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Efficacy of bacteriophage and organic acids in decreasing STEC O157:H7 populations in beef kept under vacuum and aerobic conditions: A simulated High Event Period scenario

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ABSTRACT

After High Event Periods, beef subprimals are usually removed from vacuum and treated with antimicrobials. After re-packaging, subprimals are tested to verify the presence of STEC. In this study, bacteriophage and organic acids were applied on beef contaminated with STEC O157:H7 to evaluate the efficiency of industry practices. Beef samples inoculated with STEC were treated with bacteriophage, lactic acid, and peroxyacetic acid and kept under vacuum or aerobic conditions. STEC loads were evaluated 30 min and 6 h after antimicrobial applications. Under aerobic conditions for 30 min and 6 h, phage reduced STEC in beef by approximately 1.4 log whereas organic acids led to a 0.5 log reduction. Under vacuum for 30 min, bacteriophage significantly reduced STEC by 1 log. No effects were observed when samples were treated with organic acids. Under vacuum after 6 h, bacteriophage reduced STEC loads by 1.4 log, lactic acid reduced by 0.6 log, and no effects were observed when peroxyacetic acid was applied.

1. Introduction

In the United States, the production interval when beef processing establishments experience an elevated rate of STEC contamination in trimmings is referred to as a High Event Period (HEP). In large operations, contamination of trimmings is assessed by sampling combos using the N60 or N60 plus methods (USDA-FSIS, 2014). More recently, novel methodologies using a manual sampling device (MSD) and a continuous sampling device (CSD) have been also been implemented to sample combos (Wheeler & Arthur, 2018). The United States Department of Agriculture (USDA) – Food Safety Inspection Service (FSIS) considers an establishment's process to be within a HEP if the percent positive within a specified production interval is 5% or greater (USDA-FSIS, 2014). A HEP may indicate a localized out-of-control event or a systemic breakdown of slaughter dressing operations that led to contamination of beef carcass components including primals, subprimals, and trimmings (USDA-FSIS, 2014). Previous studies have shown that bacteria from hides is the major source of contamination identified in carcasses during processing (Arthur et al., 2008; Bell, 1997; Byrne, Bolton, Sheridan, McDowell, & Blair, 2000; McEvoy et al., 2000). During hide removal, bacteria on hides are usually transferred to the carcass (Barkocy-Gallagher et al., 2003; Nou et al., 2003) whereas contamination of trimmings and primals depends on bacteria loads and the ability that establishments have to prevent cross contamination between hides and carcasses during dressing procedures (Brichta-Harhay et al., 2008). Although a decrease of STEC loads is achieved by implementing good dressing procedures and antimicrobial applications during slaughter (Brashears & Chaves, 2017), Yang, He, Badoni, Tran, and Wang (2017) showed that 62.2% of the *E. coli* found in meat are shared during processing and fabrication with mesh gloves, conveyor belts, and cutting surfaces. Therefore, if not eliminated or reduced by interventions during slaughter, higher STEC loads may be disseminated during fabrication, contaminate trimmings and primals, and increase rates of positive STEC trimming samples.

According to the USDA-FSIS, while STEC positive combos must be diverted to further processing to be heat treated, beef primals and subprimals produced from the same carcasses that generated positive trimmings must be tested before entering commerce (USDA-FSIS, 2014). After experiencing a HEP, establishments rework subprimals associated with contaminated trimmings by (1) removing them from vacuum sealed bags, (2) treating with antimicrobials, and (3) re-packaging. Subsequently, in order to ensure that subprimals are not contaminated, the pieces are (4) removed from vacuum sealed bags again, and the meat surface is swabbed to test for STEC contamination (5). Industry personnel estimates that the time spent from steps 1 to 4 may vary from 30 min to 6 h depending on processing speed and

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production volume.

In the U.S., application of organic acids to decontaminate meat surfaces is the most common intervention used to decrease bacteria loads and eliminate STEC (Wheeler, Kalchayanand, & Bosilevac, 2014). Advantages of using those acids include availability, cost, and simplicity of usage (Mataragas, Skandamis, & Drosinos, 2008). However, previous studies demonstrated that *E. coli* strains may develop resistance to commonly used organic acids including lactic acid (Anderson & Marshall, 1989; Dickson & Kunduru, 1995; Smulders & Greer, 1998). In this study, our goal was to test the effects of bacteriophage applications as an antimicrobial intervention on beef contaminated with *E. coli* O157:H7. Although bacteria can also develop resistance to bacteriophage by mutating its receptors (Oechslin, 2018), phages can also evolve by mutating their own receptors (de Sousa & Rocha, 2019). Therefore, due to this co-evolutionary race, isolating new phages to attack specific bacteria will always remain as an alternative to maintain phage interventions with a broad spectrum. In this research, we evaluated the killing efficiency of a commercial cocktail containing two *E. coli*-specific bacteriophages and peroxyacetic and lactic acids on beef contaminated with STEC, under vacuum and aerobic conditions, by simulating steps and time intervals where subprimals are reworked and retreated after a HEP.

2. Materials and methods

2.1. E. coli strains and inoculum preparation

Four strains of *E. coli* O157:H7 were used in this study to ensure that interventions were tested against different genotypes. Strains included ATCC® 35150™, ATCC® 43895™, ATCC® 43894™, and NCTC 13128. All ATCC strains were *stx*1 and *stx*2 positives whereas the NCTC strain was negative for both genes. ATCC strains were recovered by following the ATCC bacterial culture guidelines (ATCC, 2015) whereas the strain NCTC 13128 was directly recovered from a glycerol stock by streaking the content of the micro tube onto agar plates. Briefly, strains were thawed and a loop full of each stock was aseptically streaked on to 1.6% LB agar plate in duplicate. After incubating overnight at 37 °C, one isolated colony was aseptically added to 10 mL of sterile LB broth and incubated overnight at 37 °C with shaking (200 rpm). Subsequently, 1 ml was then transferred into a tube containing LB broth and cultured overnight at 37 °C. After approximately 16 h, final absorbance was verified at 600 nm (OD600) and CFU concentration per ml was estimated by using the Agilent *E. coli* cell culture biocalculator (AGILENT, 2019). Cultures were individually diluted in 0.1% sterile buffered peptone water (BPW) to achieve 1×10^7 CFU/ml. The final inoculum was prepared by combining individual cultures of all 4 *E. coli* strains.

2.2. Bacteriophage solution titer and killing efficiency

The commercial preparation PhageGuard E™ containing two bacteriophages (EP75 and EP335) was provided by MICREOS Food Safety, Inc. (Wageningen, The Netherlands). Bacteriophage EP75 is a Vi1virus, that belongs to the *Caudovirale* order, Ackermannviridae family, and subfamily Cvivirinae whereas the bacteriophage EP 335 is a Phieco32virus that belongs to the *Caudovirale* order and *Podoviridae* family (Van Mierlo, Hagens, Witte, Klamert, & Fieseler, 2019). Phage stock concentration was determined by following the double-layer agar method (Adams, 1959). Briefly, the PhageGuard E™ stock solution was serially diluted in sterile potable water and added into Luria Broth (LB) soft agar (0.6% agar) previously inoculated with individual fresh-log phase *E. coli* cultures. Soft agar was previously maintained at 43 °C and after inoculation, tubes were vortexed twice and distributed onto the lawn of plates of hard LB hard agar (1.6%). The soft agar was allowed to solidify at room temperature and subsequently and plates were incubated overnight at 30 °C. Concentration of the PhageGuard E™ was determined to be 2×10^{11} PFU/ml.

Killing efficiency of PhageGuard E™ was determined by testing the stock solution against individual *E. coli* strains. A volume of 100 µl of overnight *E. coli* cultures were spread onto LB agar plates. After plates dried, the same volume of bacteriophage solution was applied onto the lawn of the plates. Plates were allowed to dry and were incubated overnight at 30 °C. Killing efficiency was determined in quadruplicate. Bacteriophage was not applied on control plates.

2.3. Sample preparation and experimental design

Beef Rose Meat (m. *cutaneous trunci*, IMPS 194) (USDA-AMS, 2014), a cut that lies on the outside of the carcass and spans from the chuck to the flank (USMEF, 2019) was procured from a USDA-inspected facility. Beef cuts were transported under refrigeration (4 °C) to the University of Nevada, Reno's Meat Quality Laboratory. Upon arrival, aliquots from individual coolers ($n = 4$, approximately 18 kg each) were screened for generic *E. coli*. A total of 160 samples measuring 10×10 cm^2 were subsampled, fabricated from rose meat pieces, and assigned to a completely randomized design (CRD). Fixed effects included antimicrobial treatment (CI = Control Inoculated, PGE = Bacteriophage-treated, PAA = peroxyacetic acid-treated, and LA = lactic acid-treated), packaging atmosphere (V = under vacuum and NV = wrapped with O_2 permeable film), and treatment time (30 min and 6 h).

2.4. Sample inoculation, antimicrobial applications, and bacterial enumeration

Beef samples were inoculated with a cocktail containing all four *E. coli* strains. The concentration of the inoculum was 1×10^7 CFU/ml. A volume of 500 µl of the inoculum was uniformly pipetted on meat and distributed throughout the whole surface area of the sample using a sterile plastic rod. In order to simulate contamination of beef trimmings and beef cuts in a HEP scenario, samples were kept under exposure of O_2 (NV) or in vacuum conditions (V). All samples were kept under refrigeration prior to inoculation and during the trial (5 ± 2 °C). Samples wrapped with O_2 permeable film (NV) were: (1) inoculated, (2) wrapped with film and stored for 17–24 h, (3) removed from package and treated with antimicrobials (PGE, PAA, or LA), (4) wrapped with film and stored for 30 min or 6 h, and (5) removed from package and swabbed for bacteria enumeration. Vacuum packaged samples were processed in similar fashion. Samples were (1) inoculated, (2) vacuum packaged (model VMC10OP, VacPak-It, Lititz, PA, United States) and stored for 17–24 h, (3) removed from package and treated with antimicrobials (PGE, PAA, or LA), (4) vacuum packaged and stored for 30 min or 6 h, and (5) removed from package and swabbed for bacteria enumeration. All antimicrobials were applied at a volume of 500 µl by pipetting and distributing the solution throughout the sample surface. PhageGuard E™ was diluted in potable water as recommended by the manufacturer and applied at a concentration of 1×10^8 PFU/ml. Lactic acid solution (composition = 2-hydroxypropionic acid, 87.5–88.5%, *w*/w, Purac®) was applied at 5% at 50 °C, whereas PAA (Ethaneperoxoic acid, stabilized, < 43%, composition = acetic acid 40–50%, hydrogen peroxide 5–7%, and peroxyacetic acid 10–19%; Xgenx®) was applied at 400 ppm at room temperature. Both organic acids were obtained directly from a chemicals distributor and shipped directly to our laboratory. Control inoculated samples were treated with BPW. For bacteria enumeration, all surface area was swabbed (QS1000, Hygiena, Camarillo, CA, United States) and the swab homogenate was vortexed, serially diluted, and spread-plated onto LB agar plates in duplicate. Plates were incubated inverted overnight at 37 °C, and *E. coli* colonies were enumerated (CFU/cm^2). Differently than our previous experiments (Yeh et al., 2017; Yeh, de Moura, Van Den Broek, & de Mello, 2018), the homogenate obtained by swabbing the beef samples was not treated to eliminate possible residual phages or organic acids before plating. Our goal was to mimic real industry scenarios where phage separation and organic acid

neutralization are not practiced by laboratories after receiving samples from processing facilities.

2.5. Statistical analysis

The experiment was conducted as a CRD and was replicated 5 times whereas 1 replication had 1 repetition, 1 replication had 3 repetitions, and 3 replications had 2 repetitions ($n = 160$ total e.u., $n = 10$ per fixed effects combination). The following model was used: $Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \mathcal{E}_{ijkl}$, where Y_{ijkl} was *E. coli* count, μ was the grand mean across the treatments included in the experiment, α_i was the effect of antimicrobial from the grand mean specific to the *i* levels (CI, PGE, PAA, and LA), β_j was the effect of treatment time from the grand mean specific to *k* levels (30 min and 6 h), and γ_k was the effect of packaging atmosphere from the grand mean specific to *j* levels (V and NV). All interactions and respective errors were also added in the model. In addition, two nested models of interest within packaging atmosphere (γ) were identified. Therefore, data were also analyzed separately as a CRD for V and NV and the following model was used for both: $Y_{ijl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \mathcal{E}_{ijl}$. Data were analyzed using the GLIMMIX procedure of SAS® 9.3 package (SAS Institute, Inc., USA) as a $4 \times 2 \times 2$ factorial for the full model and 4×2 for each nested model. When significance ($P \leq .05$) was indicated, means separations were performed by using the LSMEANS and DIFF functions.

3. Results

Killing efficiency of PhageGuard E™ is shown in Table 1. Bacteriophages EP75 and EP335 reduced the growth of the strains ATCC® 35,150™, ATCC® 43,895™, ATCC® 43,894™, and NCTC 13128 by approximately 98.2%, 97.2%, 96.7%, and 98.3%, respectively.

A three-way interaction ($P = .035$) was observed when evaluating the full model including the three fixed effects (antimicrobial treatment, packaging atmosphere, and treatment time). When looking at individual effects, antimicrobial treatment and packaging atmosphere were highly significant ($P < .0001$) whereas effect of lysing time was not significant ($P = .226$) (Table 2). Therefore, this interaction was mainly driven by effects of antimicrobial treatment and packaging. A three-way interaction translates a two-way interaction that varies across levels of a third effect. In this study, a trend for a two-way interaction between antimicrobial treatment and treatment time ($P = .083$) was observed, thus two nested models containing these both effects were evaluated within packaging atmosphere.

Results of the nested model evaluating samples kept under vacuum (V) are shown in Table 3. Control inoculated samples (CI) treated with BPW yielded 2.07 and 2.37 log CFU/cm^2 after 30 min and 6 h, respectively. The ANOVA showed an interaction between antimicrobial treatment and treatment time ($P = .006$). When samples were treated with PGE, 1.07 and 1.41 log reductions were observed for treatment time of 30 min and 6 h, respectively. Within treatment time of 30 min, no statistical difference was observed when comparing CI, LA, and PAA, whereas PGE applications significantly reduced bacterial load. Overall, samples treated with PGE yielded the lowest bacterial load when compared to CI and organic acid treatments.

Results for samples kept under aerobic conditions (NV) are also shown in Table 3. For this model, no interaction between antimicrobial treatment and treatment time was observed ($P = .986$). Individual effect of treatment time was also not significant ($P = .432$). However, the effects of antimicrobial treatment were statistically significant at $P < .0001$. Under aerobic conditions, samples treated with organic acids showed lower *E. coli* counts when compared to CI samples. Lactic acid applications reduced bacteria loads by 0.47 whereas PAA reduced 0.53 log when compared to CI samples. Bacteriophage applications led to the optimal reduction, lowering bacteria counts by 1.41 log when compared to CI samples. Therefore, in both packaging atmosphere conditions (V and NV), bacteriophage applications showed improved control of *E. coli*, when compared to LA and PAA.

Table 1
Killing efficacy of PhageGuard E™ (Bacteriophages EP75 and EP335) for four *E. coli* O157:H7 strains.

Strain	Bacteriophage application	Average CFU (4 plates)	Killing efficiency (%)
ATCC® 35150™	[a]Control	83.5	98.2
	2×10^{11} PFU/ml	1.5	
ATCC® 43895™	Control	181	97.2
	2×10^{11} PFU/ml	5	
ATCC® 43894™	Control	407	96.7
	2×10^{11} PFU/ml	13.5	
NCTC 13128	Control	103.5	98.3
	2×10^{11} PFU/ml	1.75	

[a] No bacteriophage applied

Table 2
Significance of individual fixed effects and interactions for the full model: [a]$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \mathcal{E}_{ijkl}$.

Fixed effect	*P*-value
α (antimicrobial treatment)	< 0.0001
β *(Treatment time)*	0.226
γ *(packaging atmosphere)*	< 0.0001
$\alpha\beta$	0.083
$\alpha\gamma$	0.237
$\beta\gamma$	0.851
$\alpha\beta\gamma$	0.035

[a] Y_{ijkl} = *E. coli* counts; μ = overall mean; α_i = effect of antimicrobial from the grand mean specific to the i levels (CI, PGE, PAA, and LA); βj = effect of treatment time from the grand mean specific to j levels (30 min and 6 h); γ_k = was the effect of packaging atmosphere from the grand mean specific to k levels (V and NV). Assuming that errors have a normal distribution $\mathcal{E} \sim N(0, \sigma^2)$.

4. Discussion

The killing efficiency of the bacteriophage solution against the 4 strains was at least 96.7%. Both *caudovirales* phages were previously described by Van Mierlo et al. (2019) as STEC-specific phages with genome size of > 50 nucleotides. The EP 75 shares its nucleotide identity of 96% with the *Escherichia* phage Phaxl, 92%, is homologous to *Escherichia* phage ECML-4, and have at least 91% nucleotide identity with other *Salmonella* phages. The bacteriophage EP 335 has nucleotide identity of 96% with the *Escherichia* phage KBNP1711 and 85.47% with *Escherichia* phage NJO1. The EP 335 is also homologous to two more *Escherichia* phages and to the vB_EcoP_SU10, a bacteriophage that can infect a wide range of *E. coli* strains (Mirzaei, Eriksson, Kasuga, Haggård-Ljungquist, & Nilsson, 2014). Bacteriophages move between different host species and acquire different genetic material, which possibly, broadens their host range (Bergman, Fineran, Petty, & Salmond, 2019).

To our knowledge, this study was the first to compare the effects of bacteriophage versus organic acid solutions on the reduction of *E. coli* contamination in vacuum and aerobically-packaged beef. After a HEP, beef processors usually segregate subprimals corresponding to STEC positive trim combos to be treated with antimicrobials. These subprimals are only allowed to enter the commerce after tested negative for STEC, otherwise they must be diverted to further processing as positive trimmings. In the United States, LA and PAA are two of the most common organic acids used to decontaminate beef carcasses and subprimals. Peroxyacetic acid is a combination of hydrogen peroxide and acetic acid that when diluted in water disrupts chemiosmotic functions

Table 3
Effects of the application of organic acids and bacteriophage solutions under vacuum (V) and aerobic conditions (non-vacuum, NV) on beef contaminated with 4 *E. coli* strains (ATCC® 35,150™, ATCC® 43,895™, ATCC® 43,894™, and NCTC 13128). Log CFU/cm^2.

		Antimicrobial treatment*				
Nested model	Treatment time	CI	LA	PAA	PGE	Average**
Vacuum (V)	30 min	2.07^{A}	2.12^{Aa}	1.80^{Ab}	1.00^{B}	1.75
	6 h	2.37^{A}	1.72^{Bb}	2.30^{Aa}	0.96^{C}	1.84
	Average***	2.23^{A}	1.92^{AB}	2.06^{B}	0.68^{C}	*SEM = 0.13*
Non-vacuum (NV)	30 min	2.68	2.17	2.14	1.26	2.07
	6 h	2.71	2.29	2.21	1.31	2.13
	Average***	2.70^{A}	2.23^{B}	2.17^{B}	1.29^{C}	*SEM = 0.11*

A,B,C Means in the same row having different superscripts are significant at $P = .0061$ for V and $P < .0001$ for NV.
a,b Means in the same column within V having different superscripts are significant at $P < .0061$.
SEM = Standard error of the mean.
* CI = Control Inoculated, LA = Lactic acid at 5%, PAA = Peroxyacetic acid at 400 ppm, and Bacteriophage (PhageGuard E™ at 1×10^8 PFU/ml).
** Average of means within treatment time ($P = .432$).
*** Average of means within antimicrobial treatment ($P < .0001$).

of the membrane and inhibit bacterial biochemical metabolism (Baldry & Fraser, 1988; Leaper, 1984; Liberti, Notarnicola, & Lopez, 1999). The mode of action of LA is based on the disruption of cell regulation, which requires the bacterium to use additional amount of energy to maintain its internal optimal pH in order to avoid acid stress damage in cellular mechanisms (Desriac et al., 2013; Foster, 1995).

The efficacy of both organic acids has been reported by several authors who showed inconsistent reductions varying from no reduction to 3.5 log. Regarding PAA, Penney et al. (2007) evaluated spray applications at 180 ppm on beef and veal carcasses surfaces and observed a 3.5 log reduction of nonpathogenic *E. coli* O157:H7. Ellebracht et al. (2005) detected no > 1.2 log reduction when dipping beef trimmings in PAA solutions at 200, 500, and 1000 ppm. Both studies used only one *E. coli* O157:H7 strain (Penney et al used the strain NZRCC 3614 whereas Ellebracht et al. used a pathogenic rifampicin-resistant strain) and tested the efficiency of PAA using different application methods. Based on results presented in both studies, it was expected to observe optimal reduction when samples were submerged in PAA solutions due to a better distribution of the antimicrobial on trimmings. Conversely, Penney et al. observed lower counts of *E. coli* counts when compared to results presented by Ellebracht et al. In addition, King et al. (2005), using the same strain as used by Ellebracht et al., did not observe a significant reduction of *E. coli* when PAA was applied on contaminated carcass surfaces.

For years, LA has been widely used in the United States to decontaminate beef carcasses and subprimals (Fouladkhah, Geornaras, Yang, & Sofos, 2013). Bosilevac, Nou, Barkocy-Gallagher, Arthur, and Koohmaraie (2006) showed a 1 log reduction of *Enterobacteriaceae* counts when applying a solution of LA at 2% at 42 °C on carcasses. Castillo et al. (2001) tested the efficiency of LA on the same *E. coli* strain used by Ellebracht et al. and showed that a solution of 4% led to a 2 log reduction when a post chill spray was applied onto outside rounds. In addition, Pittman et al. (2012) testing the effectiveness of LA on five strains of STEC O157:H7, achieved a 1.6 log reduction of bacteria loads when applying LA at 5% in vacuum conditions with treatment time of 24 h. In the same study, an additional 1.2 reduction was observed after a second application of LA after beef samples were removed from vacuum. However, Uyttendaele et al. (2001) showed no reduction of two different *E. coli* strains (an acid resistant - II/45/4, and an acid sensitive - IX/8/16) when LA at 2% was applied on fresh beef surface.

In our study, when simulating a rework procedure of subprimals after a HEP (treating with antimicrobials and re-vacuum-packaging the meat), PAA did not significantly reduce bacteria loads (for 30 min, CI = 2.07^{A} vs PAA = 1.80^{A}; and for 6 h, CI = 2.37^{A} vs PAA = 2.30^{A} log CFU/cm^2). No reduction was also observed when samples were treated with LA for 30 min under vacuum conditions (CI = 2.07^{A} vs LA = 2.12^{A}). However, when treatment time was extended to 6 h, LA led to a significant 0.65 log reduction in *E. coli* counts. Overall, *E. coli* O157:H7 strains have been reported to be able to self-adapt in acid conditions, including two strains tested in this research (Tsai & Ingham, 1997). Although Pittman et al. (2012) showed a large and significant reduction (1.6 log) of one strain (ATCC 43895) combined with other strains in a cocktail, our results showed a small reduction when compared to bacteriophage applications. When the bacteriophage solution was applied, optimal *E. coli* reductions were observed when compared to both LA and PAA acids (for 30 min, CI = 2.07^{A} vs BA = 1.00^{B}; and for 6 h, CI = 2.37^{A} vs BA = 0.96^{C}).

Under aerobic conditions, treatment time did not affect *E. coli* counts. Although both organic acids significantly lowered contamination, the lowest counts were observed when beef was treated with PGE (CI = 2.70^{A}, LA = 2.23^{B}, PAA = 2.17^{B}, and BA = 1.29^{C}). Better activity of organic acids under aerobic conditions when compared to vacuum may be associated to different bacterial metabolisms. In vacuum conditions, *E. coli* metabolism possibly shifts from aerobic to anaerobic due to low availability or absence of O_2 (Unden, Becker, Bongaerts, Schirawski, & Six, 1994). Although vacuum packaging does not totally remove all O_2 from the package (Kelly, Cruz-Romero, Kerry, & Papkovsky, 2018), *E. coli* strains possible adopt a less effective metabolism alternating from aerobic to anaerobic when growing in low O_2 conditions. It is known that anaerobic metabolism and fermentation are less efficient than aerobic metabolism (Von Wulffen et al., 2016), therefore, under aerobic conditions (NV), bacteria have a higher metabolism rate. As previously mentioned, the effects of both organic acids used in this research are directly dependent on bacteria metabolism, which in optimal aerobic conditions could have led to a better bacterial load reduction when compared to the absence of antimicrobial effect observed in vacuum-packaged samples.

Optimal PGE effects on *E. coli* loads both under vacuum and in aerobic conditions were possibly achieved due phage mode of action. Lytic *caudovirales* bacteriophages attach and inject viral DNA into bacteria, hijacking the host replication and translation mechanisms to produce phage structural proteins, which are assembled into virions with genetic material. Subsequently, phages are released from the infected cell through the activity of endolysins, peptidoglycan hydrolases that burst the cell wall at the end of the cycle (Salmond & Fineran, 2015). Although bacteriophage applications against STEC in vacuum conditions has not been discussed in scientific reports yet, results of this research suggest that bacteriophage activity seems to be more effective than organic acids, even when *E. coli* adopts a less efficient metabolism when low concentrations of O_2 are available. When compared to organic acids, the bacteriophage also depends on bacteria metabolism to perform its lytic cycle. However, the bacteriophage better efficiency in

lysing host cells is possibly attributed to independent molecular mechanisms that initiate viral DNA replication. Weigel and Seitz (2006) suggest that *E. coli* bacteriophages are able to regenerate closed ends of the bacteria DNA to generate phage genome and consequently produce phage structures. Previous research suggested that *E. coli* strains are still able to replicate during cold storage under vacuum atmosphere (Dykes, Moorhead, & Roberts, 2001). Therefore, even with low metabolic rates, the active and independent bacteriophage mode of action is still able to induce the lyse of the host, differently than organic acids, which probably depend on higher bacterial metabolic rates to induce bacteria cell death. The efficacy of bacteriophage applications in lowering *Enterobacteriaceae* in meats has been demonstrated by several authors in different conditions (Duc, Son, Honjoh, & Miyamoto, 2018; Hudson et al., 2013; Hungaro, Mendonça, Gouvêa, Vanetti, & Pinto, 2013; Liu et al., 2015; Stratakos & Grant, 2018; Sukumaran, Nannapaneni, Kiess, & Sharma, 2015; Thung et al., 2017; Tomat, Casabonne, Aquili, Balagué, & Quiberoni, 2018; Yeh et al., 2017; Yeh et al., 2018; Zinno, Devirgiliis, Ercolini, Ongeng, & Mauriello, 2014), but to our knowledge, no studies evaluating the effects of bacteriophage activity on STEC under vacuum conditions were published. Results of this research suggested that bacteriophage applications targeting STEC contamination in beef are more effective than organic acids in both scenarios, under aerobic conditions and when meat is vacuum-packaged.

5. Conclusion

Bacteriophage applications led to an optimal reduction of *E. coli* O157:H7 (including *stx*1 and *stx*2 gene-positives) in contaminated beef when compared to organic acid applications. Lactic and peroxyacetic acids did not reduce *E. coli* counts under vacuum conditions and minimally decrease contamination under aerobic atmosphere. Bacteriophage applications are more effective than organic acids when decontaminating beef under aerobic conditions similar to subprimal and trimmings fabrication for example. In addition, when reworking beef subprimals associated to STEC-positive trimmings after a High Event Period, packing plants must consider using bacteriophage solutions to improve the control of *E. coli* O157:H7 contamination in vacuum-packaged beef.

Declaration of Competing Interest

The authors declare no conflict of interest associated with this research.

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