Research Journal of Medical Sciences 3 (4): 158-162, 2009
ISSN: 1815-9346

Novel Approaches of *E. coli* O157: H7 Decontamination

[1]M. Raftari, [2]F. Azizi Jalilian, [2]A.S. Abdulamir, [1]R. Son, [2]Z. Sekawy and [1]A.B. Fatimah
[1]Faculty of Food Science and Technology, [2]Faculty of Medicine and Health Science,
Putra Malaysia University, 43300, Serdang, Selangor

Abstract: Researchers in the area of microbiological meat safety, in an attempt to reduce beef carcass contamination, try carcass-washing treatments as an effective method to control pathogenic bacteria. Spray wash treatments utilizing 3 concentrations (1, 1.5 and 2%) of acetic, lactic, propionic and formic acids were performed to evaluate their efficacy in reducing numbers of *Escherichia coli* O157: H7 on meat tissues at 4±1°C. The meat was decontaminated with hot water and then inoculated with *E. coli* O157: H7, which then was spray washed with organic acids for 15 sec separately. The population of *E. coli* O157: H7 significantly ($p<0.05$) reduced after being spray washed with all treatments. The lethality effect of all organic acids according to the concentration was 2% concentration >1.5% concentration >1% concentration. Mean log reductions of *E. coli* O157: H7showed that the antibacterial effect of formic acid >lactic acid >acetic acid >propionic acid. The results of this study also indicated that formic acid is a good antibacterial agent for decontaminating animals carcass surfaces.

Key words: Beef, *Escherichia coli* O157: H7, acetic acid, lactic acid, propionic acid and formic acid

INTRODUCTION

The contamination of sterile animal muscle used as food is a direct consequence of slaughtering and dressing of animal carcasses. Wide ranges of microorganisms from different sources are transferred onto meat surfaces that are rich in nutrients (Marshall and Bal'a, 2001) Hide, hair and hooves of the animals are some of the most widespread sources of bacterial contamination of animals carcass surfaces (Mies *et al*., 2004).

Escherichia coli O157: H7 is one of the most frequent pathogen that contaminates meat. *E. coli* O157: H7 contaminate meat by contact with sewage, or contaminated skin and equipment during slaughtering (Jay *et al*., 2005).

The involvement of *E. coli* O157: H7 foodborne illnesses has been associated with the consumption of meat and meat products, especially undercooked ground beef (Jay *et al*., 2005). Meat pathogens can cause self-limiting human enteric diseases or systemic and fatal infections of the immunocompromised, the elderly and the young (Marshall and Bal'a, 2001).

E. coli O157: H7 is a Gram negative, facultative anaerobe, non-sporeforming rod shape bacterium. Diseases caused by *E. coli* O157: H7 vary from non bloody diarrhea and bloody diarrhea through haemorrhagic colitis (Adams and Moss, 2000).

With respect to health and economic problems caused by these bacteria, it is very important to reduce their initial microbial population on meat. Various intervention strategies have been developed to reduce the level of bacteria on 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).

Organic acids are Generally Recognized as Safe (GRAS) antimicrobial agents and the dilute solutions of organic acids (1-3%) are generally without effect on desirable sensory properties of meat when used as a carcass decontaminant (Smulders and Greer, 1998).

Previous studies focused on limited treatments for controlling bacteria in which results were inconsistent because of the extensive variations in conditions of experiments.

Therefore, this study attempted to compare the antibacterial effect of large number of different treatments, three concentrations of four most frequently used organic acids in previous studies as acetic, lactic, propionic and formic acids, on one of the important species of bacteria on meat.

The objective of this research was to study and compare the antibacterial effect of the studied acids at three concentrations (1, 1.5 and 2%) on the *E. coli* O157: H7 inoculated on meat at 4±1°C.

MATERIALS AND METHODS

Organic acids: Three concentrations (1, 1.5 and 2%) of four types of food grade organic acids namely Acetic Acid (100%) (AA), L-Lactic Acid (90%) (LA), Propionic

Corresponding Author: Fatimah Abu Bakar, Faculty of Food Science and Technology, University of Putra Malaysia, 43300, Serdang, Selangor

Acid (99%) (PA) and Formic Acid (90%) (FA) (Merck, Germany) were prepared by diluting of glacial form of the acids in sterile Distilled Water (DW).

Meat preparation: Fresh meat was obtained from a local butchery in Serdang, Selangor, Malaysia. Having been packed in sterile bags, the meat was transported to laboratory in a cool box. The samples were prepared immediately after transferring meat to laboratory. Several 10 g pieces of meats were procured from freshly slaughtered cow.

Bacterial strains: *Escherichia coli* O157: H7 ATCC 888402 was obtained from the American Type Culture Collection (ATCC).

Sample preparation: *E. coli* O157: H7 was cultured on standard plate count agar (Merck, Germany) and was then incubated for 24 h at 37°C. After 24 h of incubation, a number of colonies were inoculated in sterile DW and the cell concentration was adjusted to about 10^3 bacteria mL^{-1}.

The prepared 10 g pieces of meat were decontaminated by washing with hot sterile DW (80°C) for 30 sec (Chowdhury *et al*., 2006), then they were kept for few minutes to reach room temperature. At this stage, about 10^3 bacteria mL^{-1} (Benson, 2001) of *E. coli* O157: H7 was inoculated on decontaminated meat by pouring and swabbing over the meat surfaces (Dorsa *et al*., 1997). Subsequently, the inoculated meat with selected bacterium was kept for 20 min to allow attachment and absorption of bacterium however, some of the inoculated pieces of meat were kept as inoculation control (Dubal *et al*., 2004).

After 20 min, the inoculated meat was spray washed with organic acids for 15 sec individually (Bell *et al*., 1997). Once the inoculated meat was spray washed and drained, they were packed in sterile bags that were stored at 4±1°C. Another set was also prepared at the same time as a replicate.

Microbiological analyses were carried out immediately after spray washing until the 12th day of refrigeration. The surface pH of samples was measured by using flat probe pH meter (Prescisa, Switzerland) on 0, 2nd, 6th and 12th days of storage. At this step, each piece of meat (10 g) was aseptically blended with 90 mL of sterile peptone water (Merck, Germany) in a laboratory blender (AOAC, 1990). After that 1 mL of the blended sample of each inoculated meat with *E. coli* O157: H7 was transferred onto Petri dishes for pour plate culturing with standard plate count agar (Merck, Germany) individually. Again, another 1 mL of the same suspension was cultured as a duplicate. The Petri dishes were then incubated for 24 h at 37°C. After 24 h of incubation, the number of colonies was enumerated in each Petri dish.

Statistical analysis: The bacterial population (CFU mL^{-1}) was obtained from four replications performed on separate days and their means were converted to $\log_{10}$ CFU mL^{-1}. Differences between $\log_{10}$ CFU mL^{-1} of untreated beef carcass tissue and $\log_{10}$ CFU mL^{-1} of treated beef carcass tissue were calculated as log reduction (Bjornsdottir *et al*., 2006; Bell *et al*., 1997). Log reductions of treatments were compared by Analysis of Variance (ANOVA) test using the general linear models of SPSS 12.0 for windows, $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

The initial surface pH of meat decreased directly after spray washing with treatments. With progress of storage, it increased, while the pH of controls decreased. A significant ($p<0.05$) reductions were found in the population of *E. coli* O157: H7 after being exposed to all treatments. The mean log reductions of *E. coli* O157: H7 showed 1±0.5, 1.14±0.5 and 1.28±0.5 $\log_{10}$ cfu mL^{-1} reductions after being exposed to AA at 1, 1.5 and 2% concentrations (Fig. 1a) and at pH range 4.86-5.79, 4.74-5.70 and 4.65-5.66, respectively. 1.08±0.5, 1.22±0.5 and 1.35±0.5 $\log_{10}$ cfu mL^{-1} reductions after being exposed to LA at 1, 1.5 and 2% concentrations (Fig. 1b) and at pH range 4.70-5.65, 4.59-5.55 and 4.47-5.44, respectively. 0.89±0.5, 1.02±0.5 and 1.17±0.5 $\log_{10}$ cfu mL^{-1} reductions after being exposed to PA at 1, 1.5 and 2% concentrations (Fig. 1c) and at pH range 5.14-5.98, 5.02-5.93 and 4.89-5.80, respectively and 1.41±0.5, 1.58±0.5 and 1.84±0.5 $\log_{10}$ cfu mL^{-1} reductions after being exposed to FA at 1, 1.5 and 2% concentrations (Fig. 1d) and at pH range 4.39-5.46, 4.30-5.39 and 4.23-5.35, respectively. The untreated meat showed no significant change in the population at pH range 6.18-4.50.

The main goal of this study was to investigate the antibacterial effect of various organic acids applied as spray wash treatment and explore their effect on decreasing the microbial loads of bacteria efficiently on beef tissue. PH is one of the important factors, which influences the growth of bacteria. It has been well established that most microorganisms grow best at pH values around 7.0 (Jay *et al*., 2005), therefore, pH reduction is one of the inhibitor factors, which can limit the growth of bacteria. Malicki *et al*. (2004) indicated that direct bactericidal action of organic acids results from pH

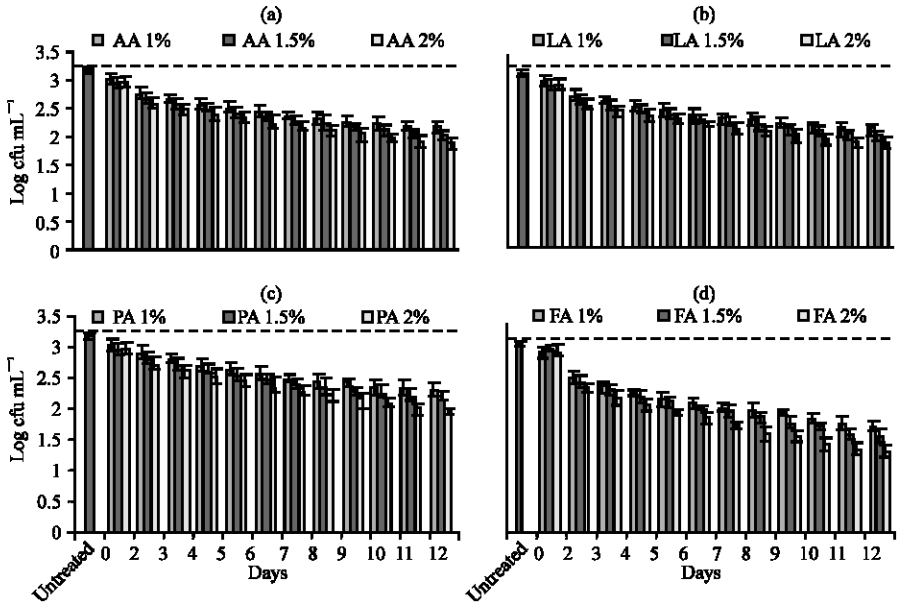

Fig. 1: Cell number reduction of *E. coli* O157: H7 on meat spray washed with AA (1-A), LA (1-B), PA (1-C), FA (1-D) stored for 12 days. A progressive lowering of *E. coli O157: H7* number was detected over time. Dashed line represents the mean of untreated

decrease within bacterial cell. They also observed that pH of fish meal decreased directly after acid addition, which resulted in reduction of *E. coli* O157: H7 population. Moreover, Shin *et al.* (2002) found that the bacteriostatic effect of propionate against *E. coli* was proportional to pH decrease in culture medium.

To date, organic acids have been found as safe antibacterial agents. Various researchers have proved the antibacterial effect of organic acids on different types of pathogenic bacteria (Castillo *et al.*, 2001; Samelis *et al.*, 2001; Bjornsdottir *et al.*, 2006).

In this study, the population of *E. coli* O157: H7 decreased after being exposed to all treatments. The reduction rate of the selected bacterium was proportional to the type and the concentration of each organic acid. Analysis of Variance (ANOVA) for log reduction of *E. coli* O157: H7 showed that there was a significant difference ($p<0.05$) between 1, 1.5 and 2% concentrations of each organic acid. Log reductions analysis showed that increase in the concentration of organic acids resulted in increasing the antibacterial effect of organic acids. These findings were similar to that of Anderson and Marshall (1990), who studied the reduction in the microbial population of *E. coli* and *S. typhimurium* exposed to 1, 2 and 3% concentrations of lactic acid. They found that population reduction of *E. coli* rose by increasing concentration of lactic acid.

The antibacterial effect of the organic acids was found to be caused mainly by the undissociated form of organic acids (Dibner and Buttin, 2002). Non-dissociated organic acids can passively diffuse through a bacterium's cell wall and once internalized into the neutral pH of the cell cytoplasm, they dissociate into anions and protons, both of which exert an inhibitory effect on bacteria (Ricke, 2003). Releasing proton ions causes the internal pH to decrease leading to disruption of proton motive force and inhibiting substrate transport mechanisms (Russell, 1991).

Analysis of Variance (ANOVA) of mean log reductions of population of *E. coli* O157: H7 showed that there is no significant difference ($p<0.05$) between lethal effect of AA, LA and PA, but there was significant difference between antibacterial effects of FA and other organic acids. The findings of the current study showed that FA treatment was the most effective in reducing the population of selected bacterium. These results were in agreement with that of Chaveerach *et al.* (2002), who indicated that formic acid showed stronger lethal effect on *Campylobacter jejuni* than propionic and acetic acids. The strong antibacterial effect of formic acid is related to its structure. Formic acid is an organic acid with shortest chain, which could be beneficial for its diffusion into the cell and cause acidification of the cytoplasm (Skrivanova *et al.*, 2006).

CONCLUSION

Taken together the population of *E. coli* O157: H7 decreased after being exposed to AA, LA, PA and FA treatments. Among the treatments, FA showed the best antibacterial effect on both bacteria. Collectively, formic acid treatment is a feasible and economical method of decontaminating meat.

REFERENCES

Adams, M.R. and M.O. Moss, 2000. Food microbiology. The royal society of chemistry: Cambridge, pp: 217-224. ISBN: 978-0-85404-611-9.

Anderson, M.E. and R.T. Marshall, 1990. Reducing microbial populations on beef tissues: Concentration and temperature of lactic acid. J. Food Safety, 10: 181-190. http://cat.inist.fr/?aModele=afficheN&cpsidt=4367526.

AOAC (Association of Official Analytical Chemists), 1990. Official Methods of Analysis. 15th Edn. Agricultural Chemicals, Contaminants, Drugs, Vol. 1.

Bell, K.Y., C.N. Cutter and S.S. Sumner, 1997. Reduction of foodborne micro-organisms on beef carcass tissue using acetic acid, sodium bicarbonate and hydrogen peroxide spray washes. J. Food Microbiol., 14: 439-448. DOI: 10.1006/fmic.1997.0108.

Benson, S.J., 2001. Microbiological application. A laboratory manual in general microbiology. The McGraw-Hill Companies, New York, pp: 257-290. ISBN: 978-0071121699.

Bjornsdottir, K., F. Breidt, R.F. McFeeters, 2006. Protective effects of organic acids on survival of *Escherichia coli* O157: H7 in acidic environments. J. Appl. Environ. Microbiol., 72 (1): 660-664. DOI: 10.1128/AEM.72.1.660-664.2006.

Chaveerach, P., D.A. Keuzenkamp, H.A. Urlings, L.J. Lipman, F. van Knapen, F. 2002. *In vitro* study on the effect of organic acids on Campylobacter jejuni/coli populations in mixtures of water and feed. Poult. Sci., 81: 621-628. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12033410.

Chowdhury, B.R., S.M. Mukherjee, R. Chakraborty and U.R. Chaudhuri, 2006. Effect of combination pre-treatment on physicochemical, sensory and microbial characteristics of fresh aerobically stored minced goat (Black Bengal) meat organs. Afr. J. Biotech., 5 (12): 1274-1283. http://www.academicjournals.org/AJB/PDF/pdf2006/16Jun/Banani%20et%20al.pdf.

Castillo, A., L.M. Lucia, D.B. Roberson, T.H. Stevenson, I. Mercado, G.R. Acuff, 2001. Lactic acid sprays reduce bacterial pathogens on cold beef carcass surfaces and in subsequently produced ground beef. J. Food Prot., 64 (1): 58-62. PMID: 11198442.

Dibner, J.J. and P. Buttin, 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Appl. Poult. Res., 11 (4): 453-463. http://japr.highwire.org/cgi/content/short/11/4/453.

Dorsa, W.J., 1997. New and established carcass decontamination procedures commonly used in the beef-processing industry. J. Food Prot., 60: 1146-1151. http://cat.inist.fr/?aModele=afficheN&cpsidt=2823672.

Dubal, Z.B., A.M. Paturkar, V.S. Waskar, R.J. Zende, C. Latha, D.B. Rawool, M.M. Kadam, 2004. Effect of food grade organic acids on inoculated *S. aureus*, *L. monocytogenes*, *E. coli* and *S. typhimurium* in sheep/goat meat stored at refrigeration temperature. J. Meat Sci., 66: 817-821. DOI: 10.1016/j.meatsci.2003.08.004.

Jay, J.M., M.J. Loessner and D.A. Golden, 2005. Modern Food Microbiology. 7th Edn. Springer Science and Business Media. Inc, New York, pp: 471-491. ISBN: 0-387-23180-3.

Malicki, A., W. Zawadzki, S. Bruzewicz, S. Graczyk and A. Czerski, 2004. Effect of formic and propionic acid mixture on *Escherichia coli* in fish meal stored at 12°C. Pak. J. Nutr., 3 (6): 353-356. DOI: 10.3923/pjn.2004.353.356.

Marshall, D.L. and M.F.A. Bal'a, 2001. Microbiology of Meat. In Meat Science and Applications. Marcel Dekker, Inc, New York, pp: 160-181. ISBN: 0-8247-0548-3.

Mies, P.D., G.R. Acuff, J.W. Savell, L.M. Lucia, B.R. Covington and K.B. Harris, 2004. Decontamination of cattle hides prior to slaughter using washes with and without antimicrobial agents. J. Food Prot., 67: 579-582. http://cat.inist.fr/?aModele=afficheN&cpsidt=15587886.

Ricke, S.C., 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. J. Poult. Sci., 82: 632-639. http://cat.inist.fr/?aModele=afficheN&cpsidt=14676118.

Russell, J.B., 1991. Resistance of *Streptococcus bovis* to acetic acid at low pH: Relationship between intracellular pH and anion accumulation. Appl. Environ. Microbiol., 57 (1): 255-259. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=182694.

Samelis, J., J.N. Sofos, M.L. Kain, J.A. Scanga, K.E. Belk and G.C. Smith, 2001. Organic acids and their salts as dipping solutions to control *Listeria monocytogenes* inoculated following processing of sliced pork bologna stored at 4°C in vacuum packages. J. Food Prot., 64 (11): 1722-1729. http://www.biomedexperts.com/Abstract.bme/11726150.

Smulders, F.J. and G.G. Greer, 1998. Integrating microbial decontamination with organic acid in HACCP programmes from muscle foods: Prospects and controversies. J. Food Microbiol., 44 (3): 149-169. DOI: 10.1016/S0168-1605(98)00123-8.

Shin, R., M. Suzuki and Y. Morishita, 2002. Influence of intestinal anaerobes and organic acids on the growth of enterohaemorrhagic *Escherichia coli* O157: H7. J. Med. Microbiol., 51: 201-206. http://www.ncbi.nlm.nih.gov/pubmed/11871614.

Skrivanova, E., M. Marounek, V. Benda and P. Brezina, 2006. Susceptibility of *E. coli*, *Salmonella* sp. and *C. perfringens* to organic acids and monolaurin. J. Vet. Med., 51: 81-88. http://www.cazv.cz/attachments/VM_51_81-88.pdf.