10, 30 and 60 mg/ml using sterile de-ionized water (pH 7.0 ± 0.2) at room temperature was applied following a modified method reported by Su and D'Souza (2012). Chlorine dip (sodium hypochlorite at 200 mg/L) at room temperature for contact time of 2 min was carried out as control treatment. Each treated fresh-cut sample (25 g) was allowed to stand for 30 min before carefully transferred into sterile plastic bags (Tufflock 170 × 150 mm. Tuffy Brands Pty. Cape Town, South Africa) under bio-safety cabinet condition and then stored at 4 and 10 °C for 6 days in a controlled chamber (LTIM 10 Lab design Engr Pty, Maraisburg. South Africa).

2.5. Microbiological analysis

Bacteria recovery population was performed every 48 h for 6 days in all the storage temperatures. Fresh-cut samples in the sterile bags were homogenized in a stomacher blender (Model No BA 6021; Seward Lab. London SE 19UG UK) for 120 s with 10 ml of sterile peptone water buffer (PWB, Biolab). Aliquots of mixtures obtained were serially diluted in saline peptone water (SP; 8.5 g/L Nacl and 1 g/L peptone) and 0.1 ml each was spread-plated on sterile petri plates containing Oxford *Listeria* agar. Inoculated plates were incubated at 37 °C for 48 h in order to obtain log reduction values as $\log_{10}$ CFU/ml.

2.6. Phage titration

Phage titration was carried out by the method described by Leverentz et al. (2003). Briefly, aliquots from phage treated samples were homogenized and filtered through 0.45-μm-pore size membrane (Acrodisk; Pall Gelman, Ann Arbor, Mich). Phage titer was then determined by the soft agar overlay method using Brain heart infusion agar (BHI Biolab). Resulting plaques were counted as expressed as $\log_{10}$ PFU/ml.

2.7. pH and colour parameters

Quality parameters of pH and Chroma values (CIE $L^* a^* b^*$) of the treated samples were evaluated after the experiment with untreated sample as control. The pH of homogenized samples was determined using penetration electrodes pH meter (Model Basic 2°; Crison Instrument, Barcelona, Spain). Colour parameters (CIE L* a* b*) was carried out using the hunter lab colorimeter (Colour flex

EZ, CFEZ 0840 Virginia USA) and hue angle calculated by the method reported by Falade and Oyeyinka (2015). All determinations were carried out three times per each treatment.

2.8. Statistical analysis

All experiments were replicated three times. Data from each treatment were statistically subjected to analysis of variance (ANOVA) using SPSS software (IBM 24 SPSS Inc., Chicago, IL, USA) and means separated using Duncan multiple range tests ($p \leq 0.05$).

3. Results and discussion

3.1. Effect of antimicrobial treatment on L. monocytogenes growth on the fresh-cut produce

Generally, *L. monocytogenes* bacteria grew on both fresh-cut samples stored at 4 and 10 °C respectively. As shown in Table 1 the pathogen grew from an initial population of 3.42–5.37 and 5.77 log CFU/g on fresh-cut tomato stored at 4 and 10 °C respectively. While it grew from initial population of 4.34–6.87 and 6.98 log CFU/g on fresh-cut melon stored at same temperatures respectively (Data on day 2 and 4 not shown).

Comparatively, treatment of inoculated samples with chlorine at 200 mg/ml caused 1–2 log reduction, which supports previous report by Ramos, Miller, Brandão, Teixeira, and Silva (2013). Although, chlorine wash has been extensively used in the fresh produce industry, its comparative effectiveness has been limited due to production of halogen-based compounds. By implication, this could trigger carcinogenic effect in human body, hence the need for alternative but non-toxic antimicrobials (Bull et al. 2011; Legay, Rodriguez, Sérodes, & Levallois, 2010; Meireles et al., 2016).

Regardless of storage condition, phage treatment significantly ($p < 0.05$) reduced *L. monocytogenes* bacteria on both fresh-cut samples. After six days of storage at 4 and 10 °C, phage treatment reduced inoculated fresh-cut tomato by 0.49 and 0.58 log CFU/ml compared to the untreated sample respectively, while it reduced inoculated fresh-cut melon by 1.49 and 1.30 log CFU/ml in 4 and 10 °C respectively. Insignificant differences in the level of reduction in each storage temperature could be that the temperature difference was not great enough.

However, differences in lytic output on both fresh produce as observed in the phage titers could be linked to variation in pH condition of the two fresh-cut samples (Fig. 1). Phage efficacy has been reported to decline with increasing acid condition of food matrix, as pH values below 5 often result in greater inactivation by phage treatment (Guenther, Huwyler, Richard, & Loessner, 2009; Leverentz et al. 2003; Oliveira et al. 2014; Perera et al. 2015). In the present study, phage treatment declined rapidly on tomato with pH (4.34 ± 0.1) compared to melon (5.89 ± 0.1) (Fig. 1).

Suggestions towards addressing this decline by some authors include, increasing phage concentration or combination of different phages as cocktail (Hagens & Loessner, 2010; Leverentz, Conway, Janisiewicz, & Camp, 2004), while other authors suggested combination with other natural antimicrobials such as bacteriocin, antagonistic bacteria and antibiotics (Hong, Choi, Lee, & Conway, 2015; Leverentz et al. 2003; Soni, Desai, Oladunjoye, Skrobot, & Nannapaneni, 2012).

In this study, combination of phage with trisodium phosphate (TSP) to reduce *L. monocytogenes* on fresh-cut produce was evaluated.

After six days of storage, TSP treatment at 10, 30 and 60 mg/ml significantly ($p < 0.05$) reduced *L. monocytogenes* population by 0.51, 0.97 and 1.71 log CFU/ml, and by 0.60, 0.84 and 1.25 log CFU/ml on fresh-cut tomato stored at 4 and 10 °C respectively. On fresh-cut melon, TSP at 10, 30 and 60 mg/ml reduced *L. monocytogenes* population by 1.64, 1.67 and 3.62 log CFU/ml, and by 1.53, 1.69 and 3.53 log CFU/ml at 4 and 10 °C respectively (Table 1). Combination of phage with TSP at 10, 30 and 60 mg/ml reduced inoculated fresh-cut tomato by 0.53, 1.05 and 2.04 log CFU/ml, and by 0.62, 1.01 and 2.00 log CFU/ml stored at 4 and 10 °C respectively. Phage combination with TSP at 10, 30 and 60 mg/ml reduced inoculated fresh-cut melon stored at 4 and 10 °C by 1.64, 2.22 and 4.63 log CFU/ml, and by 1.66, 2.13 and 4.46 log CFU/ml respectively (Table 1).

Trisodium phosphate is a 'GRAS' approved antimicrobial agent with alkaline nature, whose primary mode of action is believed to be associated with its emulsifying properties on bacterial cell wall, disruption of cellular membranes, and denaturation cellular proteins leading to cell death (Cai et al. 2015; Hoelzer et al. 2014; Sampathkumar, Khachatourians, & Korber, 2003). Variation in reduction via TSP antimicrobial treatment on both inoculated fresh-cut sample, could be linked to differences in its ability to increase the pH medium (lowering of acid condition) on each food substrate, as previous studies have shown that *L. monocytogenes* is very susceptible to alkaline conditions (Mendonca, Amoroso, & Knabel, 1994; Taormina & Beuchat, 2002).

The relative ineffectiveness of TSP at 10 mg/ml to enhance phage lysis could be due to its low concentration where effective surface

Table 1
Effect of antimicrobial treatment of phage with TSP at 10, 30 and 60 mg/ml and chlorine (control) at 200 mg/L on the growth population (log CFU/ml) of *L. monocytogenes* inoculated on fresh-cut tomato and water-melon stored for six days at 4 and 10 °C.

Fresh produce	Tomato				Watermelon			
Temperature	4 °C		10 °C		4 °C		10 °C	
Storage days	0	6	0	6	0	6	0	6
Treatment	Growth population							
Control	2.32^{a}±0.03	3.12^{d} ± 0.01	2.32^{a}±0.03	3.59^{c}±0.02	2.02^{a}±0.01	4.46^{c}±0.01	2.02^{a}±0.01	5.46^{d} ± 0.02
Lm	3.42^{a}±0.02	5.37^{d} ± 0.01	3.42^{a}±0.02	5.77^{d} ± 0.02	4.34^{a}±0.02	6.87^{d} ± 0.01	4.34^{a}±0.02	6.98^{d} ± 0.01
Lm + P	3.25^{a}±0.06	4.88^{d} ± 0.02	3.25^{a}±0.06	5.19^{d} ± 0.02	3.45^{a}±0.02	5.38^{d} ± 0.02	3.45^{a}±0.02	5.68^{d} ± 0.02
Lm + TSP_{10}	3.23^{a}±0.03	4.86^{d} ± 0.02	3.23^{a}±0.02	5.17^{d} ± 0.02	3.25^{a}±0.02	5.23^{d} ± 0.01	3.25^{a}±0.02	5.45^{d} ± 0.02
Lm + P + TSP_{10}	3.23^{a}±0.02	4.84^{d} ± 0.02	3.23^{a}±0.02	5.15^{d} ± 0.01	3.38^{a}±0.02	5.22^{d} ± 0.01	3.38^{a}±0.02	5.32^{d} ± 0.02
Lm + TSP_{30}	3.20^{a}±0.01	4.40^{d} ± 0.01	3.20^{a}±0.01	4.93^{d} ± 0.02	3.19^{a}±0.02	5.20^{d} ± 0.02	3.19^{a}±0.02	5.59^{d} ± 0.02
Lm + P + TSP_{30}	3.17^{a}±0.02	4.32^{c}±0.02	3.17^{a}±0.02	4.76^{d} ± 0.01	3.12^{a}±0.01	4.65^{d} ± 0.01	3.12^{a}±0.01	4.85^{d} ± 0.02
Lm + TSP_{60}	3.10^{a}±0.02	3.66^{d} ± 0.02	3.10^{a}±0.02	4.52^{d} ± 0.01	2.18^{a}±0.02	3.25^{d} ± 0.02	2.18^{a}±0.02	3.45^{d} ± 0.02
Lm + P + TSP_{60}	3.05^{a}±0.01	3.32^{d} ± 0.01	3.05^{a}±0.01	3.77^{d} ± 0.02	2.00^{a}±0.02	2.24^{d} ± 0.02	2.00^{a}±0.02	2.61^{c}±0.02

Control: Chlorine at 200 mg/L, Lm: *L. monocytogenes*, Lm + P: *L. monocytogenes* plus phage, Lm + TSP: *L. monocytogenes* plus trisodium phosphate. Lm + P + TSP: *L. monocytogenes* plus phage and trisodium phosphate. Values are means ± standard deviation of three replicates experiments. Mean values in the same column with the same superscripts are not significantly different ($p \leq 0.05$).

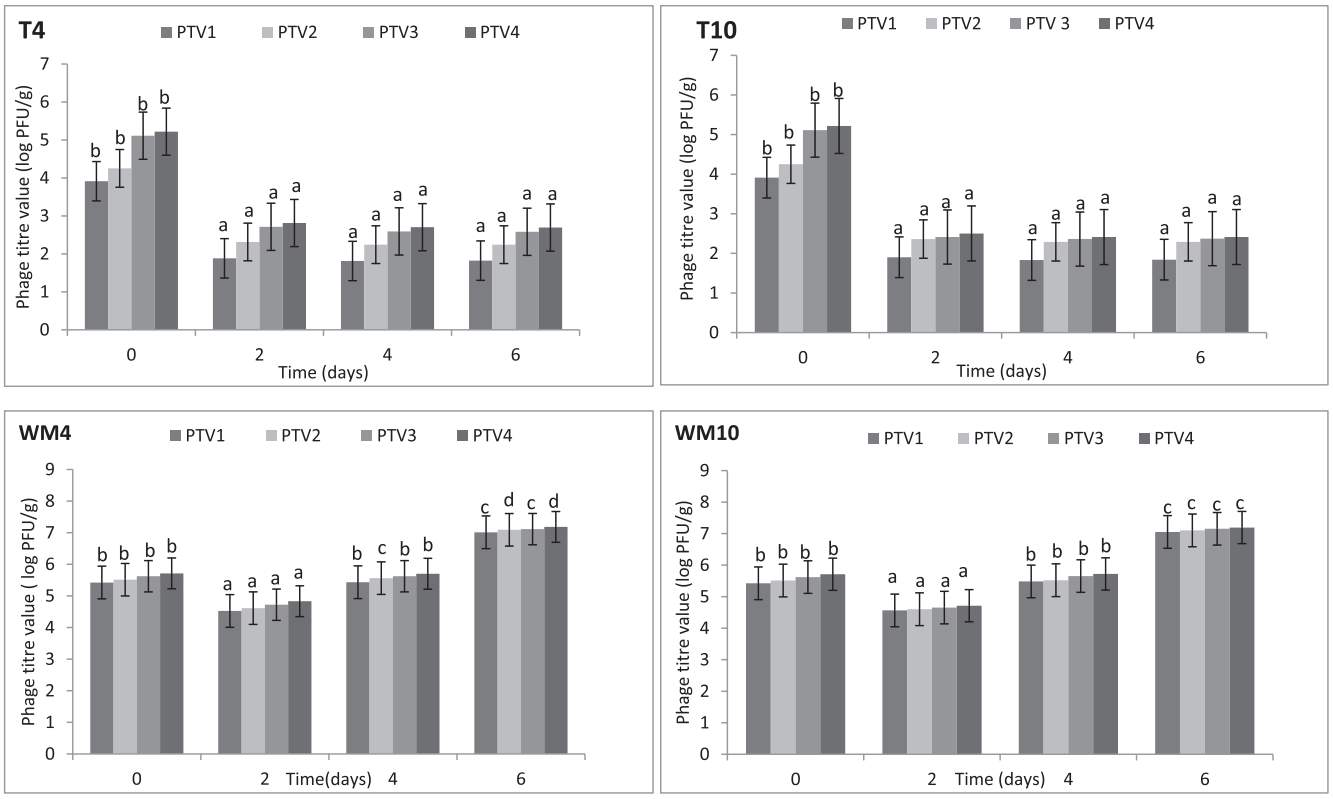

Fig. 1. Effect of anti-listeria combination of phage and TSP treatment at 10, 30 and 60 mg/ml on phage titre values (population) on inoculated fresh-cut tomato (T4 & T10) and fresh-cut melon (WM4 & WM 10) stored at 4 and 10 °C for six days. T4 & T10 represents fresh-cut tomato storage at 4 and 10 °C. WM 4 & WM 10 represents fresh-cut water-melon storage at 4 & 10 °C respectively. Data represents mean value of three replicate determinations and bars represents standard deviation of the mean where different letters A-D indicates significant differences ($p < 0.05$) among treatments for the days of storage.
PTV1: Titre values of phage treatment alone, PTV2: Titre values of phage plus TSP at 10 mg/ml, PTV3: Titre values of phage plus TSP at 30 mg/ml, PTV4: Titre values of phage plus TSP at 60 mg/ml.

tension on the cell wall could not be achieved. However, phage-TSP at 30 and 60 mg/ml showed significant ($p < 0.05$) reductions on both produce from 1 to ~ 5 log CFU/ml, with highest reduction on fresh-cut tomato and melon stored at 4 °C by phage-TSP at 60 mg/ml showing 2.05 and 4.63 log CFU/ml respectively. This phenomenon could be linked to better access for phage lysis of the pathogen via effective TSP reduction of surface tension (surfactant property) on the cell wall. Unlike gram negative bacteria, previous authors have reported the ineffectiveness of TSP on gram positive bacteria such as *L. monocytogenes* (Parish et al. 2003; del Río et al. 2006; Su & D'Souza, 2012). In retrospect, such anti-listeria ability of TSP on the tested fresh produce has often been limited to few seconds/minutes. For example, Su and D'Souza (2012) reported negligible *L. monocytogenes* reductions of 0.21 and 0.28 log CFU/ml on inoculated fresh lettuce when tested with 20 and 50 mg/ml TSP for 5 min respectively. The author however suggested longer contact time, higher concentration and combination of TSP with other antimicrobial for effective *L. monocytogenes* inactivation. In the present study, longer contact time (storage) of six days and increased TSP concentration of 60 mg/ml, combined with phage lysis, was evaluated and found to be effective on this pathogen.

Furthermore, an approximate 5 log reduction as reported requirement of phage inactivation of *L. monocytogenes* on solid matrix (fresh-cut water melon) was obtained (Carlton, Noordman, Biswas, De Meester, & Loessner, 2005; Pietracha & Misiewicz, 2016). This result further support previous findings by Hoelzer et al. (2014) who reported 2.5–5.0 log CFU/ml reduction of *L. monocytogenes* inoculated on apple and lettuce when treated with TSP.

3.2. Effect of antimicrobial treatment on pH and colour

The pH of untreated samples slightly ($p < 0.05$) increased after six days of storage. The pH increased from 4.34 to 4.47 and 4.43–4.58 on untreated fresh-cut tomato, and increased from 5.89 to 5.97 and 5.93–5.99 on untreated fresh-cut melon stored at 4 and 10 °C respectively (Tables 2 and 3). Phage treatment was insignificant on the pH value of both samples, while addition of TSP at 10, 30 and 60 mg/ml significantly ($p < 0.05$) increased the pH values.

Summarily, phage-TSP addition increased pH values from 4.96 to 6.87 and from 4.99 to 6.79 on fresh-cut tomato stored at 4 and 10 °C respectively, while similar increase from 6.08 to 7.97 and 5.95–8.79 was observed on fresh-cut melon. Increase in pH could be attributed to the alkaline nature of TSP, which lowers the acidity of the medium hence favoring phage lysis on low-acid medium (melon) than acidic medium (tomato) as shown by variations in phage titer values (Leverentz et al. 2003).

Effect of antimicrobial treatment on colour parameters CIE (L^* a* b*) of both fresh-cut produce showed slight ($p < 0.05$) decrease after storage (Tables 2 and 3). Specifically, the a^* chroma-value which represent the degree of redness for both samples decreased from 22.16 to 20.18 and 21.15–19.18 on untreated fresh-cut tomato stored at 4 and 10 °C respectively, while it decreased from 24.16 to 21.28 and 22.25–20.28 on untreated fresh-cut melon stored at same temperatures.

However, this chroma value was not affected by phage treatment on both samples, but slightly increased with addition of TSP. After storage, phage-TSP slightly ($p < 0.05$) increased a* value from 23.66 to 25.39 and 22.09–25.17 on fresh-cut tomato stored at 4 and

Table 2
Effect of antimicrobial treatment of phage and TSP at 10, 30 and 60 mg/ml on pH and colour (CIE $L^* a^* b^*$ and hue angle) of fresh-cut tomato stored for six days at 4 and10 °C.

Day	0					6				
Parameters	pH	L^*	a*	b*	Hue angle	pH	L^*	a*	b*	Hue angle
Tomato(4 °C)										
Control	$4.34^{a} \pm 0.04$	$60.84^{e} \pm 0.11$	$22.16^{a} \pm 0.04$	$19.06^{b} \pm 0.18$	$40.69^{d} \pm 0.12$	$4.47^{a} \pm 0.11$	$58.16^{e} \pm 0.12$	$20.18^{a} \pm 0.06$	$19.00^{c} \pm 0.03$	$43.27^{c} \pm 0.14$
Lm + P	$4.38^{a} \pm 0.21$	$58.42^{d} \pm 0.10$	$22.09^{a} \pm 0.06$	$18.49^{ab} \pm 0.16$	$39.87^{cd} \pm 0.11$	$4.52^{a} \pm 0.13$	$51.14^{d} \pm 0.11$	$20.15^{a} \pm 0.12$	$18.35^{bc} \pm 0.08$	$43.32^{c} \pm 0.10$
Lm + P + TSP_{10}	$4.96^{ab} \pm 0.23$	$52.34^{c} \pm 0.08$	$23.66^{ab} \pm 0.13$	$18.40^{ab} \pm 0.05$	$37.87^{c} \pm 0.25$	$5.02^{ab} \pm 0.18$	$50.46^{c} \pm 0.18$	$21.53^{ab} \pm 0.14$	$18.31^{bc} \pm 0.17$	$40.38^{bc} \pm 0.24$
Lm + P + TSP_{30}	$5.51^{b} \pm 0.28$	$48.25^{b} \pm 0.25$	$25.74^{b} \pm 0.15$	$17.23^{a} \pm 0.11$	$33.80^{b} \pm 0.16$	$5.74^{b} \pm 0.16$	$47.19^{b} \pm 0.07$	$23.36^{b} \pm 0.16$	$17.18^{b} \pm 0.22$	$36.33^{b} \pm 0.15$
Lm + P + TSP_{60}	$6.53^{c} \pm 0.05$	$44.78^{a} \pm 0.15$	$28.79^{c} \pm 0.07$	$17.01^{a} \pm 0.06$	$30.58^{a} \pm 0.08$	$6.87^{c} \pm 0.09$	$42.13^{a} \pm 0.03$	$25.39^{c} \pm 0.06$	$16.73^{a} \pm 0.28$	$33.38^{a} \pm 0.07$
Tomato(10 °C)										
Control	$4.43^{a} \pm 0.10$	$58.82^{d} \pm 0.09$	$21.15^{a} \pm 0.14$	$18.38^{b} \pm 0.25$	$40.99^{d} \pm 0.17$	$4.58^{a} \pm 0.15$	$55.27^{e} \pm 0.25$	$19.18^{a} \pm 0.17$	$17.36^{c} \pm 0.04$	$42.15^{d} \pm 0.05$
Lm + P	$4.50^{a} \pm 0.31$	$51.40^{c} \pm 0.03$	$21.05^{a} \pm 0.06$	$17.29^{ab} \pm 0.07$	$39.39^{cd} \pm 0.07$	$4.61^{a} \pm 0.17$	$48.38^{d} \pm 0.24$	$19.11^{a} \pm 0.22$	$16.27^{b} \pm 0.06$	$40.41^{d} \pm 0.07$
Lm + P + TSP_{10}	$4.99^{ab} \pm 0.32$	$50.29^{c} \pm 0.05$	$22.09^{ab} \pm 0.03$	$17.24^{ab} \pm 0.06$	$37.97^{c} \pm 0.26$	$5.11^{b} \pm 0.06$	$47.36^{c} \pm 0.21$	$20.16^{b} \pm 0.25$	$16.19^{b} \pm 0.12$	$38.76^{c} \pm 0.16$
Lm + P + TSP_{30}	$5.59^{b} \pm 0.27$	$48.19^{b} \pm 0.13$	$24.04^{b} \pm 0.22$	$16.58^{a} \pm 0.11$	$34.59^{b} \pm 0.24$	$5.68^{b} \pm 0.02$	$46.29^{b} \pm 0.10$	$22.28^{c} \pm 0.05$	$15.42^{a} \pm 0.28$	$34.69^{b} \pm 0.24$
Lm + P + TSP_{60}	$6.64^{c} \pm 0.22$	$43.18^{a} \pm 0.26$	$27.19^{c} \pm 0.21$	$16.12^{a} \pm 0.10$	$30.66^{a} \pm 0.16$	$6.79^{c} \pm 0.01$	$41.19^{a} \pm 0.11$	$25.17^{d} \pm 0.11$	$15.16^{a} \pm 0.19$	$31.06^{a} \pm 0.17$

Control: Untreated, Lm + P: *L. monocytogenes* plus phage, Lm + P + TSP: *L. monocytogenes* plus phage and trisodium phosphate.
Values are means ± standard deviation of three replicates experiments.
Mean values in the same column with the same superscripts are not significantly different ($p \leq 0.05$).

Table 3
Effect of phage and TSP at 10, 30 and 60 mg/ml on pH and colour (CIE $L^* a^* b^*$ and hue angle) of fresh-cut watermelon stored for six days at 4 and 10 °C.

Day	0					6				
Parameters	pH	L^*	a*	b*	Hue angle	pH	L^*	a*	b*	Hue angle
WM (4 °C)										
Control	$5.89^{a} \pm 0.10$	$56.84^{e} \pm 0.14$	$24.16^{a} \pm 0.19$	$21.06^{b} \pm 0.12$	$41.08^{d} \pm 0.18$	$5.97^{a} \pm 0.15$	$53.26^{e} \pm 0.12$	$21.28^{a} \pm 0.05$	$20.09^{c} \pm 0.03$	$43.35^{c} \pm 0.04$
Lm + P	$5.91^{a} \pm 0.14$	$52.42^{d} \pm 0.26$	$24.29^{a} \pm 0.27$	$20.49^{ab} \pm 0.11$	$40.15^{cd} \pm 0.25$	$6.12^{a} \pm 0.23$	$50.24^{d} \pm 0.05$	$21.35^{a} \pm 0.14$	$19.45^{bc} \pm 0.11$	$43.33^{c} \pm 0.21$
Lm + P + TSP_{10}	$6.08^{ab} \pm 0.21$	$48.34^{c} \pm 0.16$	$26.76^{ab} \pm 0.18$	$20.40^{ab} \pm 0.21$	$37.32^{c} \pm 0.11$	$6.17^{ab} \pm 0.04$	$46.36^{c} \pm 0.11$	$24.43^{ab} \pm 0.17$	$19.33^{bc} \pm 0.08$	$38.35^{bc} \pm 0.15$
Lm + P + TSP_{30}	$7.19^{b} \pm 0.13$	$46.25^{b} \pm 0.08$	$27.84^{b} \pm 0.09$	$19.33^{a} \pm 0.26$	$34.77^{b} \pm 0.06$	$7.74^{b} \pm 0.16$	$43.29^{b} \pm 0.08$	$25.26^{b} \pm 0.22$	$18.28^{b} \pm 0.25$	$35.89^{b} \pm 0.07$
Lm + P + TSP_{60}	$7.53^{c} \pm 0.05$	$42.78^{a} \pm 0.17$	$30.79^{c} \pm 0.03$	$19.21^{a} \pm 0.19$	$31.96^{a} \pm 0.21$	$7.97^{c} \pm 0.22$	$40.23^{a} \pm 0.18$	$27.19^{c} \pm 0.12$	$18.23^{a} \pm 0.14$	$33.84^{a} \pm 0.19$
WM (10 °C)										
Control	$5.93^{a} \pm 0.27$	$55.72^{d} \pm 0.25$	$22.25^{a} \pm 0.18$	$20.28^{b} \pm 0.21$	$42.35^{d} \pm 0.20$	$5.99^{a} \pm 0.16$	$52.27^{e} \pm 0.24$	$20.28^{a} \pm 0.03$	$17.36^{c} \pm 0.20$	$40.56^{d} \pm 0.17$
Lm + P	$5.95^{a} \pm 0.16$	$50.36^{c} \pm 0.24$	$22.31^{a} \pm 0.09$	$19.26^{ab} \pm 0.13$	$40.80^{cd} \pm 0.18$	$6.18^{a} \pm 0.27$	$48.38^{d} \pm 0.06$	$20.11^{a} \pm 0.08$	$18.17^{b} \pm 0.08$	$40.41^{d} \pm 0.04$
Lm + P + TSP_{10}	$6.19^{ab} \pm 0.07$	$49.29^{c} \pm 0.06$	$24.11^{ab} \pm 0.16$	$18.34^{ab} \pm 0.05$	$37.26^{c} \pm 0.09$	$6.41^{b} \pm 0.08$	$47.36^{c} \pm 0.17$	$22.26^{b} \pm 0.25$	$17.39^{b} \pm 0.14$	$42.09^{c} \pm 0.18$
Lm + P + TSP_{30}	$7.59^{b} \pm 0.03$	$45.29^{b} \pm 0.05$	$25.14^{b} \pm 0.22$	$17.48^{a} \pm 0.25$	$34.81^{b} \pm 0.17$	$7.88^{b} \pm 0.05$	$46.29^{b} \pm 0.11$	$23.38^{c} \pm 0.18$	$16.32^{a} \pm 0.18$	$34.92^{b} \pm 0.09$
Lm + P + TSP_{60}	$8.14^{c} \pm 0.15$	$42.28^{a} \pm 0.13$	$28.22^{c} \pm 0.26$	$17.32^{a} \pm 0.06$	$31.54^{a} \pm 0.27$	$8.79^{c} \pm 0.25$	$41.19^{a} \pm 0.15$	$26.07^{d} \pm 0.09$	$16.13^{a} \pm 0.22$	$31.74^{a} \pm 0.22$

WM: Watermelon, Control: Untreated, Lm + P: *L. monocytogenes* plus phage, Lm + P + TSP: *L. monocytogenes* plus phage and trisodium phosphate.
Values are means ± standard deviation of three replicates experiments.
Mean values in the same column with the same superscripts are not significantly different ($p \leq 0.05$).

10 °C respectively, while slight increase from 26.76 to 27.19 and 24.11–26.07 on fresh-cut melon stored at same temperatures was observed. Changes in this chroma value could be linked to biosynthesis of the red pigment compound of the carotenoid-lycopene on both samples with respect to pH changes by TSP addition. This result supports previous findings of Rizk, El-Kady, and El-Bialy (2014) where increase and stability of lycopene pigment in tomato with pH increases was observed.

Furthermore, other authors have reported that the colour of certain inoculated fresh produce was not affected when treated with phage (Perera et al. 2015), and with TSP at 40–150 mg/ml (Zhuang & Beuchat, 1996). Increased use of phage-based products in agriculture (bio-pesticide), food safety (biocontrol) and diagnostic (biotherapy) applications have been reported (Doffkay, Dömötör, Kovács, & Rákhely, 2015; Sillankorva et al., 2012), and this potentially represents a promising, safer and eco-friendly alternative to common sanitizers in fresh produce industry. Furthermore, the present study further validate a potential decontamination technique with respect to quality and safety of fresh produce.

4. Conclusions

Recent interest on the use of phage to inhibit pathogens of public health importance such as *L. monocytogenes* can be further improved by combination with other 'GRAS' approved antimicrobials. From the results obtained in this study, TSP at higher concentration, longer contact time and in combination with phage was very effective. This could serve as a potential inactivating hurdle against *L. monocytogenes* contamination of fresh produce without deleteriously affecting its keeping quality compared to traditional use of chlorine.

Although, results obtained are valid in the present experimental condition on the nature of fresh produce, storage condition, bacterial inoculum size, type and concentration of antimicrobial. However, further investigation into the effect of this combination treatment on other commonly contaminated fresh produce and on the morphology of the pathogen is needed.

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