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Effects of Citric Acid and Lactic Acid on the Survival of Escherichia Coli O157:H7 Grown On Meat and Carrot

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Abstract: *Outbreaks of Escherichia coli O157:H7 infections associated with meat, vegetables, fruits and root crops have occurred with increasing frequency in the recent years. This study was carried out to investigate the effects of citric acid and lactic acid on the survival of E. coli O157:H7 grown on meat and carrot. Five hundred grams each of meat and carrot samples obtained from the market were washed with sterile distilled water and then cut into small pieces using a sterile knife. The substrates were divided into 10g and packed in polythene bags and kept inside freezer for 7days after which they were thawed at $4^{O}C$ for 24 hours before use. The samples were inoculated with E. coli O157:H7 cells at concentration of 10^{5}cfu/g. The survival of E. coli O157:H7 cells were determined by dipping the meat and carrot samples separately in solutions containing citric acid and lactic acid at concentrations of 0, 3.0, 3.8, 4.6, 5.4 and 6.2 for the period of 0, 10, 20, 30, 40, 50 and 60 min. The effects of the combination of the two acids were also determined by dipping the two substrates in solution containing both acids at the same concentrations for 0, 10, 20 and 30 min. After acid treatments the numbers of E. coli O157:H7 cells that survived were determined by plate count method. The results show that at pH levels of 6.2 and 5.4, citric acid was not effective ($p>0.05$) in reducing numbers of E. coli O157:H7 attached to meat and carrot surfaces at dipping times of up to 60 min. However, at pH levels of 4.6, 3.8 and 3.0, citric acid reduced counts of the organism ($p>0.05$) with increase in dipping time for up to 40 min. The results show that at pH values of 6.2 and 5.4, lactic acid was also ineffective ($p>0.05$) in reducing numbers of E. coli O157: H7 attached to the meat and carrot surfaces at dipping times of 60 min, while at pH value of 5.4, reduction started occurring at dipping time of 50 min. There was reduced effectiveness of the acids in decontaminating E. coli O157:H7 from the surface of meat compared to that of carrot. Additive interactive rather than synergistic effect were seen when citric acid – lactic acid treatment was applied on both meat and carrot. It is clear from this study that both acids could not completely eliminate E. coli O157:H7 from food samples however citric acid and not lactic acid may have more value as surface decontaminant of food than lactic acid.*

I. Introduction

Depending on the virulence factors they possess, virulent Escherichia coli strains cause either non-inflammatory diarrhoea (watery diarrhoea) or inflammatory diarrhoea (dysentery with stools usually containing blood, mucus and leukocytes). Although there are so many serotypes of E. coli, E. coli O157:H7 is the predominant serotype and the one associated most frequently with human infections worldwide (CDC, 1995). E. coli O157:H7 is an important zoonotic pathogen that causes both outbreaks and sporadic cases of diseases such as haemorrhagic colitis and haemolytic uraemic syndrome, which leads to kidney failure and thrombocytopenic purpura that can be fatal (Moxley, 2004). The pathogenicity of E. coli O157:H7 appears to be associated with a number of virulence factors, including the production of several cytotoxins (Griffin and Tauxe, 1991; Karmali et al., 1993).

According to the (CDC, 1995), most outbreaks of E. coli O157:H7 have been associated with eating undercooked, contaminated ground beef and other foods of bovine origin like unpasteurized dairy products. In recent years E. coli O157:H7 has been identified in outbreaks of food-borne illness linked to fresh produce such as salad vegetables. Acidification is one of the important measures commonly employed to control growth and survival of spoilage and pathogenic microorganisms in food (Browning et al., 1990). However, various acid foods such as apple cider (Besser et al., 1993), mayonnaise (Weagant et al., 1994) and yoghurt (Morgan et al., 1993) have been implicated in the outbreaks of food-borne disease caused by E. coli O157:H7. The pathogen's survival in acid foods is particularly important, since several outbreaks have been associated with low levels of E. coli O157:H7 surviving in acid foods (Booth and Kroll, 1989; Buchanan and Doyle, 1997). Survival in these foods is extended greatly when stored at refrigeration temperature (Zhao et al., 1993). Since E. coli O157:H7 has also been of public health concern for food procedures and consumers, this study was to determine the effects of citric acid and lactic acid on the survival of E. coli O157:H7 grown on meat and carrot.

II. Materials And Methods

Preparation of Inoculums. The inoculum used as test microorganism was isolated from beef muscle. After two successive transfers of the test organism onto TSB at 37^0C for 18-24 hours. The activated culture was serially diluted with TSB to obtain a cell concentration that is approximately 10^9cfu/ml after incubationat 37^0C for 18-24hours and plate counts made (Grant and Patterson, 1995). The procedure described by Tsai and Ingham (1997) was used to prepare acid adapted cells of E. coli O157:H7. This paper should be consulted for necessary details.

Preparation of Meat and Carrot Samples. Meat and carrot samples were obtained at Terminus market in Jos metropolis, transported to the laboratory, washed with sterile distilled water and then cut into small pieces using sterile knife. The substrates were divided into 10g and packed in plastic bags, and kept inside freezer for 7 days for decontamination of bacterial cells (Silla and Torres, 1996). Samples were thawed at 4^OC for 24 hours before use. Samples were inoculated by dipping for 5 min in a beaker containing attachment suspension using a procedure described by Marshall et al. (1991). The attachment suspension was maintained at room temperature. After dipping, samples were removed from the beaker and placed on sterile Petri dishes and used immediately.

Preparation of Acid Dip Solutions. Dip solutions were prepared according to the method described by Els et al. (1996). Dip solutions were prepared using series of phosphate buffers. In order to obtain the buffers, 0.1M of citric acid and lactic acid was separately diluted to pH values of 0, 6.2, 5.4, 4.6, 3.8 and 3.0. (0 represents samples in TBS without acidulants and serves as control). The pH values were checked with pH meter. Both acid solutions were also combined at different concentrations in order to determine interaction effects between them. The solutions were held at room temperature during the experiment.

Experimental Protocol. The effects of acidity on the survival of E. coli O157:H7 cells were determined by dipping duplicate inoculated meat and carrot samples for 0, 10, 20, 30, 40, 50 and 60min in solutions containing various concentrations of citric acid and lactic acids. Combined effects between citric acid and lactic acid were determined by the method of Krogstad and Moellering (1986). Five replicates each of meat and carrot samples were dipped in solutions containing both acids for 0, 10, 20, and 30 min. After dipping in solutions for appropriate time, samples were transferred aseptically to sterile Petri dishes and then enumerated for the presence of E. coli O157:H7 using a selective agar medium sorbitol MacConkey agar this was followed by biochemical and serological test (March and Ratnam, 1989; Cheesbrough, 1991). Counts of the organism were used to determine the population of survivors after acid treatments. Each experiment was repeated 3 times in 5 replicates. Data were analyzed according to the analysis of variance (ANOVA) procedures and means separated using Duncan new Multiple Range Test.

III. Results And Discussion

The effects of citric acid against E. coli O157:H7 attached to meat and carrot samples are represented in Figures 1 and 2 respectively. The results show that at pH levels of 6.2 and 5.4, citric acid was not effective ($p>0.05$) in reducing numbers of E. coli O157:H7 attached to meat and carrot surfaces at dipping times of up to 60min. However, at pH levels of 4.6, 3.8 and 3.0, citric acid reduced counts of the organism ($p>0.05$) with increase in dipping time for up to 40min. Exposure of both meat and carrot inoculated with E. coli O157:H7 in citric acid after 40min for all concentrations (except at pH values of 6.2 and 5.4) gave no further reduction ($p>0.05$) in the numbers of E. coli O157:H7. Thus, dipping in these solutions for times longer than 40min had little or no merit.

The effect of lactic acid on E. coli O157:H7 attached to meat and carrot samples are shown in Figures 3 and 4 respectively. The results show that at pH values of 6.2 and 5.4, lactic acid was also ineffective ($p>0.05$) in reducing the numbers of E. coli O157:H7 attached to meat and carrot surfacesat dipping times of 60min, while at pH value of 5.4, reduction started occurring at dipping times of 50min. The result also that even at pH 4.6, 3.8 and 3.0, lactic did not reduce the numbers of the organism until after dipping time of 30min. Thus, at pH levels of 4.6, 3.8 and 3.0, lactic could only reduce counts by 0.7, 0.9 and 1.1 $\log_{10}$cfu/g respectively from meat and by 1.2, 1.3 and 1.4 $\log_{10}$ cfu/g respectively from carrot, after dipping the substrates for 60min in the various solutions.The survival curves in Figures 1–4 indicate that there is reduced effectiveness of the acidulants in decontaminating E. coli O157:H7 from the surface of meat compared to that of carrot.

Results obtained after dipping pieces of meat and carrot for 30 min in solutions containing a mixture of both citric and lactic acids are shown in Tables 1 and 2 respectively. Dipping solutions containing pH 6.2 did not differ significantly ($P>0.05$) from the control in reducing the numbers of E. coli O157:H7. Meaning that addition of lactic acid to citric acid reduced the effectiveness of citric acid at pH level of 6.2. When the pH level was reduced to 5.4, the combined effect of citric acid – lactic acid treatment reduced counts similarly ($p>0.05$)

to that of citric acid alone. Additive interactions were also seen when the pH levels of citric acid–lactic acid treatment were reduced to 4.6, 3.8 and 3.0 for both meat and carrots.

The results from this investigation confirm previous studies on the effectiveness of citric acid and lactic acid as anti-bacterial treatment of raw food and food products when applied at low pH values (Oh and Marshall, 1995). Likewise, other investigators have found acids like lactic acid as an effective surface sanitizer of animal carcasses (Ockerman et al, 1994) and catfish (Ingham, 1989). Citric acid is also one of the acidulants most commonly used in the manufacture of canned foods such as meat and vegetable products (McCormick, 1983). In addition to its use as a preservative in acid foods such as canned vegetables, mayonnaise and salad dressing, citric acid is also incorporated into foods to reduce microbial growth (Graham and Lund, 1986). This explains the interest aroused in the use of these acidulants as a surface sanitizer and as an antibacterial agent to control the growth of E. coli O157:H7 inoculated into foods. Although the counts of E. coli O157:H7 in the food samples were reduced remarkably at low pH values, the pathogen was not completely destroyed. This observation supports the work of many investigations (Counter and Kotrola, 1995; Clavero and Beuchat, 1996) who reported that E. coli O157:H7 is capable of surviving in acid environment.

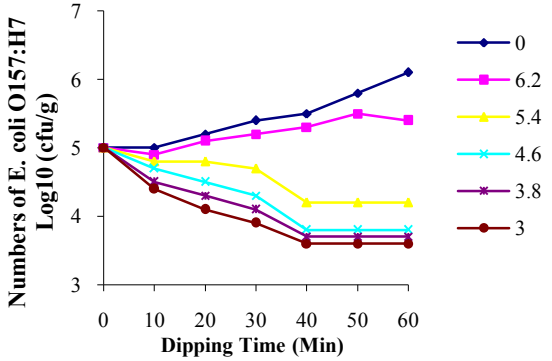

Fig. 1: Numbers (log_{10} (cfu/g) of *E. coli* O157:H7 that survived on Beef(meat) after dipping for up to 60min in Solutions Containing different Concentrations of Citric Acid (CA).

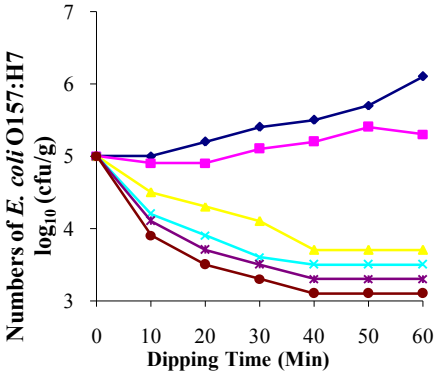

Fig. 2: Number (log_{10} (cfu/g) of *E. coli* O157:H7 that survived on CarrotAfter dipping for up to 60min in Solutions containing different Concentrations of Citric Acid (CA).

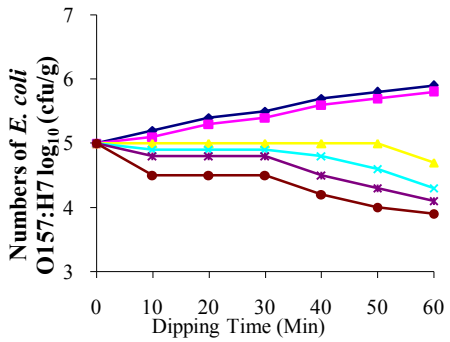

Fig. 3: Numbers (log_{10} (cfu/g) of *E.coli* O157:H7 that survived on Beef (meat) after dipping for up to 60min in Solutions Containing different Concentrations of Lactic Acid (LA)

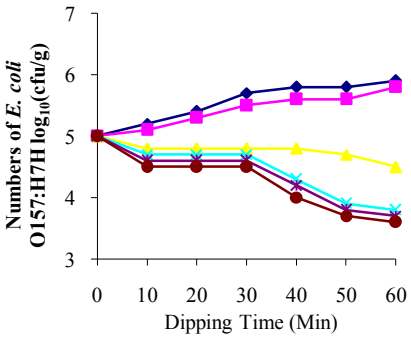

Fig.4: Numbers (log_{10} (cfu/g) of *E. coli* O157:H7 that survived on Carrot after dipping for up to 60min in Solutions Containing different Concentrations of Lactic Acid (LA).

Table 1: Numbers (log_{10} cfu/g) of E. coli O157: H7 that survived on Beef (meat) after dipping in Solutions containing Combination of Lactic Acid and Citric Acid at Various Concentrations.

pH of Dip Solution LA+CA	Dipping time (min)			
	0	10	20	30
Control	5.0 ^{abc}	5.0 ^{abc}	5.2^{ab}	5.3^{ab}
6.2	5.0 ^{abc}	5.0 ^{abc}	5.1^{abc}	5.2^{ab}
5.4	5.0 ^{abc}	4.8 ^{bdce}	4.7^{bdce}	4.7^{bdce}
4.6	5.0 ^{abc}	4.7 ^{bcde}	4.5^{cdef}	4.4^{efg}
3.8	5.0 ^{abc}	4.5^{cdef}	4.3^{efg}	4.1^{b}
3.0	5.0 ^{abc}	4.4^{efg}	4.1^{b}	3.9^{a}

Means having the same superscript letter are not significantly different (p>0.05) at the 95% confidence level using DMRT.
CA = Citric acid, LA = Latic acid

Table 2: Numbers (log_{10}cfu/g) of E. coli O157: H7 that survived on Carrot after dipping in Solutions Containing Combination of Lactic Acid and Citric Acid at Various Concentrations.

pH of Dip Solution LA+CA	Dipping time (min)			
	0	10	20	30
Control	5.0 ^{abc}	5.0 ^{abc}	5.3 ^{ab}	5.4^{adc}
6.2	5.0 ^{abc}	4.9 ^{da}	5.1 ^{abc}	5.2 ^{ab}
5.4	5.0 ^{abc}	4.5^{def}	4.3^{efg}	4.0 ^{b}
4.6	5.0 ^{abc}	4.2^{ef}	4.0^{b}	3.6 ^{gf}
3.8	5.0 ^{abc}	4.1^{b}	3.6 ^{gf}	3.5 ^{gf}
3.0	5.0 ^{abc}	3.9^{a}	3.5 ^{gf}	3.2 ^{g}

Means having the same superscript letter are not significantly different (p>0.05) at the 95% confidence level using DMRT.
CA = Citric acid, LA = Latic acid

The inability of the citric acid and lactic acid to completely destroy E. coli O157:H7 in the food samples investigated in this study may be related to duration of exposure time. In vitro studies (Oh and Marshall, 1993) used much longer exposure time (24 h) compared to the relative brief exposure used in the present study. The reduced effectiveness of lactic acid on the reduction of E. coli O157:H7 counts from the food samples compared to citric acid is in agreement with the report by Abdul Raouf et al. (1993) who stated that the relative inhibitory activity of organic acids on E. coli O157:H7 was of the order: acetic > lactic > citric.The difference in effectiveness of the two acidulants in reducing the counts of E. coli O157:H7 may also be related to their anionic forms, which are capable of sequestering metal ions needed for bacterial growth (Silla and Torres, 1996). This supposition is supported by the difference observed between citric acid and lactic acid in terms of physiological characteristics: citric has more carboxylic groups (three) than lactic acid (only one) and so can bond a greater number of metal ions.

Therefore this indicates that the metal–chelation activity of citric acid is higher than that of lactic acid and consequently has more inhibitory ability against E. coli O157:H7 than lactic acid. Graham and Lund (1986) also observed inhibition of the growth of Clostridium botulinum that was attributable to the chelation of metal ions by citric acid, apart from its effect on pH. In addition, Graham and Lund (1986) indicated that the inhibitory effect of citric acid and, perhaps, any other sequestering acid, was affected by the ion content of the medium. The concentrations of ions vary with different culture media and even different commercial brands of agar used to reconstitute the culture media, with a consequent effect on the recovery of bacterial cells (Sikes et al., 1993). Therefore, given the different ionic charges found in different foods, it would be interesting to compare the inhibiting effect of various acidulants for each food in order to make a better selection of the appropriate acid for each case.

The resistance of E. coli O157:H7 to acidulants is also related to the nature of the surface to which the organism is attached. The result from this study revealed that E. coli O157:H7 cells which were attached to meat surface were found to be less resistant to sanitizers than those attached to carrots. The ability of microorganisms to become more resistant to a sanitizer and other antimicrobial agents once they become attached to surface has been documented (Malu et al., 1990). Thus the surface to which E. coli O157:H7 becomes attached plays an important role in the efficacy of sanitizers. It is known also that the activities of acidulants and other antimicrobial agents are reduced by protein materials (Oh Marshall, 1995). Meat muscle is approximately 18-20% protein while carrot is approximately 1% protein. This partially explains why E. coli O157:H7 cells attached to meat are more resistant to the 2 acidulants than those attached to carrot. Therefore, a combination of

pH level, exposure time, and metal–chelation activity of the acidulants, food composition and surface factors of the substrate may affect the effectiveness of sanitizers used in this study.

Contrary to other findings (Oh and Marshall, 1995), a synergistic interaction was not observed in the present study in the citric acid and lactic acid inhibition of E. coli O157:H7. The interaction observed was judged to be additive because the combination response was less than two logs from the most active single compound alone. This was true even though at pH 6.2, lactic acid reduced the effectiveness of citric acid. In another study, lactic acid and another sanitizer known as glycerol monolaurate acted synergistically because they were added directly to a product slurry rather than being applied as a surface dip, and longer exposure times (24h at 35^{o}C or 21d at 4^{o}C) were also used. Thus citric acid and lactic acid may act synergistically during long–term storage rather than as immediate sanitizers.

IV. Conclusion

Although the acidulants could not completely eliminate E. coli O157:H7 from food samples, citric acid and not lactic acid even at high concentrations, citric acid may have more value as surface decontaminant or sanitizer of foods than lactic acid. As outbreaks of E. coli O157:H7 infection have been associated with low levels of the pathogen surviving in acid foods, it is important that food safety agencies should equally be concerned about preventing or limiting growth of the pathogen between the farm and the plate.

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