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Effect of Acid Resistance of *Escherichia coli* O157:H7 on Efficacy of Buffered Lactic Acid To Decontaminate Chilled Beef Tissue and Effect of Modified Atmosphere Packaging on Survival of *Escherichia coli* O157:H7 on Red Meat

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ABSTRACT

The present study examined the effect of pH-independent acid resistance of *Escherichia coli* O157:H7 on efficacy of buffered lactic acid to decontaminate chilled beef tissue. A varied level of acid resistance was observed among the 14 strains tested. Eight strains were categorized as acid resistant, four strains as acid sensitive, and two strains demonstrated acid-inducible acid resistance. The survival of an acid-resistant (II/45/4) and acid-sensitive (IX/8/16) *E. coli* O157:H7 strain on chilled beef tissue treated with 1 and 2% buffered lactic acid, sterile water, or no treatment (control) was followed. A gradual reduction of *E. coli* O157:H7 was noticed during the 10 days of storage at 4°C for each of the treatments. Decontamination with 1 and 2% buffered lactic acid did not appreciably affect the pathogen. Differences in the pH-independent acid resistance of the strains had no effect on the efficacy of decontamination. The effect of modified atmosphere packaging (MAP) on survival of *E. coli* O157:H7 in red meat was also studied. MAP (40% CO_2/60% N_2) or vacuum did not significantly influence survival of *E. coli* O157:H7 on inoculated sliced beef (retail cuts) meat compared to packing in air. The relative small outgrowth of lactic acid bacteria during storage under vacuum for 28 days did not affect survival of *E. coli* O157:H7. Neither lactic acid decontamination nor vacuum or MAP packaging could enhance reduction of *E. coli* O157:H7 on beef, thus underlining the need for preventive measures to control the public health risk of *E. coli* O157:H7.

Escherichia coli O157:H7, first recognized as a pathogen in 1982, causes diarrhea, bloody diarrhea (hemorrhagic colitis), and renal failure (hemolytic uremic syndrome) in humans. Cattle are a known natural reservoir of this organism, and food of animal origin can be contaminated with the pathogen during slaughtering or processing. The serious nature of the illness caused by *E. coli* O157:H7 makes prevention of human infection a high priority for the food industry *(20).* Continuous hygiene is the most effective pathogen-intervention strategy available. However, occasional contamination does occur. Decontamination procedures can minimize the risk of bacterial pathogens on meat products from contaminated raw carcasses *(11).*

The effectiveness of organic acid washes on the inactivation of pathogenic and nonpathogenic bacteria associated with beef tissue has been extensively studied. However, the efficacy of lactic acid spray for eliminating *E. coli* O157:H7 is controversial. In studies involving multiple pathogens, *E. coli* O157:H7 was shown to be the most resistant toward acid treatment *(21).* Brackett et al. *(2)* reported that acid sprays of up to 1.5% (vol/vol) acetic, citric, and lactic acid did not appreciably affect the pathogen. Conner et al. *(6)* reduced the *E. coli* O157:H7 population by only 0.1 log CFU/g using a 2% acid spray. It was also found that a 3% lactic acid solution at 20°C had no effect on the number of *E. coli* O157:H7 recovered and that only a 1-log reduction was achieved at 55°C *(21),* whereas Dorsa et al. *(12–14)* obtained a lower bacterial count after treatment of beef tissue with lactic acid. Differences in the resistances of *E. coli* O157:H7 to acid washing were observed *(7).* Inducible resistance mechanisms of acid tolerance response or acid shock response could increase the resistance of *E. coli* O157:H7 to acidic conditions *(15).* Therefore, it is important to assess acid-resistance characteristics of the strains involved *(1).* The present study examined the effect of acid resistance of *E. coli* O157:H7 on efficacy of buffered lactic acid to decontaminate chilled beef tissue.

Good hygienic practices should be applied to prevent cross-contamination and dissemination of *E. coli* O157:H7 during storage and processing. The principal method of extending storage life of subprimal cuts or retail cuts is the maintenance of the chill chain. It is essential to keep the temperature of fresh meat products below 4°C. For wholesale trade, refrigeration is frequently combined with modified atmosphere packaging (MAP). Storage of meat in vacuum packs or carbon dioxide-enriched atmospheres (residual oxygen, <1%) leads to a microbial association dominated by lactic acid bacteria

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(LAB) *(8).* Thus, numerous hurdles are present to inhibit *E. coli* O157:H7 during long-term storage and transport of MAP meat. The present study examined the effect of MAP on survival of *E. coli* O157:H7 in red meat.

MATERIALS AND METHODS

Microorganisms and inoculum preparation. The *E. coli* O157:H7 strains used in this study were nine isolates from cattle feces obtained from the Department of Veterinary Food Inspection and Public Health, University of Gent, and five clinical strains obtained from the Department of Microbiology, University of Brussels. Acid-adapted (A) and unadapted (NA, not adapted) exponential-phase cells of each strain were prepared, after prior activation in nutrient broth pH 7.0 (NB; Oxoid, Hampshire, UK) for 18 h at 37°C, by cultivation in NB acidified to pH 5.0 with HCl and NB pH 7.0, respectively, for 4 h at 37°C according to the method described by Leyer et al. *(18).* To prepare inocula for the chilled beef tissue decontamination and MAP of beef retail cuts, two selected isolates from cattle feces (strain IX/8/16, an acid-sensitive strain, and strain II/45/4, an acid-resistant strain) were activated in NB pH 7.0 for 18 h at 37°C.

Determination of acid-resistance characteristics of *E. coli* O157:H7 strains. Acid resistance was monitored by adding cells suspensions of A and NA cultures of each *E. coli* O157:H7 strain to 4 ml of sterilized minimal medium (glucose, 4 g/liter; dipotassium phosphate, 7 g/liter; monopotassium phosphate, 2 g/liter; sodium citrate, 0.5 g/liter; magnesium sulphate, 0.1 g/liter; and ammonium sulphate, 1 g/liter) acidified with DL-lactic acid (85%, wt/vol; Sigma Chemical Co., St. Louis, Mo.) to pH 3.85 to give ca. 7.5 log CFU/ml. Cells were statically incubated in the minimal medium pH 3.85 for 60 min at 25°C. Samples were taken immediately following inoculation and mixing by vortexing (0 min) and after 20, 40, and 60 min. If necessary, samples were diluted in physiological peptone saline (8.5 g/liter NaCl [Vel, Belgium]; 1 g/liter peptone [Oxoid]) and spiral plated in duplicate on modified Levine's eosin methylene blue agar *(5).* Plates were incubated at 37°C for 24 h prior to enumeration.

Decontamination of beef carcass tissue using a lactic acid/sodium lactate buffer. Chilled beef tissue was obtained from the surface of prerigor carcasses of cows slaughtered at the abattoir of the University of Gent. The beef tissue was cooled on ice and immediately transported to the laboratory for inoculation and decontamination.

Surfaces of 25 cm^2 (5 by 5 cm) were marked and inoculated with 0.1 ml (deposit and spread out with a Drigalsky spatula) of an appropriate dilution of the activated culture to obtain ca. 5 log CFU/cm^2. Separate inoculations were performed for each strain: strain IX/8/16 (acid sensitive) and strain II/45/4 (acid resistant). Inoculated beef tissue was kept at 4°C for 1 h to allow the pathogens to attach to the beef tissue. Then, the chilled beef tissue was decontaminated by application of 1 ml (droplets from a 1-ml pipette at ca. a 2-cm distance from the beef tissue) of a 1 and 2% lactic acid/sodium lactate buffer (pH 3.0) at 25°C to a 25-cm^2 inoculated surface of beef tissue. Application of sterile water was used as a control treatment. Untreated inoculated beef tissue was also included in the experiment. All inoculated and decontaminated (1 and 2% buffered lactic acid) beef tissue, as well as the inoculated control (water) and untreated beef tissue, were kept at 4°C for 10 days. Samples (25 cm^2) were taken at days 0, 1, 2, 4, 7, and 10, and *E. coli* O157:H7 was enumerated on modified Levine's eosin methylene blue agar. The experiment was for both the acid-sensitive and acid-resistant strain performed in duplicate.

Survival of *E. coli* O157:H7 under MAP beef gravy medium. Beef gravy medium, a simulation medium for red meat, was prepared as described by Juneja et al. *(17),* autoclaved for 15 min at 121°C, and poured in 9-mm-diameter petri dishes until completely filled. Appropriate dilutions of *E. coli* O157:H7 strain IX/8/16 and strain II/45/4 activated in NB pH 7.0 for 24 h at 37°C were mixed to obtain a cocktail of the two strains. A 0.1-ml volume of the inoculum was spread out using a sterile Drigalsky spatula on the surface of the beef gravy medium to obtain ca. 10^5 CFU/cm^2. Inoculated plates were packed under vacuum or MAP (40% CO_2/60% N_2) (gas/product ratio: 2 cc/1 g) in a plastic bag of high oxygen barrier (Sidamil, UCB Transpac, Belgium) with a MULTIVAC A300/42 (Haggenmuller KG, Wolfertshwenden, Germany). As a control treatment, inoculated beef gravy medium was kept in air. Vacuum, MAP, and air-packed samples were stored at 4°C for 7 days. After 0, 1, 2, 3, 5, and 7 days, samples were taken, and *E. coli* O157:H7 was enumerated on modified Levine's eosin methylene blue agar, in duplicate. The experiment included duplicate samples per sampling day.

Survival of *E. coli* O157:H7 in MAP beef. Raw beef (*Musculus semitendinosus*) was obtained immediately after cutting up a postrigor carcass from the abattoir of the University of Gent and transferred to the lab in a cooled insulated box. Using a disinfected slicing machine, slices of ca. 0.5 cm thick were made. On each slice, 25-cm^2 (5 by 5 cm) surfaces were indicated with a marker. Appropriate dilutions of *E. coli* O157:H7 strain IX/8/16 and strain II/45/4 activated in NB pH 7.0 for 24 h at 37°C were mixed to obtain a cocktail of the two strains. A 0.1-ml volume of the inoculum was spread out using a sterile Drigalsky spatula on the marked 25-cm^2 surface of the chilled beef to obtain ca. 10^5 CFU/cm^2. Inoculated beef slices were individually packed under vacuum or MAP (40% CO_2/60% N_2) (gas/product ratio: 2 cc/1 g) in a plastic bag of high oxygen barrier (Sidamil) with a MULTIVAC A300/42. As a control treatment, inoculated beef slices were kept in air. MAP and air-packed samples were stored at 4°C for 7 days, and vacuum-packed samples were kept at 4°C for 28 days.

After 0, 1, 2, 3, 5, and 7 days of storage at 4°C (as well as after 14, 21, and 28 days for vacuum), samples were taken, and *E. coli* O157:H7 was enumerated on modified Levine's eosin methylene blue agar, in duplicate. Total mesophilic count (TMC) was determined by plating on plate count agar (Oxoid) and incubating for 3 days at 30°C; LAB were determined by plating on deMan Rogosa Sharpe agar (Oxoid) and incubated for 5 days at 30°C. The experiment included duplicate samples per sampling day.

RESULTS AND DISCUSSION

Determination of acid-resistance characteristics of *E. coli* O157:H7 strains. A varied level of resistance to lactic acid was observed among the five clinical strains tested. Two strains were categorized as acid sensitive (EH 302 and EH 402), because both A and NA cells showed marked sensitivity and decrease of populations (ca. 1.0 to 1.4 log) in the minimal medium. Two strains were categorized as acid resistant (EH 372 and EH 385), because both A and NA cells showed resistance toward reduced pH. Strain EH 321 demonstrated acid-inducible acid tolerance: A cells were acid resistant, while NA cells were sensitive. Strains isolated from cattle showed a similar variation in resistance toward lactic acid. Six out of nine strains were categorized as acid resistant: less than a 0.5-log reduction was obtained after 60 min in minimal medium, pH 3.85, for both A and NA cells. Of these, strain II/45/4 (Fig. 1a) was used in

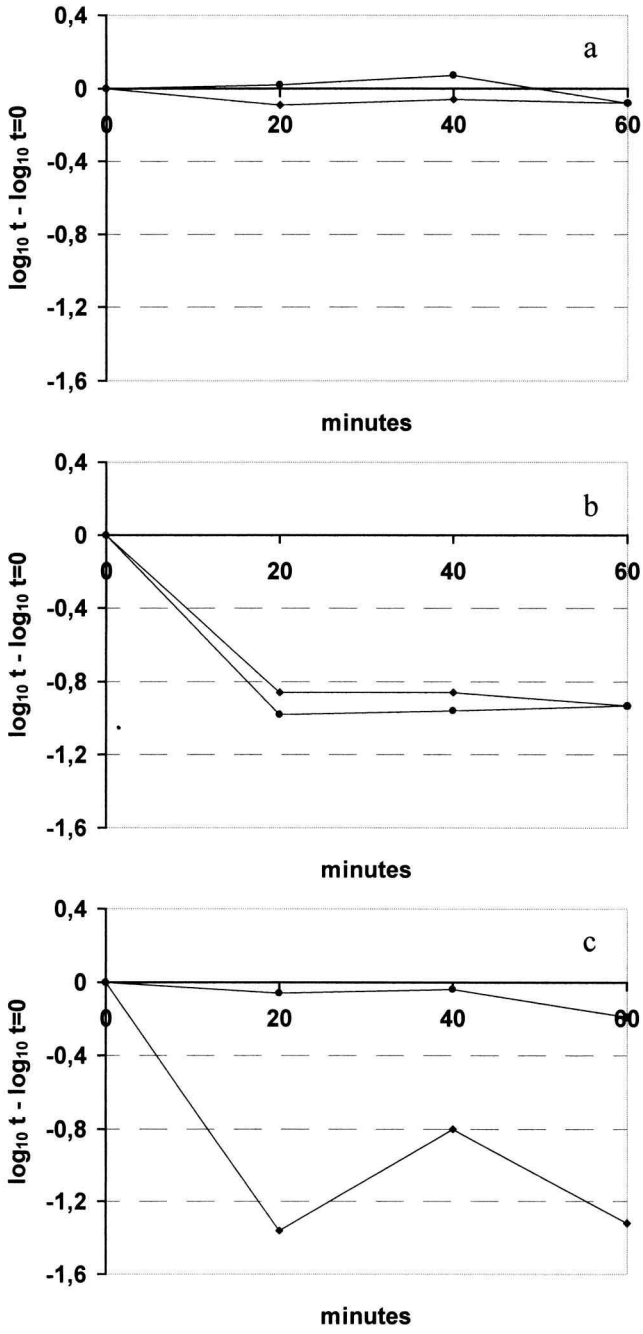

FIGURE 1. *Survival in minimal medium pH 3.85 of acid-adapted (●) and nonadapted cells (◆) of* E. coli *O157:H7 strain II/45/4 (a, acid resistant), strain IX/16/22 (b, acid sensitive), and strain IX/16/22 (c, acid-inducible acid tolerance).*

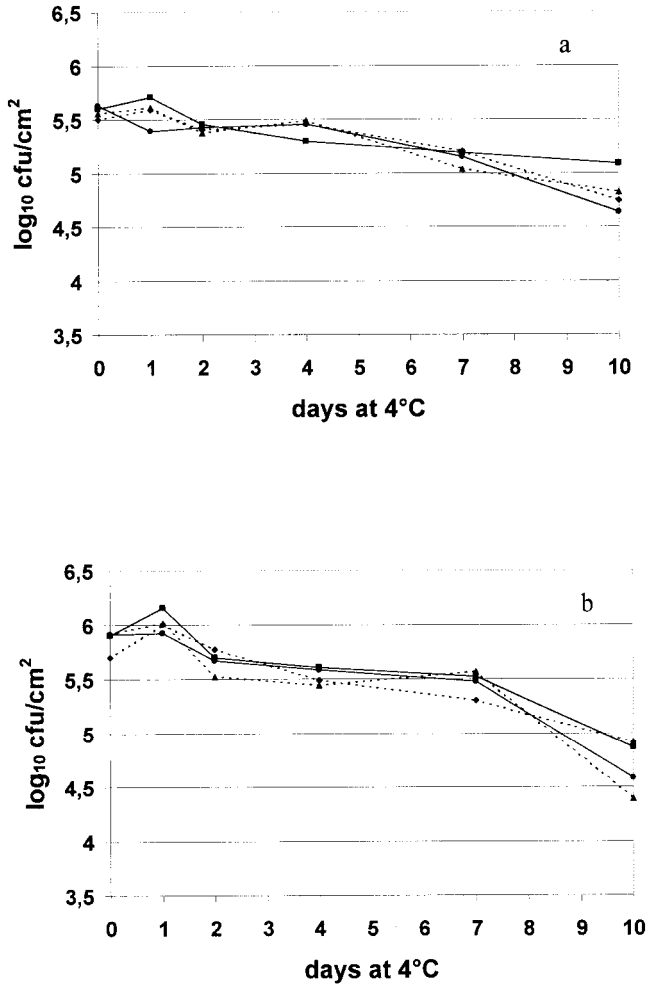

FIGURE 2. *Survival of* E. coli *O157:H7 strain II/45/4 (a, acid resistant) and strain IX/16/22 (b, acid sensitive) on beef carcass tissue stored at 4°C under air after decontamination with 1% buffered lactic acid (- -◆- -) , 2% buffered lactic acid (—●—), sterile water (- -▲- -), and no treatment (—■—).*

further experiments. Strain IX/8/16 (Fig. 1b), an acid-sensitive strain, was also used in further experiments: both A and NA cells showed a 1.0-log reduction after 20 min in minimal medium, pH 3.85. Strain IX/16/22 was the single cattle isolate that demonstrated acid-inducible acid tolerance (Fig. 1c).

Buchanan and Edelson *(4)* concluded that pH-dependent and pH-independent acid-tolerant phenotypes may exist among enterohemorrhagic *E. coli.* Strain-to-strain variability in acid resistance and the ability to induce acid tolerance were also reported by Berry and Cutter *(1).* Prior characterization of acid resistance of *E. coli* O157:H7 strains is important for the subsequent decontamination studies with organic acids.

Decontamination of chilled beef tissue using a lactic acid/sodium lactate buffer. The survival of an acid-resistant *E. coli* O157:H7 strain (II/45/4) on chilled beef tissue treated with 1 and 2% buffered lactic acid, sterile water, or no treatment (control) was similar up to 7 days of storage at 4°C. Small differences (within 0.5 log unit) occurred after 10 days of storage (Fig. 2a). On beef tissue treated with 2% buffered lactic acid, the highest reduction of *E. coli* O157:H7 was observed—about 0.89 log CFU/cm^2. The reduction in cell numbers decreased in the order beef tissue treated with 1% buffered lactic acid (0.79 log CFU/cm^2), sterile water (0.72 log CFU/cm^2), and control (0.54 log CFU/cm^2). In the case of an acid-sensitive strain (IX/8/16), also a gradual reduction of surviving *E. coli* O157:H7 was noticed up to 7 days of storage at 4°C for each of the treatments. After 10 days at 4°C, reductions of the initial population noticed were 1.55, 1.02, 0.90, and 0.79 log CFU/cm^2 for treatment with sterile water, 2% buffered lactic acid, control, and 1% buffered lactic acid, respectively (Fig. 2b). Decontamination with 1 and

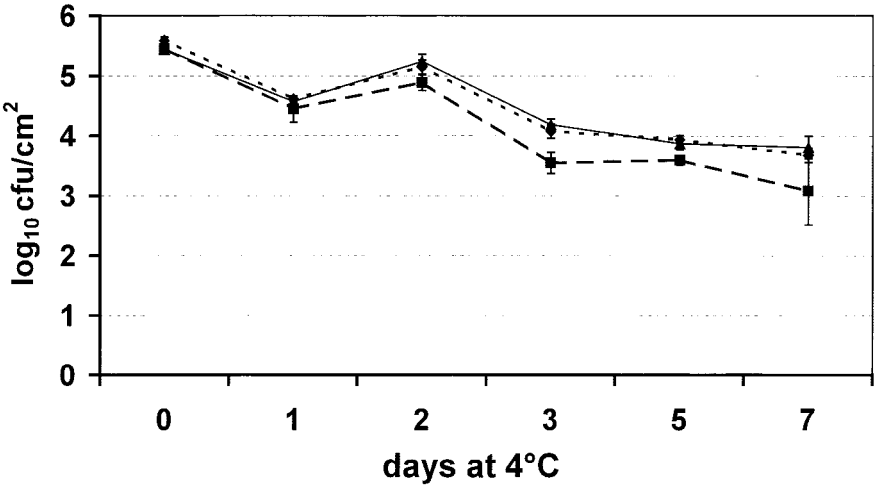

FIGURE 3. *Survival of* E. coli *O157:H7 (strains II/45/4 and IX/16/22) on sliced beef packed under MAP (40% CO_2/60% N_2) (—▲—) , vacuum (- -■- -), and air (- -◆- -) and stored at 4°C.*

2% buffered lactic acid did not appreciably affect the pathogen, probably because the bacterial cells were already too firmly attached to the chilled beef tissue before treatment. This confirms data of Brackett et al. *(2)* that acid sprays had little effect on the number of *E. coli* O157:H7 present on chilled raw beef. Other studies, however, conclude that the use of a lactic acid wash during the processing of beef tissue does reduce populations of *E. coli* O157:H7 and offers some residual efficacy in suppressing pathogen proliferation during refrigerated storage *(12–14).* These researchers have used beef tissue from prerigor carcasses immediately transported to the lab to minimize cooling and applied decontamination before or shortly after inoculation of the pathogen, which may have limited adhesion of the cells. Higher concentrations of buffered lactic acid will be needed to decontaminate chilled beef tissue. On chilled chicken legs, the numbers of *Enterobacteriaceae* were reduced when treated with various concentrations (2, 5, 7.5, and 10%) of lactic acid/sodium lactate buffer systems combined with MAP, and a residual effect of treatment with buffered lactic acid was noted *(24).* The antimicrobial effect was most pronounced at the higher concentrations of buffered lactic acid (7.5 and 10%).

Differences in the pH-independent acid resistance of the *E. coli* O157:H7 in the present study had no effect on the outcome of the lactic acid treatment. Nor did Berry and Cutter *(1)* obtain differences in log reduction due to 2% acetic acid spray washes on prerigor beef tissue for NA cells of an acid-resistant and an acid-sensitive strain. Although it is well established that the inherent or inducible acid resistance of a strain could increase the resistance of *E. coli* O157:H7 to acidic conditions, the advantage of acid-adapted cells or pH-independent acid-resistant cells is not fully confirmed in challenge testing. Deng et al. *(9)* found that A cells retain higher viability than NA cells in only two of nine foods tested. They also demonstrated there was essentially no difference in growth characteristics of A and NA cells in tryptic soy broth acidified at the same pH with a given acid *(10).* Acid shock and acid adaptation had little or no impact on the ability of *E. coli* O157:H7 to survive in apple juice or orange juice *(19).* Nevertheless, Berry and Cutter *(1)* indicated that adaptation to acidic conditions by *E. coli* O157:H7 can negatively influence the effectiveness of acid spray washing of beef tissue. It is recommended to have information on acid-resistance characteristics of *E. coli* O157:H7 strains for decontamination trials and survival studies in acid foods, but reduced pH and organic acids are only part of the hurdle technology commonly applied for prolonged preservation of foods. In foods, microorganisms are exposed to a number of stress conditions, and growth and survival are dependent on a number of factors, which can eliminate the advantage of acid resistance or acid adaptation of the strain in a particular situation.

Survival of *E. coli* O157:H7 under MAP beef gravy medium and sliced beef. MAP of meat is commonly applied to increase shelf life. Survival of *E. coli* O157:H7 on beef gravy medium showed a decrease of the pathogen with 0.87 to 1.0 log unit after 1 day at air, vacuum, and MAP (Fig. 3). Afterward, a slightly more rapid decrease was noted with vacuum packing than with MAP or storage at air.

TABLE 1. *Survival of* E. coli *O157:H7 on the surface of red meat (beef) packed in modified atmosphere (40% CO_2/60% N_2) during storage at 4°C for 7 days (contamination in log_{10} CFU/cm²)*

Microbiological parameter	Days of storage at 4°C					
	0	1	2	3	5	7
TMC	<1.0	1.26	<1.0	<1.0	1.67	<1.0
LAB	<1.0	<1.0	<1.0	<1.0	1.70	<1.0
outgrowth (day *N*–day 0)	—	—	—	—	>0.70	—
E. coli O157:H7	4.48	4.01	3.98	3.83	3.69	3.55
reduction (day *N*–day 0)	—	−0.47	−0.50	−0.65	−0.79	−0.93

TABLE 2. *Survival of* E. coli *O157:H7 on the surface of red meat (beef) kept in air during storage at 4°C for 7 days (contamination in log_{10} CFU/cm²)*

Microbiological parameter	Days of storage at 4°C					
	0	1	2	3	5	7
TMC	<1.0	1.08	<1.0	1.11	0.90	2.27
LAB	<1.0	1.0	<1.0	1.23	<1.0	2.20
outgrowth (day *N*–day 0)	—	—	—	>0.23	—	>1.20
E. coli O157:H7	4.17	4.05	4.00	3.84	3.67	3.88
reduction (day *N*–day 0)	—	−0.12	−0.17	−0.33	−0.50	−0.29

After 3 and 7 days' storage under vacuum, pathogen numbers dropped with 1.9 and 2.4 log units, respectively. At the same time intervals, reductions noted when stored under MAP and air were 1.2 and 1.4 log units (after 3 days) and 1.6 and 1.9 log units (after 7 days), respectively. As there were no competitive flora present on the beef gravy medium, reductions were attained because of the combination of cold temperature storage (4°C) and atmosphere applied. Results indicate that vacuum packaging accomplishes a significant reduction of *E. coli* O157:H7, while MAP does not enhance reduction of the pathogen if compared to storage at air.

However, these observations were not confirmed by survival data of *E. coli* O157:H7 from experiments of inoculated sliced meat packed under MAP (40% CO_2/60% N_2) (Table 1), air (Table 2), and vacuum (Table 3) and stored at 4°C. These results showed that the highest reduction of *E. coli* O157:H7 was obtained under MAP (0.93 log unit in 7 days), while lower but similar reductions were obtained under vacuum and air—0.38 and 0.29 log unit, respectively. Nevertheless, the competitive flora (dominated by LAB) were lower after 7 days at 4°C when packed under MAP (both TMC and LAB <1.0 log CFU/cm²) than under vacuum (TMC: 1.47 log CFU/cm², LAB: 1.18 log CFU/cm²) or air (TMC: 2.27 CFU/cm², LAB: 2.20 log CFU/cm²). The initial contamination for all atmospheres was low (TMC and LAB both ca. 1 log CFU/cm² after 1 day at 4°C). This indicates that the relative small outgrowth of LAB does not affect survival of *E. coli* O157:H7, as was also confirmed by the following. During prolonged storage of vacuum-packed meat, *E. coli* O157:H7 showed good survival (reduction of 0.94, 1.12, and 1.09 log CFU/cm² after 14, 21, and 28 days, respectively, at 4°C), although LAB developed to 1.12, 3.56, and 2.89 log CFU/cm² after 14, 21, and 28 days, respectively, at 4°C. High levels of LAB are required (>5 × 10^7 CFU/ml) to exert antagonistic action toward *E. coli* O157:H7 *(3)*. Vold et al. *(22)* concluded that exclusion of oxygen suppresses outgrowth of *E. coli* O157:H7, probably because the growth of endogenous LAB was more pronounced in the anaerobically stored samples.

The present study showed that MAP or vacuum did not significantly influence survival of *E. coli* O157:H7 on inoculated sliced beef compared to packing in air. Also, little effect of MAP for use with fresh produce (maximum 10% CO_2) was obtained on growth and survival of *E. coli* O157:H7 *(16)* and, regardless of packaging method (air, vacuum, or nitrogen packaging), Chinese-style sausages stored at 5°C demonstrated a slow decrease of viable *E. coli* O157:H7 as the storage period extended *(23)*.

As neither decontamination with a 1 or 2% lactic acid buffer nor vacuum packing or MAP could enhance reduction of *E. coli* O157:H7 on chilled beef tissue, measures to prevent contamination of meat (implementation of good hygiene and hazard analysis critical control point in all stages of food production) or other decontamination procedures with significant heat treatment such as hot water or steam *(11)* are essential to control the public health risk of *E. coli* O157:H7 contamination on chilled beef tissue.

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TABLE 3. *Survival of* E. coli *O157:H7 on the surface of red meat (beef) packed in vacuum during storage at 4°C for 7 days (contamination in log_{10} CFU/cm²)*

Microbiological parameter	Days of storage at 4°C								
	0	1	2	3	5	7	14	21	28
TMC	1.00	1.10	1.53	1.24	2.68	1.47	1.18	3.70	3.54
LAB	1.00	<1.0	1.43	1.10	2.65	1.18	1.12	3.56	2.89
outgrowth (day *N*–day 0)	—	—	0.43	0.10	1.65	0.18	0.12	2.56	1.89
E. coli O157:H7	5.28	5.13	5.07	4.91	4.75	4.90	4.34	4.16	4.19
reduction (day *N*–day 0)	—	−0.15	−0.21	−0.37	−0.53	−0.38	−0.94	−1.12	−1.09

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