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Full Length Research Paper

Survival and growth of *Esherichia coli* O157:H7 in sausage (SOGOK)

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The survival and growth of *Esherichia coli* O157:H7 populations on sausage (SOGOK) after acids spray washes were studied. The efficacy of acid spray washes using 3 and 5% acetic, citric and lactic acid with storage at 5 and 15°C for 12 and 6 days for controlling *Esherichia coli* O157:H7 attached to sausage was evaluated. Acid spray washes of sausage with 3 and 5% acetic, citric and lactic acid affected populations of *Esherichia coli* O157:H7 thereby decreasing them. No significant change in all the surface pH of sausage was observed during storage. This study emphasized that *Esherichia coli* O157:H7 populations decreased as acetic, citric, and lactic acid concentrations increased.

Key words: *Esherichia coli* O157:H7, survival, growth, sausage, acetic, citric, lactic, acid.

INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) cause hemorrhagic colitis and are often associated with devastating or life-threatening systemic manifestations (Ferens and Hovde, 2011). The most severe sequelae, the hemolytic uremic syndrome (HUS), results from shiga toxins (Stxs) produced by the bacteria in the intestine and act systemically on sensitive cells in the kidneys, brain, and other organs (Gyles, 2007). *Escherichia coli* O157:H7 is the causative agent of intestinal disorders ranging from mild infection to severe, bloody diarrhea (hemorrhagic colitis). Complications of infection include hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura. Most isolates associated with these syndromes have been shown to produce shiga toxins (Stx1, Stx2, or both; formerly referred to as SLTI and SLTII or verotoxins 1 and 2, respectively) (Calderwood et al., 1996). Other serotypes of *E. coli* can produce similar toxins, but serotype O157:H7 has been more commonly linked to epidemic and sporadic disease than has any other serotype (Griffin, 1995).

The application of food preservatives such as organic acids is based on the production of relative bland products having “traditional attributes” without at the same time exposing the consumer to unacceptable levels of food-borne pathogens or their toxins. The increasing frequency of *E. coli* O157:H7 outbreaks, associated with the consumption of acidic foods (Besser et al., 1993; Morgan et al., 1993; Zhao and Doyle, 1994; Tilden et al., 1996; Williams et al., 2000), has drawn attention to the acid resistance properties of this pathogen. Unlike most food borne pathogens, *Escherichia coli* O157:H7 is uniquely tolerant to acidic environments.

Enterohaemorrhagic *Escherichia coli* (EHEC) is transmitted to humans by food, drinking and swimming water, animal and environmental contact, or directly from person to person. Foodborne transmission is mostly associated with ground beef but outbreaks have also been associated with other forms of beef, fermented sausage, contaminated vegetables and fruits as well as unpasteurized dairy products (Karch et al., 2005). Transmission may also occur through contamination of soil and exposure in a rural environment (O’Brien et al., 2001) or through direct contact with animals (Crump et al 2002). Large EHEC outbreaks have been reported from Japan (Michino et al, 1999), Australia (Paton, 1996), Argentina (Gomez et al, 2005), the United States (Bell, 1994), Canada (Orr, 1994) and Europe (Sartz et al., 2008).

In continental Europe, sporadic cases and larger outbreaks are less frequent than in the United States and the United Kingdom (Caprioli and Tozzi, 1998). In Scandinavian countries, EHEC infections were rare and

mostly travelassociated or imported, until a Swedish outbreak in 1995 to 1996 with 110 cases of *E. coli* O157 infections in which the source was never traced (Ziese, 1996). Four large outbreaks with 11 to 37 cases in which the source of infection was not identified and limited outbreaks associated with unpasteurized dairy products and animal contact have also been described (Anon, 2006). In 1999, an outbreak with 37 cases among hospital staff was registered. The source of infection was probably lettuce (Welinder-Olsson, 2004). Recently, an outbreak involving 120 cases was registered in southwestern Sweden in which contaminated lettuce was implicated as the source of infection (Anon, 2005). Infections with *E. coli* O157 became notifiable by law in Sweden in 1996, and all other EHEC serotypes became notifiable in 2004. In addition to that, a combination of microbiological and epidemiological results established a link between sausage consumption and the outbreak in 30 out of a total of 39 investigated cases (Sartz et al., 2008).

Sausage (SOGOK) is one of the common Egyptian foods which was derived from ground beef and used as fast food. The survival of infectious pathogens in foods carried out in recent years have focused on or included *E. coli* O157:H7 as a primary target organism of food animal carcass decontamination interventions, which are of increasing commercial use in North America (Bacon et al., 2000; Castillo et al., 2002; Huffman, 2002; Smulders and Greer, 1998; Sofos and Smith, 1998). Studies have shown that the pathogen may survive decontamination of meat with lactic or acetic acid (Brackett et al., 1994; Conner et al., 1997; Cutter and Siragusa, 1994; Samelis and Sofos, 2003), suggesting that survivors can exist and may potentially adapt to the residual organic acid *in situ* in commercial meat processing environments (Samelis and Sofos, 2003).

Many of the studies have been done to determine the effect of environmental conditions on the survival and growth of pathogenic *E*. *coli* O157: H7. Many of the investigators determined the effect of different factors on the survival and growth of *E*. *coli* O157: H7 (Raghubeer and Matches, 1990). There are a number of considerations to make when carrying out survival studies. Strain to strain variability has been shown to be important for some conditions. The pH-dependent and pH independent stationary-phase acid tolerant phenotypes may exist among enterohaemorrhagic *E*. *coli*. In addition to that, the preadaptation of cells is important for survival studies with acid foods (Buchanan and Edelson, 1996). The aim of this study is to describe the survival and growth of *Esherichia coli* O157:H7 in sausage.

MATERIALS AND METHODS

Preparation of sausage (SOGOK) samples

Sausage (SOGOK) is a common food all over the world, eaten at breakfast, lunch, and dinner depending on locale. Traditionally, sausage consists of meat stuffed into the scraped intestines of an animal (frequently the animal it originated from). The intestines are scraped to ensure cleanliness and also to form a thin outer membrane that will keep the meat encased. The meat is ground and then forced into the long membrane, which is twisted periodically to form elongated tubes of meat.

Samples used in all experiment consisted of tube-shaped pieces (approximately 10 cm × 2.0 cm) of ground meat. Whole sausage samples were obtained from local markets and brought directly to the study's laboratory. Each tube weighted 25.0 (±1.0) g in a sterile polyethylene bag, flooded or wiped with 75% ethanol for 20 to 30 min. Subsequently, ethanol was substituted with sterile distilled water for 2 to 4 min. Acid spray treatments of sausage samples were performed with 5 and 10% (vol/vol) acetic, citric or lactic acid. 1 ml of individual acid was sprayed on the entire inoculated sides of sausage.

Bacterial strains and preparation of inoculum

Three wildtype strains of *E*. *coli* O157:H7 (one chicken and two ground beef isolates) were used in this study. The cultures were maintained on tryptic soy agar (TSA, Difco) at 5°C. A loopful of each culture was removed from TSA and inoculated into 10 ml tryptic soy broth (TSB, Difco) at 37°C. Three consecutive 24 h culture transfers to 10 ml TSB were performed. The third 24-h transfer was made up to 100 ml of TSB. Cultures of the three isolates were combined in equal volumes to serve as a mixture to be used as inocula for the experiments.

Procedure for inoculating sausage samples

For inoculating sausage, two populations of *E*. *coli* O157:H7 were applied. A high-population suspension (10^6 CFU ml^{-1}) of *E*. *coli* O157:H7 was prepared by adding 10 ml of the undiluted 3-isolates mixture to 10 L of 0.1% peptone water, and a low-population suspension (10^3 CFU ml^{-1}) was prepared by adding 10 ml of the diluted (10 ml from three- isolates cultures in 250 ml peptone [0.1%]) 3-isolates mixture to 10 L of 0.1% peptone water.

On the other hand, each sausage sample included control which was inoculated with 1.0 ml of inoculum added on both sides of the sausage. Both high and low-population suspensions were prepared to obtain a final concentration on sausage (10^6 CFU ml^{-1} in high population and 10^3 CFU ml^{-1} in low population).

pH measurement

The surface pH of sausage samples was measured at 10 to 20 min after application of acetic, citric and lactic acids at each time of microbiological analysis using a model Jenway 3020 pH meter.

Table 1. Survival rate of *E. coli* O157:H7 populations on sausage (SOGOK) at 5°C.

Acid	Acid concentration (%)	Inoculum	Time of storage (days) log10/ml						
			0	2	4	6	8	10	12
Acetic	3	Control[a]	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Low[b]	5.60	5.56	5.46	4.95	3.69	3.07	1.46
		High[c]	6.27	5.46	4.95	4.20	3.77	3.01	1.99
	5	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Low	5.46	4.95	4.20	3.95	2.69	2.09	+[d]
		High	6.28	5.46	4.20	3.80	3.07	2.30	+
Citric	3	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Low	6.63	6.50	5.46	4.95	4.20	3.95	2.60
		High	6.69	6.60	5.66	5.47	4. 95	3.69	2.69
	5	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Low	5.66	5.47	5.20	4.95	4.20	3.77	2.07
		High	6.46	6.26	5.66	5.07	4.50	3.95	2.09
Lactic	3	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Low	6.29	6.07	5.69	5.32	4.77	4.00	3.10
		High	6.46	6.09	5.77	5.50	4.95	4.69	3.55
	5	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Low	6.22	5.95	5.69	4.95	4.03	3.47	2.77
		High	6.40	6.09	5.87	5.46	4.95	3.01	2.99

a, Un-inoculated samples; b, low-populations suspension (10^3 cfu/ml^{-1}) of *E. coli* O157:H7; c, high-populations suspension (10^6 cfu/ml^{-1}) of *E. coli* O157:H7; +[d], growth in mTSB after 24 h of enrichment.

Storage conditions

Sausage samples (25 g) were placed in sterile polyethylene bags. The samples were kept at 5 and 15°C for up to 0, 2, 4, 6, 8, 10 and 12 days, and 0, 2, 4 and 6 respectively. For microbiological analysis, samples were taken at the end of an interval of 2 days.

Microbiological analyses

Sausage samples

At each sampling time, duplicates of 50 g sausage from each sample were analysed. Samples were combined with 225 ml sterile 0.1% peptone water (Difco) (pH 7.0) in sterile polyethylene bag and mixed. The resulting sausage slurries were serially diluted in 0.1% peptone water. Aliquots of 0.1 ml were plated in duplicates onto nutrient agar (Oxoid) and modified sorbitol MacConkey agar (mSMA, Oxoid) to facilitate detection of *E. coli* O157:H7 populations inoculated onto sausage and for differentiating test isolates used from other organisms which might be present in sausage samples. Plates were incubated at 37°C for 20 to 24 h before *E. coli* O157:H7 colonies were counted. Samples yielding colonies eventually confirmed by biochemical and serological tests to *E. coli* O157:H7 were recorded positive.

To evaluate the absence of viable *E. coli* O157:H7 populations in the negative samples, aliquots of 25 ml of the enrichments were combined with 225 ml of modified Trypticase soy broth (mTSB) {30 g Trypticase soy broth (BBL)}, 1.5 g bile salts no. 3 (Difco), 1.5 g K_2HPO_4, Acriflavine 0.01 g (Sigma) and 10 g casamino acids (Difco)}. Samples were incubated at 120 rpm (37°C) for 18 to 24 h. Enriched cultures (100 µl) were plated on mSMA and incubated for 18 to 20 h at 37°C.

Statistical analyses

Data were subjected to the statistical analysis system (SAS Institute, Cary, N.C.) for analysis of variance and Duncan's multiple range tests. Each value presented the mean of eight values (duplicate values from duplicate samples analysed from two independent trials).

RESULTS

At 5°C

E. coli O157:H7 populations on sausage (SOGOK) treated with 3% acetic, lactic and citric acid decreased significantly throughout 12-days of storage (Table 1).

Additional significant decreases were noted at 5% acetic, citric and lactic acid, though death was more rapid

Table 2. Changes in pH values of sausage (SOGOK) during storage at 5°C.

Acid	Acid concentration (%)	Inoculum	Changes in pH values						
			0	2	4	6	8	10	12
Acetic	3	Control	6.10	6.02	5.95	5.81	4.95	4.34	3.85
		Low	6.39	6.23	5.93	5.79	5.20	4.80	4.15
		High	6.36	6.29	6.20	5.95	5.35	5.00	4.60
	5	Control	6.51	6.11	5.89	5.33	4.79	4.15	3.83
		Low	6.10	5.34	5.18	4.39	4.12	3.55	3.06
		High	6.02	5.30	5.12	4.35	4.14	3.50	3.01
Citric	3	Control	6.67	5.88	4.49	4.12	3.45	3.21	2.90
		Low	6.49	5.92	4.57	4.40	4.14	3.93	3.45
		High	6.42	5.76	4.23	4.10	4.00	3.90	3.60
	5	Control	6.50	6.19	5.79	4.44	4.25	3.93	2.70
		Low	6.51	6.25	5.89	5.68	5.40	4.21	3.56
		High	6.55	6.28	5.90	5.69	5.31	4.22	3.47
Lactic	3	Control	6.50	5.99	4.99	4.02	3.85	3.41	3.00
		Low	6.45	6.22	5.98	5.12	4.70	4.03	3.45
		High	6.43	6.20	5.97	5.16	4.80	4.20	3.60
	5	Control	6.38	6.29	5.89	4.86	3.75	3.53	3.20
		Low	6.61	6.15	5.86	4.98	4.30	3.81	3.36
		High	6.59	6.19	5.80	4.99	4.21	3.90	3.47

as the acid concentration increased and reached zero level of *E. coli* O157:H7 population on sausage treated with 5% acetic acid at small and large inoculums.

As shown in Table 1, results indicated that the organic acids have great effects on survival and growth of *E. coli* O157:H7 at acetic, citric, lactic acid respectively. Control samples of *E. coli* O157:H7 grow in sausage untreated with organic acids. However, decrease in the non-significant population was observed.

On the other hand, non-significant or little changes in pH values of sausage surface were observed during storage at 5°C treated with 3 and 5% acetic, citric and lactic acid at small and large inoculum of *E. coli* O157:H7 (Table 2).

At 15°C

Significant decrease in *E. coli* O157:H7 populations which occurred within 6 days storage with acetic, citric and lactic acid at 3% were noted. Additional significant decreases were noted at 5% acetic, citric and lactic acid at 15°C (Table 3). The most rapid decrease at 15°C in populations of *E. coli* O157:H7 was observed at 3 and 5% acetic, citric and lactic acids respectively. This indicates that as the acids rate increased, the *E. coli* O157:H7 population decreased. Non-significant decreases in *E. coli* O157:H7 populations of control samples were observed. Non-significant or little changes in pH values of sausage surface were observed during storage at 15°C treated with 3 and 5% acetic, citric and lactic acid at small and large inoculum of *E. coli* O157:H7 populations (Table 4).

DISSCUSION

The application of organic acids sprays is one approach to decontaminate beef carcasses at commercial slaughter houses. The fate of *E. coli* O157:H7 on ground beef treated with organic acids has been studied in other laboratories. Many investigators studied the survival and growth of *E. coli* O157:H7 in sausage (Charimba et al., 2010; Montet et al., 2009; Porto-Fett et al., 2008; Palanichamy et al., 2008; Muthukumarasamy and Holley, 2007; Naim et al., 2004, 2003; Calicioglu et al., 2002; Pond et al., 2001; Glass et al., 1992). This study's results indicate that the use of higher acids concentration should result in a higher reduction of the bacterial load at all populations. However, at 5 and 15°C, the reduction of *E. coli* O157:H7 populations on sausage (SOGOK) spray treated with organic acids at 3% increased. Sausage sprayed treatment with 3 and 5% acid indicated that there was an incredibly rapid decrease in *E. coli* O157:H7 populations and it reached zero levels after 6 days of storage at 15°C. On the other hand, significant decreases at 5% acetic, citric and lactic acid were observed during storage at 5°C treated with 3 and 5% acetic, citric and lactic acid at small and large inoculum of *E. coli* O157:H7.

Table 3. Survival rate of *E. coli* O157:H7 populations on sausage (SOGOK) at 15°C.

Acid	Acid concentration (%)	Inoculum	Time of storage (days) log10/ml			
			0	2	4	6
Acetic	3	Control[a]	0.00	0.00	0.00	0.00
		Low[b]	5.50	3.56	2.46	+[d]
		High[c]	6.06	3.86	2.95	+
	5	Control	0.00	0.00	0.00	0.00
		Low	5.21	3.95	2.80	+
		High	6.42	4.06	3.03	+
Citric	3	Control	0.00	0.00	0.00	0.00
		Low	5.34	4.10	3.80	1.67
		High	6.10	4.46	4.10	1.89
	5	Control	0.00	0.00	0.00	0.00
		Low	5.64	4.01	2.93	+
		High	6.36	4.15	3.06	+
Lactic	3	Control	0.00	0.00	0.00	0.00
		Low	5.24	4.32	3.25	1.86
		High	6.41	4.82	3.50	1.95
	5	Control	0.00	0.00	0.00	0.00
		Low	5.42	4.46	3.10	+
		High	6.33	4.56	3.26	1.20

a, Un-inoculated samples; b, low-populations suspension (10^3 cfu/ml^{-1}) of *E. coli* O157:H7; c, high-populations suspension (10^6 cfu/ml^{-1}) of *E. coli* O157:H7; +[d], growth in mTSB after 24 h of enrichment.

Table 4. Changes in pH values of sausage (SOGOK) during storage at 15°C.

Acid	Acid concentration (%)	Inoculum	Changes in pH values			
			0	2	4	6
Acetic	3	Control	6.30	5.60	5.45	4.71
		Low	6.12	5.32	5.11	4.23
		High	6.10	5.33	5.10	4.21
	5	Control	6.41	5.49	5.70	5.57
		Low	6.30	5.21	4.94	4.43
		High	6.22	5.10	4.98	4.32
Citric	3	Control	6.23	5.35	4.43	4.21
		Low	6.13	5.33	4.23	4.12
		High	6.10	5.50	4.45	4.09
	5	Control	6.47	5.43	4.28	4.13
		Low	6.52	5.32	4.23	4.09
		High	6.43	5.23	5.80	4.12
Lactic	3	Control	6.22	5.83	4.45	4.09
		Low	6.34	5.87	4.23	4.10
		High	6.15	5.66	4.25	4.08
	5	Control	6.49	5.24	4.34	4.09
		Low	6.23	5.12	5.32	4.00
		High	6.13	5.10	5.31	4.05

Similarly, Raftari et al. (2009) reported that the population of *E. coli* O157:H7 and *Staphylococcus aureus* decreased after being exposed to acetic, lactic, propionic and formic acids treatments. In addition to that, the effectiveness of the ascorbic, propionic, citric, lactic and acetic acid ranging from 0.1 to 24% reduced bacterial population in red meat and it was observed that the bacterial reduction ranging from 1 to 4 log10 CFU cm^2 was greatest with higher concentrations and combinations if the acids temperature was elevated, or if bacteria were attached to a depose tissue (Anderson et al., 1987; Dickson and Anderson, 1992). Also, Cutter and Sirgausa (1994) recorded that while spray treatments with organic acids reduce *E. coli* O157:H7 populations on red meat, neither lactic, citric or acetic acids at concentrations up to 5% reduce the pathogen to zero levels.

Moreover, Anderson et al. (1977) reported that spraying beef with 3% acetic acid at 10 to 15°C resulted in a 99.9% reduction in total aerobic bacteria populations. Additional studies revealed that spraying beef with 3% acetic acid increased shelf-life, as indicated by total microbial populations, by 18 to 21 days (Anderson et al., 1979). Anderson et al. (1987) and Dickson and Anderson (1992) likewise observed that bacterial reductions were greatest with higher concentrations of acids. Non-significant decrease in *E. coli* O157:H7 populations was observed with the control samples which were treated with distilled water. Other researchers have reported that water reduced bacterial populations on meat (Anderson and Marshall, 1989; Cutter and Siragusa, 1994; Greer and Dilts, 1992). Spray treatments with water may effectively reduce bacterial populations by physically removing cells from the meat surface (Cutter and Siragusa, 1994).

Other investigators reported greater effect on reducing populations of *E. coli* O157:H7 than would a single application (Dickson, 1991). However, Brackett et al. (1994) suggest that acid alone has less effect on reduction of *E. coli* O157:H7 population than other factors. Moreover, *E. coli* O157:H7 was recorded to be unexpectedly resistant to acidic conditions. Zhao et al. (1993) noted that *E. coli* 0157:H7 were able to survive at 8°C for as long as 31 days in apple cider at pH 3.6 to 4.0. Similarly, Abdul-Raouf et al. (1993) reported that *E. coli* O157:H7 was quite resistant to acidic conditions of beef slurries acidified by citric or lactic acid. In addition to that, other researchers have used low organic acid concentrations from 0.5 to 2.0 with effectiveness for controlling *E. coli* O157:H7 on beef (Abdul-Raouf et al., 1993; Brackett et al., 1994; Dickson, 1991; Hamby et al., 1987; Podolak et al., 1996). Other investigators reported that various intervention strategies have been developed to reduce the level of bacteria on the surface of animals' carcass such as washing and sanitizing with hot water, chlorinated water, food grade acids and salts (Dubal et al., 2004; Smulders and Greer, 1998).

The results of this study indicated that non-significant population decrease was observed at control samples of *E. coli* O157:H7 grown in sausage untreated with organic acids. On the other hand, non-significant changes in pH values of sausage surface were observed during storage at 5 and 15°C treated with 3 and 5% acetic, citric and lactic acid at small and large inoculum of *E. coli* O157:H7 populations. The entire pH of the untreated samples changed little throughout the storage period at 15°C.

The untreated meat showed no significant changes in the populations of *E. coli* O157:H7 and *S. aureus* at pH ranges of 6.18-5.17 and 6.12-4.86 respectively, (Raftari et al., 2009). Finally, the conclusions made by Greer and Dilts (1992) was confirmed that the use of organic acids for controlling meat borne pathogens varied between different studies and may be attributed to differences in acid concentrations, methods for acid delivery, temperature of acids, contact time, sampling techniques, tissue type, or organisms.

Conclusion

A study on the survival and growth of *E. coli* O157: H7 on sausage treated with organic acids, acetic, citric and lactic acids was applied at two concentrations of 3 and 5% and storage at 5°C for 12 days and at 15°C for 6 days respectively. The *E. coli* O157: H7 inoculum was inoculated at low (10^3 × $\log_{10}$ CFU/ml) and high (10^6 × $\log_{10}$ CFU/ml) of *E. coli* O157:H7 populations. *E. coli* O157:H7 populations decreased more at 15°C in two concentrations of acids than at 5°C. At 3% acid concentration, the *E. coli* O157:H7 populations decreased, while at 5% acid concentration, the populations decreased rapidly. In addition to that, the *E. coli* O157:H7 populations exhibited more inhibition or decrease at a low inoculum level (10^3 CFU/ml^{-1}) than at high level of inoculum (10^6 CFU/ml^{-1}) at two storage temperatures tested (5 and 15°C).

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