December, 2002

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Food Research International 36 (2003) 823–830

FOOD RESEARCH INTERNATIONAL

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Behavior of *Escherichia coli* O157:H7 in tomato and processed tomato products

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Received 14 January 2003; accepted 2 April 2003

Abstract

The survival of *E. coli* O157:H7 in acid foods for weeks and its prolonged survival in refrigerated acid foods are well documented. This prompted the study to evaluate survival of *E. coli* O157:H7 in tomato and processed tomato products. The pH of the various products ranged from 4.2 to 4.8 and some products contained preservatives such as vinegar. Samples were separately inoculated with a mixture of four *E. coli* O157:H7 strains previously isolated from hamburger meat at lower and higher initial inoculum levels, incubated at 4 and 25 °C and assayed for survival at regular intervals. In fresh whole tomato, growth was detected until day 4 and complete elimination was observed at day 7 at room temperature incubation. At refrigeration temperature, counts decreased slightly but survival was noted until day 10. In processed whole tomato, similar patterns were seen at room temperature incubation, but elimination was not observed until day 16. At refrigeration temperatures counts at day 16 were higher than the initial inoculum level. In processed tomato juice, the test strains kept on growing for over 20 days at room temperature storage, whereas at refrigeration temperature, the initial number was more or less maintained until day 23. In processed tomato sauce, incubated at room temperature, a sharp decline in number until day 6 was followed by a sharp increase in number until day 16. At refrigeration temperature, the test strain survived until day 16 with slight decrease in number. The test strains were eliminated in ketchup within 2 days at room temperature, whereas elimination was not achieved until day 8 at refrigeration temperature. In pasta sauce and snack sauce, although elimination was observed at days 11 and 7, respectively, at room temperature incubation, the test strains survived for about 22 days at refrigeration temperature without much decrease in count. Patterns were similar at higher levels of initial inoculation, but it took longer to achieve elimination of the test strains. This study demonstrated that *E. coli* O157:H7 can survive markedly in various tomato products, and the survival is notably prolonged at refrigeration temperatures.

Keywords: *Escherichia coli* O157:H7; Tomato products; Survival; Inhibition

1. Introduction

Since its identification as a human pathogen in 1982 (Linton, McClements, & Paterson, 1999), *Escherichia coli* O157:H7 has been considered to be an important pathogen that can cause serious illness with symptoms ranging from bloody diarrhea to hemolytic uremic syndrome (Karnali, 1989). Most outbreaks have been linked to the consumption of foods from animal origin and transmission of this pathogen occurs primarily through the consumption of certain foods including undercooked beef (Doyle & Schoeni, 1987), raw milk (Borczyk, Karmali, Loir, & Duncan, 1987), cheeses made from unpastuerized milk, and fermented meats (Tilden et al., 1996). Raw vegetables were also implicated in more recent outbreaks (Beuchat & Ryu, 1997). The pathogen is demonstrated to grow in different types of vegetables (Abdou-Raouf, Beuchat, & Ammar, 1993; Beuchat, 1999; Del Rosario & Beuchat, 1995) and in apple juice (Karnali, 1989; Zhao, Doyle, & Besser, 1993). More recently, numerous other less likely foods have been implicated in outbreaks, including acid or acidified foods such as un-pasteurized apple cider and apple juice, mayonnaise, and mayonnaise-based salad dressings, yogurt, salami and mustard (Besser et al., 1993; Erickson, Stamer, Hayes, McKenna, & Van Alestine, 1995; Morgan, Newman, Hutchenson, Walker, Rowe, & Majid, 1993; Tilden et al., 1996).

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0963-9969/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0963-9969(03)00077-2

In contrast to other food-borne pathogens, *E. coli* O157:H7 is more tolerant of some organic acids and it can survive well in acidic food and beverages (Koodie & Dhople, 2001). Studies determined that it can survive in refrigerated acid foods for weeks (Weagant, Bryant, & Bark, 1994), and that survival is prolonged under refrigeration when compared to room temperature storage as observed in retail mustards (Mayerhauser, 2001).

Organic acids present in processed foods allow *E. coli* to adapt to acidic conditions and thus tolerate pH levels that would normally inactivate it (Duffy et al., 2000). *E. coli* O157:H7 is a hardy pathogen that can survive long periods of time in water, especially at cold temperatures (Wang & Doyle, 1998). Unfortunately, storage at lower temperatures has been shown to enhance the survival of *E. coli* at low pH (Linton et al., 1999).

As cross-contamination of acid foods with *E. coli* O157:H7 is possible in the home kitchen environment and food service establishments, this study was initiated to assess the fate of *E. coli* O157:H7 in fresh and processed whole tomato and other retail tomato-based processed products such as tomato juice, tomato sauce, ketchup, snack sauce and pasta sauce at different levels of initial inoculation and storage at room and refrigeration temperatures.

2. Materials and methods

2.1. Tomato and tomato products

Fresh whole tomato and other processed tomato products, such as whole tomato, tomato juice, tomato sauce, ketchup, snack sauce (for pizza stuffing) and pasta sauce were purchased from local stores in Washington, DC. Except canned whole tomato, which was only peeled, the other processed products had the following ingredients. Tomato juice consisted of water, tomato paste, salt and vitamin C. Tomato sauce contained tomato paste, water and salt. Ketchup contained water, tomato paste, corn syrup, vinegar, salt, onion powder, spices and natural flavor. Snack sauce consisted of water, tomato paste, soybean oil, salt, modified food starch, dried onions, high fructose corn syrup, spices and natural garlic flavor. Pasta sauce contained water, tomato paste, soybean oil, high fructose corn syrup, salt, dried onions, extra virgin olive oil, romano cheese, spices and natural flavor.

2.2. Strains used and preparation of inocula

Four strains of *E. coli* O157:H7, previously isolated from Hamburger meat were used for inoculation study: MF-1847, 93-30, 536 and 45956-52B. All test strains were obtained from the US Department of Agriculture, Eastern Regional Research Center, Wyndmoor, PA. Strains were cultured at 37 °C in Tryptic Soy Broth (DIFCO) with 6% yeast extract (TSYB). Cultures were transferred to TSYB by loop at three successive 24-h intervals before cells were harvested by centrifugation (2000 $\times g$, 15 min, 21 °C) and re-suspended in sterile 0.1% peptone water. Volumes of each strain were combined to give approximately equal populations. Mixed-strain cell suspensions were diluted in 0.1% peptone water to give desired populations.

2.3. Inoculation of samples

Fresh whole tomatoes were dipped in 90% ethanol for 2 min, thoroughly rinsed with sterile water and each tomato was aseptically cut into four pieces. The pieces were homogenized using a stomacher lab blender and aseptically divided into ten sub-samples of 200 ml volume in sterile 500 ml screw-capped bottles. Appropriate dilutions of the test strain suspension were used to individually inoculate the sub-samples by adding 2 ml into 200 ml of a sample. Four sub-samples were inoculated at low inoculum level (final concentration of 10^3–10^4 cfu/ml). After thorough mixing, two sub-samples were incubated at room temperature (25 °C) and two at refrigeration temperature (4 °C). The other four sub-samples were inoculated with the test strains to give a final inoculum level of 10^6 cfu/ml. These were similarly processed and incubated as in the previous case. Two un-inoculated sub-samples served as control to enumerate any prior *E. coli* O157:H7 contaminants. These were maintained at room temperature. Determination of initial inoculum level of our test strains was made on Sorbitol MacConkey Agar (SMAC) (DIFCO). Processed whole tomato was homogenized as described above and similar procedure was followed for inoculation and incubation. The other samples did not require stomaching, but otherwise were similarly processed and incubated. Initial inoculum level was determined by inoculating 0.1 ml of appropriate dilution on Tryptic Soy Yeast Agar (TSYA) and SMAC. Each tomato sample was also assayed for pH and contaminants prior to inoculation with the test strains.

2.4. Enumeration of E. coli O157:H7

Inoculated sub-samples were assayed for test strains immediately after inoculation and at 2-day intervals thereafter. A volume of 10 ml of the inoculated sub-samples was diluted in 90 ml peptone water (0.1%) to give 10^{-1} dilution. After further serial dilution, volumes of 0.1 ml of appropriate dilutions were plated on TSYA and SMAC in duplicates at 24h intervals. Control sub-samples were similarly plated on SMAC to check for any contaminant *E.* coli O157:H7. Counting was done after an overnight incubation at 37 °C. When no survival of *E. coli* O157:H7 was detected by direct plating, an enrichment procedure was done by transferring 10 ml of the inoculated sample into 90 ml of TSYB and incubating overnight at 37 °C followed by plating on TSYA and SMAC.

2.5. Statistical analysis

Counts were reported as mean values of duplicate sub-samples. Coefficient of variation (CV) was calculated to determine if significant variations (CV > 10%) existed between counts of duplicate sub-samples.

3. Results and discussion

The pH of the tomato samples varied between 4.2 and 4.8. No *E. coli* O157:H7 contaminants were detected at the lowest dilution (10^{-1}) used in this study. There were no significant variations in counts of duplicate sub-samples in all cases (CV < 10%). In fresh whole tomato (pH 4.8), *E. coli* O157:H7 increased by about 1 log unit at room temperature incubation during the first day when initially inoculated at high level (Fig. 1). The count stabilized until the fourth day but decreased markedly thereafter. At day 10, the count was slightly lower than the initial inoculum level, but no complete elimination was noted. At refrigeration storage, the higher initial count did not show any marked variation throughout the 10 days. At lower initial inoculation level, however, increase by two log units was observed until day 4 at room temperature storage. A fast rate of decline was noted thereafter followed by a complete elimination at day 7. Except a slight decrease in count for the first five days, elimination was not seen during storage at refrigeration temperature. At room temperature storage, our test strains were eliminated within 7 days in those sub-samples with lower levels of initial inoculum. Such low initial inoculum levels (ca. 10^3 cfu/ml) are generally considered to be similar to contaminations via unclean utensil or hands under normal food handling conditions in the house hold (Svanberg, Sjogren, Lorri, Svennenholm, & Kaifser, 1992). On the other hand, at refrigeration storage, although they were not able to increase in number, their survival time was markedly extended.

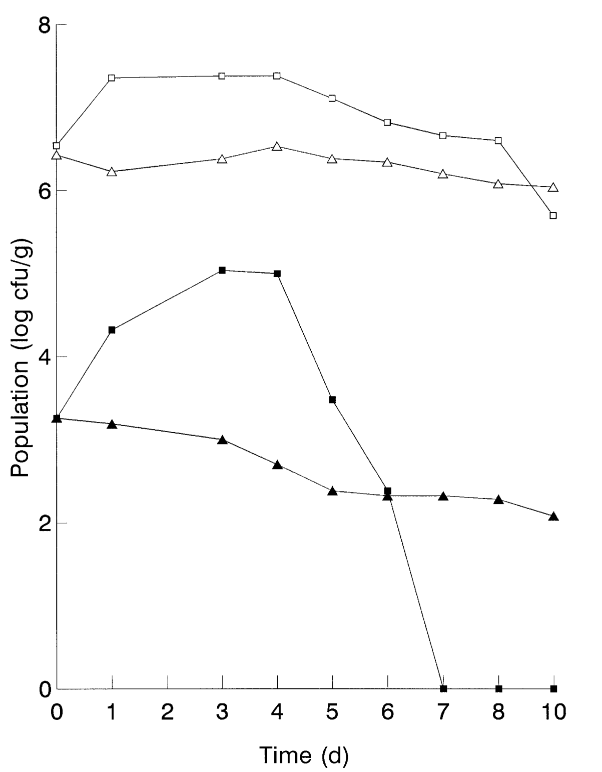

Fig. 1. Survival and elimination of *E. coli* O157:H7 in fresh whole tomato at high (open symbols) and low (closed symbols) inoculum levels incubated at room (squares) and refrigeration (triangles) temperatures.

In processed whole tomato (pH 4.6) maintained at refrigeration temperatures, *E. coli* O157:H7 did not show marked variation in count during 16 days of incubation when initially inoculated at higher level (Fig. 2A). However, at lower level of inoculation, a sharp increase in count was observed until day 5 and the count later started to decline after day 10. The gap between counts on TSYA and SMAC was slightly wider after 5 days of incubation indicating small extent of injury to the different strain components of the inoculum. Generally injured cells fail to grow on media such as SMAC due to the presence of selective agents. At refrigerated incubation, the test strains were not eliminated and the final counts were about or higher than the initial inoculum level. At room temperature incubation and higher initial inoculum level, however, a steady decrease in the test strains was noted after an initial growth during the first 2 days (Fig. 2B). No test strain was detected on SMAC at day 16, whereas counts higher than 10^2 cfu/ml were made on TSYA at the same incubation time. These could be injured cells which managed to recover on TSYA. At lower initial inoculum level, the test strains grew in processed whole tomato until day 5. In contrast to low temperature storage, the test strains increased by 4 log units at room temperature storage within 6 days. The count declined slightly thereafter, but counts at day 16 were markedly higher than the initial inoculum level.

Processed tomato juice (pH 4.4), maintained at refrigeration temperatures, supported survival of the test strains for over 3 weeks both at higher and lower initial inoculum level (Fig. 3A). Counts on SMAC were, however, markedly lower than those on TSYA indicating some level of injury particularly after day 12. At room temperature incubation and higher initial inoculum level, a general trend in count reduction was observed (Fig. 3B). Counts on SMAC were always

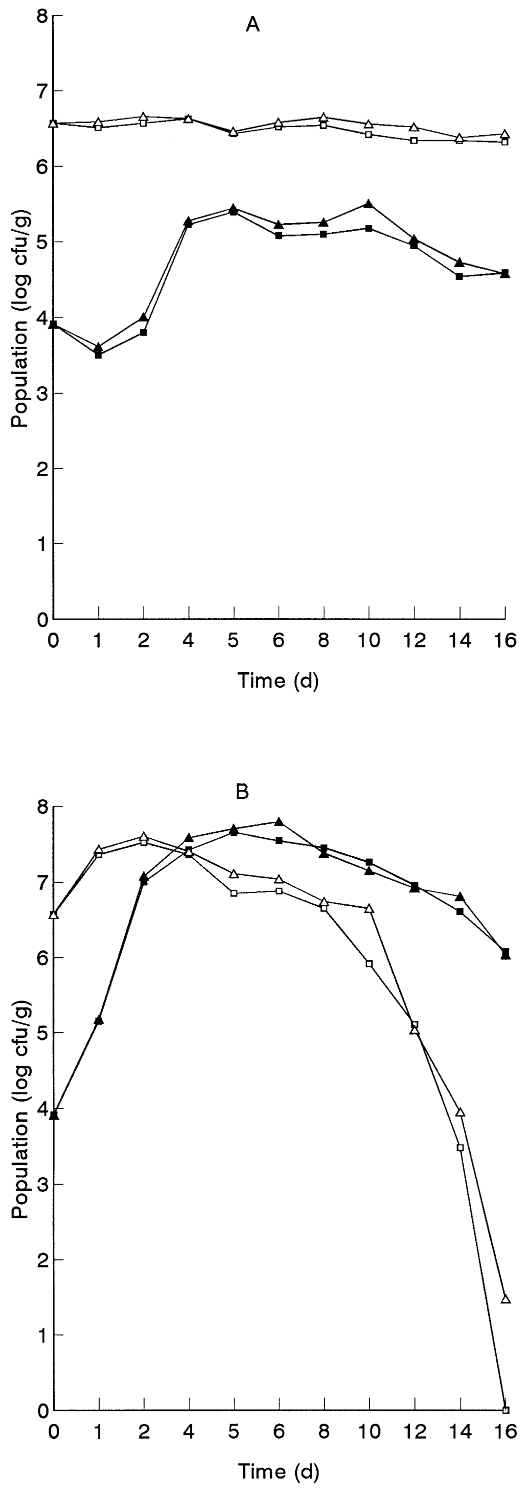

Fig. 2. Survival and elimination of *E. coli* O157:H7 in processed whole tomato at high (open symbols) and low (closed symbols) inoculum levels and incubated at refrigeration (A) and room (B) temperatures as enumerated on TSYA (triangles) and SMAC (squares).

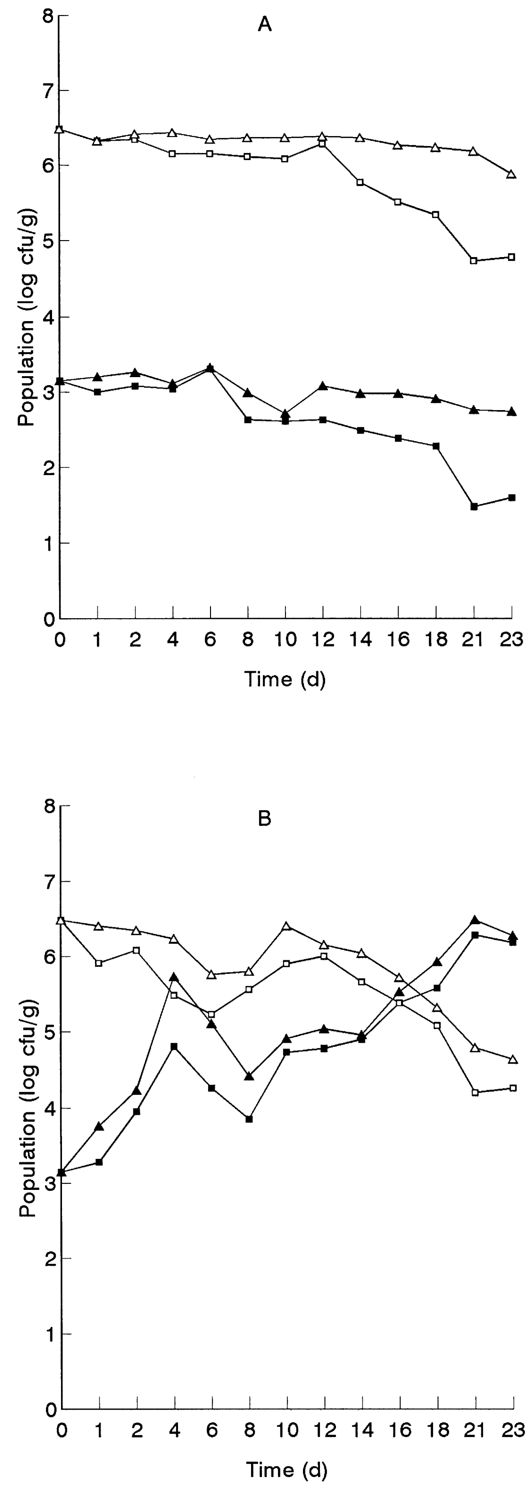

Fig. 3. Survival of *E. coli* O157:H7 in processed tomato juice at high (open symbols) and low (closed symbols) inoculum levels and incubated at refrigeration (A) and room (B) temperatures as enumerated on TSYA (triangles) and SMAC (squares).

lower at all counting periods indicating a certain level of injury. At lower inoculum level, there was a general increase in counts although a sharp decrease was noted after day 4 followed by a steady increase after day 8. Some members of the culture mixture might have reached their peak counts earlier whereas others might have maintained growth at a lower rate until they reached counts of over 10^6 cfu/ml at day 21. This would, however, be confirmed by studying the growth dynamics of each strain separately under similar conditions. The implication, here, is that contamination levels that may occur under normal food-handling conditions could result in growth of *E. coli* O157:H7 and its extended survival in tomato juice stored at room temperature.

In processed tomato sauce (pH, 4.6) maintained at lower temperatures, the count of the test strains did not show marked difference for 16 days when initial inoculum level was high (Fig. 4A). At lower initial inoculum level, only a slight decline in count was observed and counts at day 16 were still higher than 10^2 cfu/ml. Although some degree of injury was indicated by difference in counts between those on SMAC and TSYA, survival of the test strains was not challenged until day 16 at both inoculum levels. At room temperature incubation, a sharp decline in counts was observed until day 8 at both levels of initial inoculation (Fig. 4B). This decline in growth was also accompanied by some degree of injury as observed by differences in counts on TSYA and SMAC until day 8. However, marked growth was detected thereafter. Although most studies report on survival of *E. coli* O157:H7 at acidic conditions, its growth is also documented in acidic foods such as apple cider (Koodie & Dhople, 2001). Growth of this pathogen was also observed in TSB at pH levels as low as 2.0 (Koodie & Dhople, 2001) and at 4.6 (Glass, Loefferlholz, Ford, & Doyle, 1992). Our mixed culture strains decreased in count until day 6. By this time, some surviving strains could have developed acid adaptation to the environment resulting in acid tolerance, which, in turn, would have enabled the pathogen to increase by about 4 log units in the next 8 days of incubation. Growth by about 5 log units in 20 h at pH 4.5 in TSB was also reported by Deng, Ryu, and Beuchat (1999).

In tomato ketchup (pH, 4.2) stored at refrigeration temperature, our test strains could survive for over 18 days when initial inoculum level was high although the decline in count was sharp. At low initial inoculum level, survival was noted until day 8. At both initial inoculum levels, gaps in counts on SMAC and TSYA were noticeably wide indicating severe injury starting from day 1 (Fig. 5A). At room temperature, rate of elimination of our test strains in tomato ketchup was markedly fast, even at higher initial inoculum level, with counts falling by 2 log units every day until complete inhibition was reached on day 6. At low initial inoculum level, complete elimination was attained on day 2 (Fig. 5B). Ketchup contained preservatives such as vinegar and spices that can play an inhibitory role as observed in mustard products (Mayerhauser, 2001).

In snack sauce (pH, 4.6) stored at refrigeration temperature, the test strains could survive at both initial inoculum levels for over 22 days. Counts did not show any marked decrease for about 11 days (Fig. 6A). At

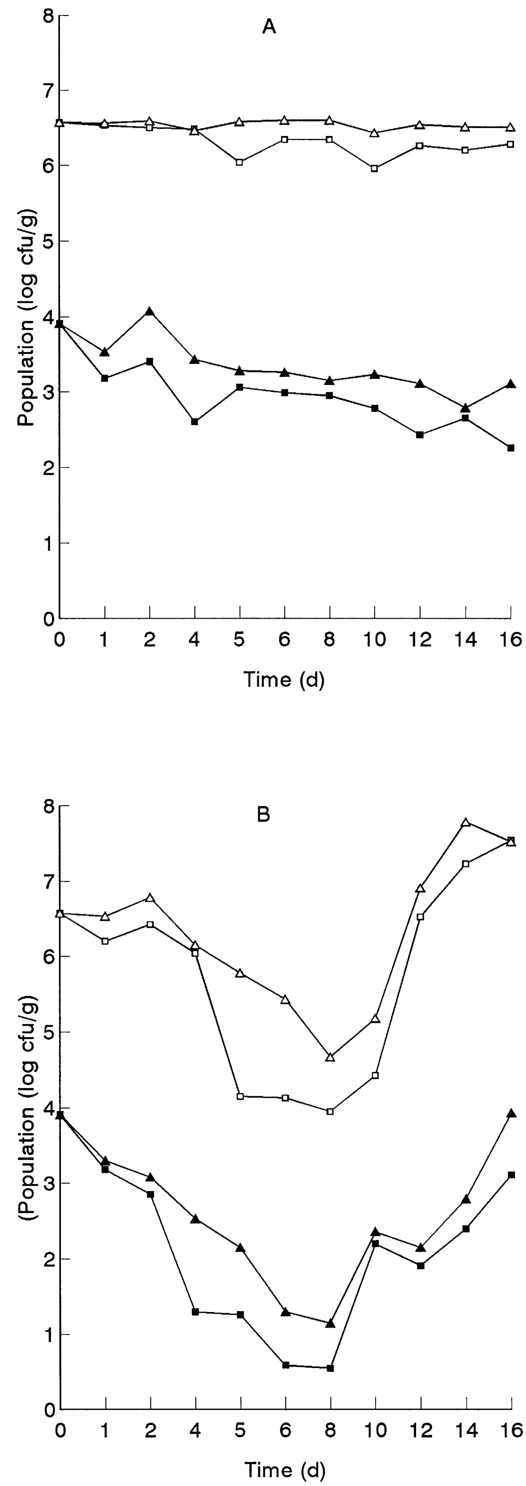

Fig. 4. Survival and growth of *E. coli* O157:H7 in processed tomato sauce at high (open symbols) and low (closed symbols) inoculum levels and incubated at refrigeration (A) and room (B) temperatures as enumerated on TSYA (triangles) and SMAC (squares).

room temperature storage, however, elimination of the test strains was observed at day 7 irrespective of the initial inoculum level. Inclusion of high fructose corn syrup and spices, among other additives, could contribute to the

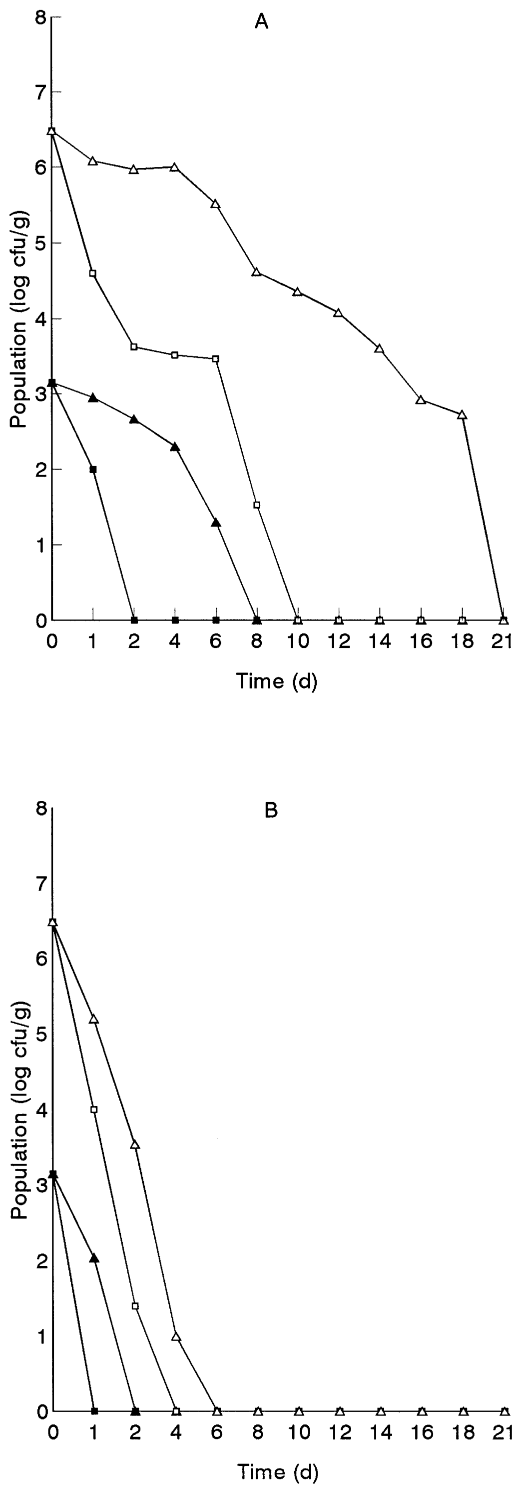

Fig. 5. Survival and elimination of *E. coli* O157:H7 in ketchup at high (open symbols) and low (closed symbols) inoculum levels and incubated at refrigeration (A) and room (B) temperatures as enumerated on TSYA (triangles) and SMAC (squares).

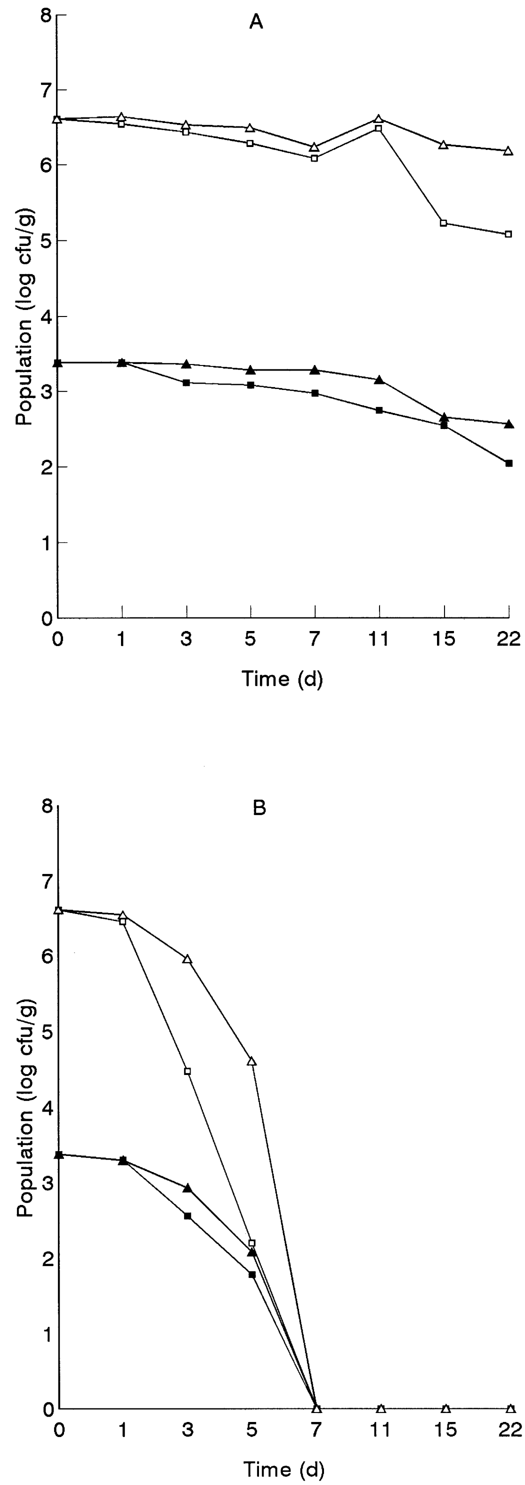

Fig. 6. Survival and elimination of *E. coli* O157:H7 in snack sauce at high (open symbols) and low (closed symbols) inoculum levels and incubated at refrigeration (A) and room (B) temperatures as enumerated on TSYA (triangles) and SMAC (squares).

inhibition of our test strains. Differences in counts on SMAC and TSYA were noticeably wide (Fig. 6B). Similar observations were also made in the pattern of survival and elimination of the test strains in pasta sauce (pH 4.6). Additives in pasta sauce were also similar to those in snack sauce. While counts did not decrease noticeably until 22 days in pasta sauce at refrigeration storage at both initial inoculum levels

(Fig. 7A), elimination of our test strains was achieved at day 11 at room temperature storage (Fig. 7B). Injury to our test strains was much heavier at room temperature storage than refrigeration storage as depicted by wide gaps between counts on SMAC and TSYA. The stronger inhibition observed in snack sauce and pasta sauce at room temperature storage could be due to the synergistic action of low pH, added preservatives and osmotic stress from supplementary syrups, salt and water-binding starch.

This study has shown that *E. coli* O157:H7 can survive much longer under refrigeration storage than storage at room temperatures even in products with low pH and added preservatives. The intrinsic properties of tomato and the processed products do not warrant elimination of this pathogen, particularly at cold storage, despite the inclusion of preservatives and other food additives. According to Marques, Worcman-Barninka, Lannes, and Landgraf (2001), *E. coli* O157:H7 survived in fruit pulp with a low pH stored under refrigeration and they stated that acid adapted organisms would be better equipped to outlast these acid challenges. This may explain survival of our test strains in all tomato products considered in this study, particularly in those with lower pH and added preservatives (ketchup, snack sauce and pasta sauce). Other workers also found out that, at lower pH values, the presence of organic acids enhanced survival of *E. coli* O157:H7 at 4 °C (Connor & Kotrola, 1995). This is particularly important in the tomato products considered in our study because of the recommendation by the manufacturers to refrigerate products after opening.

Inhibition of *E. coli* O157:H7 was achieved within hours in mustard products (pH 3.17–3.63) stored at refrigeration temperatures (Mayerhauser, 2001), although it was shown to survive for over 20 days in orange juice (pH 3.6) stored at 3 °C (Linton et al., 1999). Similar to the findings in our study, better survival of *E. coli* O157:H7 at refrigeration temperatures and its inhibition at room or higher temperatures was demonstrated in soy sauce (Masuda, Mara-Kudo, & Kumagai, 1998), apple cider (Weagant et al., 1994), salad dressings (Raghubeer, Ke, Campbell, & Mayer, 1995) and dairy foods (Guraya, Frank, & Hassan, 1998). According to Buchanan and Doyle (1997), acid tolerance in *E. coli* is a complex phenomenon, both growth phase-dependant and inducible. *E. coli* cells in the stationary phase of growth are substantially more acid tolerant than cells in the exponential phase. An acid tolerant state can persist for extended periods (⩾28 days) if the cells are stored at refrigeration temperatures (Cheville, Arnold, Buchreiser, Cheng, & Kaspar, 1996).

Some of the tomato products considered in this study do not require further heating before consumption and, thus, could be sources of infection by *E. coli* O157:H7 to consumers. Most of the tomato products are heated during subsequent preparation of food. However, development of tolerance to acidic conditions by *E. coli* O157:H7 during storage has been shown to confer some cross-protective effect against heat stress (Cheville et al.,

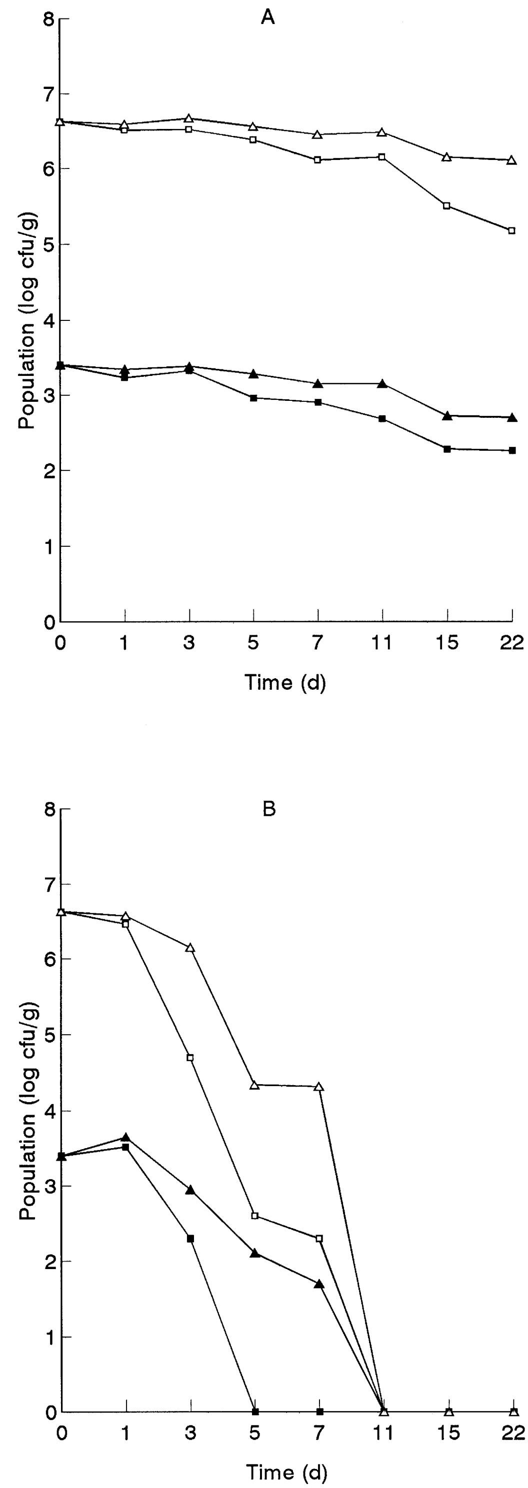

Fig. 7. Survival and elimination of *E. coli* O157:H7 in pasta sauce at high (open symbols) and low (closed symbols) inoculum levels and incubated at refrigeration (A) and room (B) temperatures as enumerated on TSYA (triangles) and SMAC (squares).

1996). This would mean that some *E. coli* O157:H7 might survive subsequent heating processes. Survival is particularly important as illness can result from ingestion of a minimal dose of 2–2000 cells (Buchanan & Doyle, 1997).

Acknowledgements

M.A. acknowledges the financial assistance obtained from the Leadership Alliance, Office of the Associate Provost of Howard University and the Fulbright Fellowship.

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