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SURVIVAL OF ACID ADAPTED *ESCHERICHIA COLI* O157:H7 IN SOME ACIDIC FOODS

ASİDE ADAPTE EDİLEN *ESCHERICHIA COLI* O157:H7'NİN BAZI ASİDİK GIDALARDAKİ CANLILIĞI

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ABSTRACT: This study examined the effect of adaptation to acid on the survival of *Escherichia coli* O157:H7 in Ayran, turnip juice, apple juice and orange juice.

E. coli O157:H7 was adapted to acid at pH 4.5 in tryptic soy broth (TSB) for 2 h. Commercial products of Ayran (pH 3.95), turnip juice (pH 3.6), orange juice (pH 3.0) and apple juice (pH 3.3) were inoculated with acid adapted or nonadapted cells of *E. coli* O157:H7. Survival of the inoculated *E. coli* O157:H7 in these commercial food products during storage periods was examined. Orange juice, apple juice and turnip juice were stored at 4°C or 20°C. Ayran was stored at 4°C. In general, low temperature enhanced the survival of *E. coli* O157:H7 in the commercial juices tested ($p<0.05$). Acid adaptation enhanced the survival of *E. coli* O157:H7 in turnip juice and apple juice stored at 4°C. It was found that survival of the acid adapted and nonadapted *E. coli* O157:H7 in orange juice during storage at 4°C or 20°C and Ayran during storage at 4°C did not show any significant differences ($p<0.05$).

This study showed that adaptation to acid might increase survival of *E. coli* O157:H7 in some acidic foods. Therefore acid adaptation is an important mechanism in *E. coli* O157:H7 and should be considered in food challenges studies.

Key words: *E. coli* O157:H7, acidic foods, acid adaptation.

ÖZET: Bu çalışmada *Escherichia coli* O157:H7'nin aside adaptasyonunun Ayran, şalgam suyu, elma suyu ve portakal suyundaki canlılığına etkisi araştırılmıştır.

Escherichia coli O157:H7 pH 4,5'te Tryptic Soy Broth besiyerinde 2 saat tutularak aside adapte edilmiştir. Ticari olarak satılan Ayran (pH 3,95), şalgam suyu (pH 3,6), portakal suyu (pH 3,0) ve elma suyu (pH 3,3) aside adapte edilen ve edilmeyen *Escherichia coli* O157:H7 kültürü ile inokule edilmiştir. Bu ürünlerde inkübasyon süresince *Escherichia coli* O157:H7'nin canlılığı saptanmıştır. Elma, portakal ve şalgam suyu 4°C ve 20°C'de depolanırken Ayran yalnızca 4°C'de depolanmıştır. Genel olarak aside adaptasyondan bağımsız olarak düşük sıcaklık, test edilen tüm örneklerde *Escherichia coli* O157:H7'nin canlılığını artırmıştır. Aside adaptasyon 4°C'de depolanan şalgam suyunda *Escherichia coli* O157:H7'nin canlılığını artırmıştır ($p<0,05$). Ayrıca aside adaptasyon 4°C'de depolanan elma suyunda da *Escherichia coli* O157:H7'nin canlılığını artırmıştır ancak bu etki istatistiksel olarak önemli bulunmamıştır ($p<0,05$). 4°C ve 20°C'de depolanan portakal suyu ve 4°C'de depolanan elma suyunda ise aside adapte edilen ve edilmeyen *Escherichia coli* O157:H7 kültürlerinin canlılıkları arasında önemli bir fark tespit edilememiştir.

Bu çalışma *Escherichia coli* O157:H7'nin asidik gıdalarda uzun süre canlı kalabileceğini ve aside adaptasyonun bazı asidik gıdalarda bu patojenin canlılığını artırdığını göstermiştir. Sonuç olarak aside adaptasyon *Escherichia coli* O157:H7'nin asidik gıdalarda canlı kalmasını sağlayan önemli bir mekanizma olup gıda güvenliği çalışmalarında mutlaka göz önüne alınmalıdır.

Anahtar kelimeler: *E. coli* O157:H7, asidik gıdalar, aside adaptasyon.

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INTRODUCTION

E. coli O157:H7 belongs to the group of enterohaemorrhagic *E. coli* (EHEC). It was identified in 1982 as an important human pathogen (1). It is highly infectious bacterial pathogen in humans and has a very low infective dose (<100 cells). *E. coli* O157:H7 causes hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) in humans (2).

Altough *E. coli* O157:H7 occurs naturaliy in cattle and other food animals, many raw foods that have been directly exposed to animal faecal contamination or indirectly exposed via faecally contaminated water supplies may also be contaminated. Examples of foods implicated in outbreaks of *E. coli* O157:H7 infections include hamburgers, roast beef, fermented sausage, cooked maize, mayonnaise, lettuce and seed sprouts (3). Food poisoning outbreaks involving acidic foods have drawn attention to the acid tolerance properties of this bacterium. The resistance of *E. coli* O157:H7 to acidic conditions would possible be a result of a genetically induced acid tolerance response (ATR).

ATR is a phenomenon by which microorganisms shows an increased resistance to environmental stress after the exposure to a moderate acid environment. Acid adaptation and increased resistance to acid stress have been observed in various organisms including *Listeria*, *E. coli* and *Salmonella* (4, 5).

ATR is described as a two stage process that begins with an initial preshock exposure to a mild pH in the range of 4.5-5.5 and then followed by acid challenge exposure to a pH below 4.0. The ATR system was found to require protein synthesis that represents a newly described genetic response to environmental stress (6).

The ability of foodborne pathogens including *S. typhimurium*, *E. coli* O157:H7, *L. monocytogenes*, *Aeromonas hydrophila* and *Shigella flexneri* to adapt to acidic conditions is a concern of food safety (7, 8, 9). Leyer and Johnson (10) demonstrated that acid adapted *S. typhimurium* survived better than nonadapted cells during milk fermentation and had an increased resistance to inactivation by organic acids commonly present in cheese, including lactic, propionic and acetic acids.

We have recently demonstrated that tolerance to severe acid stress (pH 3.0) can be induced in *E. coli* O157:H7 following a 2 h adaptation to mild acid (pH 4.5) a phenomenon termed the ATR (11). In an attempt to determine the industrial significance of the ATR, we have examined the survival of acid adapted and nonadapted cells of *E. coli* O157:H7 in Ayran, turnip juice, apple juice and orange juice. All of these juices have a relatively low pH value and are popular drinks in Turkey.

MATERIALS AND METHODS

Bacterial Strain and Media

E. coli O157:H7 932 strain (a clinical isolate) was provided by M. P. Doyle (Center for Food Safety and Quality Enhancement. Department of Food Science and Technology, The University of Georgia, Griffin, Georgia, USA).

Stock culture was stored at +4°C in tryptic soy agar (TSA, Oxoid) and subcultured every month. Culture was activated from stock culture after two successive transfers of the test organism in tryptic soy broth (TSB, Oxoid) at 37°C for 24 h. This activated culture was used in experimental studies.

Acid Adaptation of Test Organism

To prepare the acid adapted cells of *E. coli* O157:H7 the procedure described by Tosun and Gönül (11) was adapted. Cells were grown overnight at 37°C in TSB, pH 7.0 and ten millilitres of the cultures was centrifuged at 5000 rpm. After the supernatant was discarded, the cell pellets were suspended in 10 ml of pH 4.5 TSB (pH adjusted with 6N HCl) for acid adapted cells and other cell pellets were suspended in 10 ml of pH 7.0 TSB for nonadapted cells. Cultures were incubated at 37°C for 2h (adaptation time). After this time acid adapted and nonadapted cells were then diluted 10 times by using 0.1% peptone water.

Survival Studies in Food Samples

Commercial products of Ayran (pH 3.95), turnip juice (pH 3.6), apple juice (pH 3.3) and orange juice (pH 3.0) were purchased from a local supermarket and stored at 4°C prior to use. Prior to inoculation the pH of each juice was determined. Food samples did not yield bacterial colonies when plated on Sorbitol Mac Conkey agar (SMAC, Oxoid).

In order to perform survival study, 100ml of each food samples was transferred to a sterile 250 ml erlenmayer flask and inoculated with the prepared acid adapted or nonadapted inoculum of *E. coli* O157:H7 to achieve an initial population of ~10^6 cfu/ml. Samples were then stored at 4°C or 20°C. Ayran samples were stored at 4°C. During the storage period, viable populations of *E. coli* O157:H7 were determined periodically as specified in Results and Discussion.

Enumeration of *E. coli* O157:H7

Viable cells were enumerated immediately before inoculation and after different storage times. For the enumeration of *E. coli* O157:H7, samples were serially diluted with 0.1% peptone water. The viable populations of *E. coli* O157:H7 in food samples was then determined by plating 0.1ml of the serially diluted samples on SMAC agar and incubated at 37°C for 24 or 48h. *E. coli* O157:H7 formed colourless colonies on SMAC agar.

Statistical Analysis

Experiments were run in duplicates. Data were analysed by SPSS 9.0 statistical software (SPSS Inc., Chicago, IL, USA) for analysis of variance and Duncan's multiple range test.

RESULTS AND DISCUSSION

Survival of Acid Adapted *E. coli* O157:H7 in Ayran

Ayran samples inoculated with acid adapted and nonadapted cells were stored at 4°C for 33 days (Figure 1).

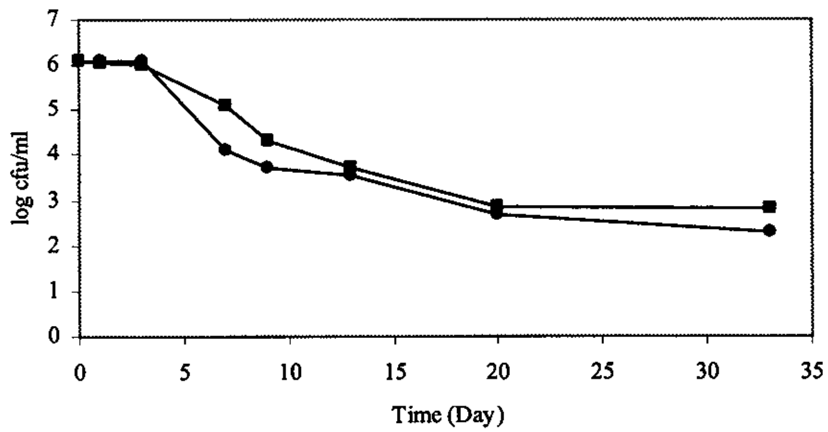

Figure 1. Survival of acid adapted and nonadapted *E. coli* O157:H7 in Ayran during storage at 4°C. Symbols: n , acid adapted; l , nonadapted. Each datum point represents the enumeration on two plates of platings conducted in duplicate in this figure and subsequent figures.

In the three days of incubation little inactivation of both acid adapted and nonadapted cells was observed. After 7 days of incubation control culture decreased 92 fold and acid adapted cell culture decreased 11 fold compared with their initial cell numbers. After this day control and acid adapted cells had similar acid tolerance. However at 33 days of incubation acid adapted cells survived about 3 fold better than the nonadapted cells which was not significant ($p<0.05$). At day 33, acid adapted cells were detected at 670 cfu/ ml while nonadapted cells were detected at 200 cfu/ ml.

As there is no other study with *E. coli* O157:H7 in Ayran, Hsin- Yi and Chou (12) observed that acid adaptation reduced the survival of *E. coli* O157:H7 in yoghurt (pH 3.9) and yakult (a fermented milk product, pH 3.6). But Gahan et al. (13) indicated that acid adaptation enhanced the survival of *L. monocytogenes* in yoghurt (pH 3.9) stored at 4°C. Essentially we have recently demonstrated that acid adapted *E. coli* O157:H7 showed maximum acid resistance at pH 3.0 (11). Effects of acid adaptation on the survival of *E. coli* O157:H7 decreased if challenge pH is above 3.0. pH of Ayran was 3.95. Both control and acid adapted cultures survived in Ayran for about 1 month. This data showed that the pH of Ayran was not a factor for survival of *E. coli* O157:H7.

E. coli O157:H7 does not show any special heat treatment resistance. Prevention of post pasteurisation is essential in dairy industry. HACCP procedure and the in-house control plan of the dairy factory must be adjusted to prevent all cross-contamination possibilities of heat treated products with raw, unheated milk.

Survival of Acid Adapted *E. coli* O157:H7 in Turnip Juice

Turnip juice is a popular drink in some region of Turkey and made of turnip radish, water, salt and black carrot. Figure 2 shows the behaviour of acid adapted and control cells in *E. coli* O157:H7 inoculated turnip juice.

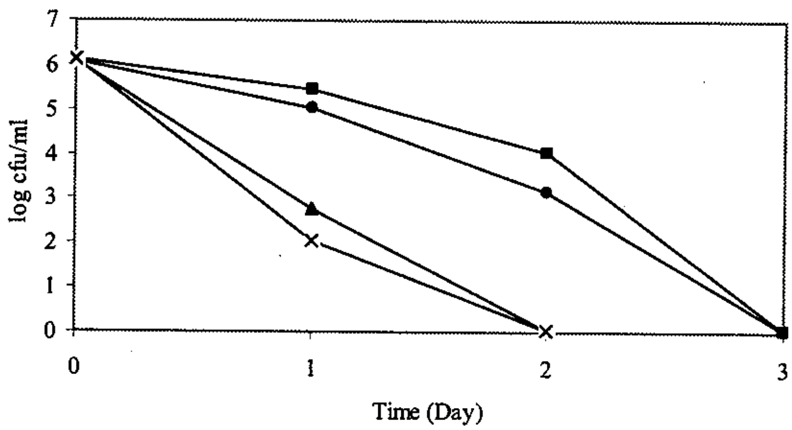

Figure 2. **Survival of acid adapted and nonadapted *E. coli* O157:H7 in turnip juice. Symbols: n, acid adapted (4°C); l, nonadapted (4°C); x, acid adapted (20°C); s, nonadapted (20°C).**

At 4°C, acid adapted cells survived much better than nonadapted cells. 1.1×10^4cfu/ ml were detected in the adapted population compared with 1.4×10^3cfu/ ml in the control cells at the two days of incubation. *E. coli* O157:H7 survived much better in turnip juice stored at 4°C than 20°C ($p<0.05$). Both acid adapted and nonadapted *E. coli* O157:H7 cells were not detected after three days of incubation in turnip juice stored at 4°C and after two days of incubation in turnip juice stored at 20°C. The pH of turnip juice was 3.6 and *E. coli* O157:H7 died off very rapidly in turnip juice compared with other food samples tested. Beuchat et al. (14) demonstrated that carrot juice had lethal and inhibitory effects on *L. monocytogenes*. Antimicrobials present in carrot may kill or prevent the growth of *E. coli* O157:H7 in turnip juice. This effect may also be researched in the future.

Survival of Acid Adapted *E. coli* O157:H7 in Apple Juice

Apple juice inoculated with acid adapted and nonadapted cells was stored 4°C or 20°C for 29 days (Figure 3). Acid adapted *E. coli* O157:H7 cells survived much better than nonadapted cells in apple juice stored at 4°C. But it was not statically significant ($p<0.05$). After 26 days nonadapted population was completely inactivated but acid adapted cells were detected at 15cfu/ml after 28 days.

Nonadapted cells of *E. coli* O157:H7 was recovered in greater numbers than acid adapted cells during storage at 20°C and 490 cells /ml were detected in nonadapted population compared with 470 cells/ ml in the acid adapted cells at the 8 days of incubation. Viable acid adapted or nonadapted organisms were no longer detected at 14 days.

Acid adaptation and cold storage increased survival of *E. coli* O157:H7 in apple juice like turnip juice. The enhanced survival of pathogens in a chilled acidic foods reported in this study corroborates results obtained by Tsai and Ingham (15). They reported that adaptation to acid and low temperature enhanced the survival of *E. coli* O157:H7 and *Salmonella* strains in ketchup. Miller and Kaspar (16) found that *E. coli* O157:H7 survived in apple juice (pH 3.7- 4.1) for 14 to 21 days at 4°C.

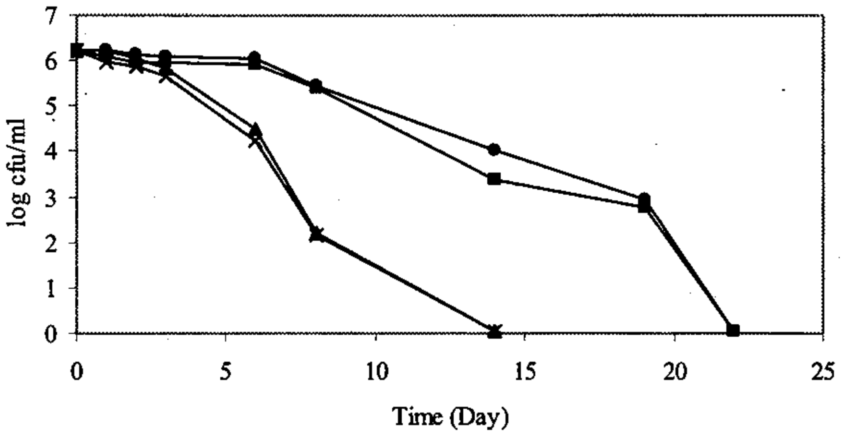

Figure 3. Survival of acid adapted and nonadapted *E. coli* O157:H7 in apple juice. Symbols: ₙ, acid adapted (4°C); ı, nonadapted (4°C); x, acid adapted (20°C); ₛ, nonadapted (20°C).

Leyer et al. (10) also observed enhanced acid tolerance of acid adapted *E. coli* O157:H7 to acidic conditions of apple cider. Control cells survived only 28h while acid adapted cells survived 82 h at 6°C. However their procedure for inducing acid adaptation was different from ours.

Fresh-pressed apple juice and apple cider have been implicated in outbreaks of food borne illness caused by *E. coli* O157:H7 (17, 18). These outbreaks also showed that *E. coli* O157 was relatively acid tolerant and could survive in acidic foods and fresh vegetable products.

Survival of Acid Adapted *E. coli* O157:H7 in Orange Juice

We evaluated whether acid adaptation would affect the survival of *E. coli* O157:H7 in orange juice or not. Acid adaptation did not enhance the survival of *E. coli* O157:H7 in orange juice ($P<0.05$). Nonadapted *E. coli* O157:H7 survived much better than the acid adapted cells in orange juice both stored at 4°C or 20°C. Storage at 4°C enhanced survival of *E. coli* O157:H7 in orange juice. At 4°C after 19 days, nonadapted cells were detected at $9.1x10^2$cfu/ml and acid adapted cells were detected at $5.9x10^2$cfu/ml. *E. coli* O157:H7 was no longer detected in orange juice stored at 4°C for 22 days and stored at 20°C for 14 days (Figure 4).

Ryu and Beuchat (19) indicated that acid adaptation did not enhance the survival of *E. coli* O157:H7 in three different brands of orange juice. Similarly, we did not detect effects of acid adaptation on survival of *E. coli* O157:H7 in orange juice. Parish (20) reported that seven of eight samples of unpasteurised orange juice (pH 3.9-4.2) contained *E. coli* and outbreaks of enterotoxigenic *E. coli* infection associated with orange juice have been reported by Singh et al. (21). Thus, the possibility of extended survival and growth of *E. coli* O157:H7 in orange juice cannot be dismissed.

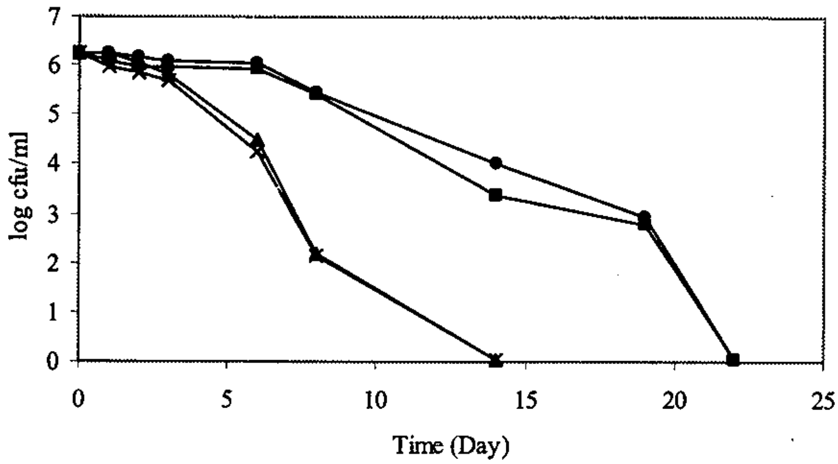

Figure 4. **Survival of acid adapted and nonadapted *E. coli* O157:H7 in orange juice. Symbols, n , acid adapted (4°C); n, nonadapted (4°C); x, acid adapted (20°C); s , nonadapted (20°C).**

CONCLUSION

E. coli O157:H7 poses a risk to the dairy industry because dairy cattle are a reservoir and source of this microorganism. Outbreaks associated with the consumption of raw milk and yoghurt has been documented by several authors (22, 23, 24). Although yoghurt is usually considered intrinsically safe because of its acidity and pH value, results of the present study demonstrated that *E. coli* O157:H7 may survive in Ayran. Shelf life of Ayran is about 10 days. If milk used for Ayran manufacture is contaminated with *E. coli* O157:H7 after pasteurisation process or during the manufacturing this microorganism may survive until Ayran is consumed. *E. coli* O157:H7 could survive in apple and orange juice for about 1 month at 4°C and acid adaptation may also enhance survival of *E. coli* O157:H7 in turnip and apple juice stored at 4°C. However *E. coli* O157:H7 is not particularly heat resistant and pasteurisation process for fruit juices may kill *E. coli* O157:H7, which produce heat resistance toxin and this toxin, may still be active in insufficiently heated foods (25). Especially *E. coli* O157:H7 is a potential risk factor for fresh-pressed fruit juices. In these juices *E. coli* O157:H7 may survive for a long time and cause illness after consumption and acid tolerance properties of this bacterium may enhance survival at low pH.

Because the infective dose of *E. coli* O157:H7 is very low (<100 cells of the organisms) even low levels of contamination in foods are of concern. Therefore any *E. coli* O157:H7 control measures implemented in the food chain should aim not only to reduce the hazards, but to actually remove it.

Acid adaptation is an important mechanism for the survival of *E. coli* O157:H7 in foods and in food processing environment. An understanding of the mechanism of adaptations to acid stress is important in order to design effective food safety systems and to prevent food borne disease.

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