Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2015) 39: 485-492
© TÜBİTAK
doi:10.3906/vet-1401-96

Determination of the survival levels of acid-adapted *Escherichia coli* O157:H7 in sucuk (Turkish-type fermented sausage)

Fatma ÖZTÜRK[1,*], Abdulkadir HALKMAN[2]
[1]Department of Fishing and Processing Technology, Faculty of Fisheries, İzmir Kâtip Çelebi University, Çiğli, İzmir, Turkey
[2]Department of Food Engineering, Faculty of Engineering, Ankara University, Dışkapı, Ankara, Turkey

Received: 29.01.2014 • **Accepted/Published Online:** 15.04.2015 • **Printed:** 28.08.2015

Abstract: This study aimed to determine the time taken for *Escherichia coli* O157:H7 to adapt to different types of acids and the effect of acid adaptation on the survival level of *E. coli* O157:H7 in sucuk (Turkish-type fermented sausage). *E. coli* O157:H7 cells were adapted to acids in a tryptic soy broth (TSB) set to pH 5.5 for 1-, 2-, 3-, and 4-h periods using hydrochloric, acetic, lactic, and citric acid. The acid tolerance of acid-adapted and nontreated cells was determined in TSB of pH 3.5 and then acid-adapted and nontreated *E. coli* O157:H7 (10^5 CFU/g) was added to sucuk. In the adaptation trial in which acetic acid was used, the highest acid resistance was seen in the 3-h adaptation samples, and in the lactic acid trials, the highest acid resistance was seen in the 3- and 4-h adaptation samples. In the adaptation trials with hydrochloric acid, it was observed that the duration of the adaptation period did not increase the resistance. It was determined that the survival rate of *E. coli* O157:H7 in sucuk was significantly increased through acid adaptation. As a result, it was understood that acid-adapted *E. coli* O157:H7 can maintain its viability in sucuk for at least 15 days.

Key words: *Escherichia coli* O157:H7, acid adaptation, acid tolerance, Turkish-type fermented sausage, sucuk

1. Introduction

Escherichia coli O157:H7 is a foodborne pathogen that causes hemorrhagic colitis and hemolytic uremic syndrome (1,2). Inadequately cooked meat and unpasteurized milk have led to a large number of infections resulting from *E. coli* O157:H7 (3,4). There have also been reports of *E. coli* infections originating from acidic foods such as apple cider, yogurt, and mayonnaise (5,6). The increase of infections originating from these kinds of foods, which are generally accepted as safe, has led to a rise in the number of questions about the safety of acidic foods.

The acid tolerance response (ATR) occurs when pathogen bacteria gain resistance to highly acidic media by the synthesis of acid shock proteins, which is controlled by specific genes. This process results from exposure to moderately acidic (pH 5.0–6.0) media over a certain period of time (7–12).

ATR allows *E. coli* O157:H7 to maintain its vitality in both acidic and fermented foods by gaining resistance to acids; thus, it can survive for an extended period of time in acidic foods such as apple cider and mayonnaise (6), fermented meat, and dairy products (7,13).

Sucuk (Turkish-type fermented sausage) is a traditional meat product popular with Turkish people, and there are other very similar fermented sausages such as saucisson, chorizo, salami, summer sausage, pepperoni, teewurst, and mettwurst widely consumed in many other countries (14). Globally, dried fermented meat products such as sucuk are considered to be a safe food group for humans.

Factors such as antimicrobial metabolites produced by starter cultures, low pH, and low water activity in fermented sausages made from raw ingredients inhibit the growth of foodborne pathogens (15–17). However, in recent years, it was determined that *E. coli* O157:H7 could maintain its viability in these 'safe' foods for a long time (18).

The purpose of the study was to determine the time taken for *E. coli* O157:H7 to adapt to different types of acids and the effect of acid adaptation on the survival level of *E. coli* O157:H7 in sucuk (Turkish-type fermented sausage).

2. Materials and methods

2.1. Types and growth conditions of bacteria

The *E. coli* O157:H7 KUEN 1461 strain supplied by the culture collection of the Food Engineering Department of Ankara University was used in this study. Stock cultures of *E. coli* O157:H7 were kept at –20 ± 2 °C in tryptic soy broth (TSB, Merck) including glycerol (20%; v/v). For the trials,

* Correspondence: fatmayaman26@hotmail.com

100 µL of stock culture was added to 10 mL of TSB and incubated for 24 h at 37 °C to activate the bacteria. This activation process was repeated twice.

A lyophilized commercial starter culture (Lyocarni RBL-73 from Sacco; http://www.saccosrl.it/pdfSchedeTecniche/M92RBL730UK0.pdf) was used for the sucuk production. The culture contained *Staphylococcus carnosus*, *Staphylococcus xylosus*, and *Lactobacillus curvatus* strains and was kept at −20 ± 2 °C.

2.2. Preparation of acid-adapted *E. coli* O157:H7 cells

The method defined by Cheng et al. (19) was slightly modified and used in the preparation of the acid-adapted cells. First, 5 µL of active *E. coli* O157:H7 was transferred into 50 mL of TSB and incubated for 14 h at 37 °C. After incubation, 9 mL of the activated culture was centrifuged at 5000 rpm (Hettich EBA 12; Germany) for 10 min.

The suspended pellets were washed twice with 0.85% NaCl solution. The pellets were transferred into 10 mL of TSB that was set to pH 5.5 using 6 N HCl (37%; Merck), 5 M acetic acid (100%; Merck), 3 M lactic acid (85% DL-lactic acid; Sigma), and 1 M citric acid (99.5%; Merck) solutions and then incubated for 1, 2, 3, and 4 h at 37 °C.

For the preparation of the nontreated cells, 5 µL of active culture was transferred to 50 mL of TSB and incubated for 14 h at 37 °C. After incubation, the cultures were centrifuged and the pellet was suspended in 10 mL of TSB at pH 7.0.

2.3. Acid tolerance of *E. coli* O157:H7

To determine the resistance of *E. coli* O157:H7 cells to strongly acidic conditions, samples of 50 mL of TSB (pH 3.5) were inoculated with acid-adapted and nontreated *E. coli* O157:H7 cultures using HCl, acetic, lactic, and citric acids and incubated at 37 °C.

For the HCl trials, after 0, 1, 2, 3, 4, 5, 24, and 48 h of incubation, 0.1 mL of culture was transferred to tryptic soy agar (TSA, Merck) with the incubation periods being 0, 30, 60, 90, and 120 min for the organic acids. All results were given as CFU/mL after incubation for 24 h at 37 °C.

2.4. Preparation of bacteria cultures used in the production of sucuk

First, 5 µL of active *E. coli* O157:H7 was transferred into 50 mL of TSB and incubated for 14 h at 37 °C. After incubation, 9 mL of activated culture was centrifuged at 5000 rpm (Hettich EBA 12; Germany) for 10 min. The suspended pellets were washed twice with 0.85% NaCl solution. The pellets were transferred into 10 mL of TSB that was set to pH 5.5 using 3 M lactic acid (85% DL-lactic acid; Sigma) solution and then incubated for 3 h at 37 °C. After incubation, the cultures were centrifuged and the pellet was suspended in 9 mL of FTS. For the preparation of the nontreated cells, 5 µL of active culture was transferred to 50 mL of TSB and incubated for 14 h at 37 °C. After incubation, the cultures were centrifuged and the pellet was suspended in 9 mL of FTS. The solution was added to the sucuk batter so that the final concentration was 10^5 CFU/g.

2.5. Production of sucuk

Beef and sheep tail fat (25%) was cut into small pieces and then minced through a 3-mm plate. The mixture was irradiated at 25 kGy in the irradiation facility of the Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Ankara, and kept at −20 ± 2 °C until it was to be used.

The mixture was defrosted overnight and then 1.6% NaCl, 1.2% garlic, 0.5% sucrose, 0.5% chili pepper, 0.6% sweet paprika, 0.6% black pepper, 0.8% cumin, 0.04% $NaNO_3$, and 0.01% $NaNO_2$ were added. A commercial starter culture was added to the sucuk batter and homogenized using a laboratory-type mixer. After this stage, the sucuk batter was divided between the 2 trial groups; acid-adapted *E. coli* O157:H7 (10^5 CFU/g) was added to one group and the nontreated *E. coli* O157:H7 (10^5 CFU/g) was added to the other group as a control. These experiments were carried out in duplicate. The inoculated sucuk batter was kept in a refrigerator at 4 °C overnight to ensure adequate diffusion throughout the meat.

A laboratory mixer was used to stuff 50–60 g of the sucuk batter into collagen casings 35 mm in diameter. The sucuk batter samples were ripened in an automatically adjustable air-conditioned room under conditions of 85%–90% relative humidity at 22 °C, 80%–85% relative humidity at 22 °C, and 65%–70% relative humidity at 20 °C sequentially for 3, 3, and 2 days, respectively (20). After this ripening process, the sucuk samples were stored at 4 °C.

2.5.1. Bacteriological analysis of sucuk

Ripened sucuk samples (10 g) were transferred into Stomacher bags containing 90 mL of Maximum Recovery Diluent (Merck) and homogenized for 1 min at 235 rpm in the Stomacher (Seward Stomacher®400 Circulator; UK). Sorbitol MacConkey Agar (Merck) was used for the *E. coli* O157:H7 and incubated at 37 °C for 24 h. Baird Parker Agar (Merck) was used for the *Staphylococcus* spp. and incubated at 37 °C for 48 h. For the lactic acid bacteria (LAB), De Man Rogosa Sharp Agar (Merck) was used and incubated at 30 °C for 72 h. Standard microbiological methods were applied in the analyses (21).

2.5.2. Chemical and physical analyses of the sucuk samples

The 10-g samples were homogenized in 100 mL of distilled water and the pH was measured by pH meter (WTW, Inolab Level 2; Germany). In order to determine the dry matter, approximately 5 g of sucuk was weighed and dried until a constant weight was obtained at 105 °C (22).

2.6. Statistical analysis

A variance analysis with repeated measurement (ANOVA) was carried out on the experimental results. The Duncan test was used to determine the differences between the groups. Statistical analyses were performed using SPSS 15 for the variance analyses and CMSTAT for the Duncan tests.

3. Results

3.1. Acid adaptation

The survival rates of *E. coli* O157:H7 adapted to HCl at pH 5.5 for 1, 2, 3, and 4 h and those of the nontreated cells at pH 3.5 are shown in Table 1. In TSB (pH 3.5), no noticeable decrease in the number of cells in the two groups was observed in the first 5 h of incubation. Therefore, the difference among groups was not found to be significant ($P > 0.01$). In hours 24 and 48 of incubation, the number of nontreated *E. coli* O157:H7 cells was lower than that of the acid-adapted cells. In conclusion, it was determined that *E. coli* O157:H7 gained resistance to the acid via acid adaptation and the difference between adaptation periods was not significant ($P > 0.01$).

The acid-adapted and nontreated cells in the acetic acid were incubated for 120 min in TSB (pH 3.5) and their survival levels were determined (Table 2). The acid adaptation at pH 5.5 increased the resistance of *E. coli* O157:H7 to acetic acid ($P < 0.01$) and the highest acid resistance was detected in those cells exposed to a 3-h adaptation period ($P < 0.01$).

The results regarding the survival level at pH 3.5 of acid-adapted and nontreated *E. coli* O157:H7 cells for 1, 2, 3, and 4 h in TSB (pH set to 5.5 by lactic acid) are

Table 1. The survival level of HCl adapted and nontreated *E. coli* O157:H7 in pH 3.5 TSB (log CFU/mL).

Incubation (h)	Acid-adapted period (h)				Nontreated
	1	2	3	4	
0	7.08^{Aa} ± 0.04	7.03^{Aa} ± 0.05	7.02^{Aa} ± 0.02	7.04^{Aa} ± 0.05	7.21^{Aa} ± 0.10
1	6.93^{Aa} ± 0.06	6.99^{Aa} ± 0.06	6.97^{Aa} ± 0.04	7.03^{Aa} ± 0.05	7.20^{Aa} ± 0.09
2	6.92^{Aa} ± 0.05	6.93^{Aa} ± 0.01	6.89^{Aa} ± 0.11	6.95^{Aa} ± 0.04	7.17^{Aa} ± 0.09
3	6.92^{Aa} ± 0.05	6.92^{Aa} ± 0.02	6.87^{Aa} ± 0.10	6.95^{Aa} ± 0.05	7.14^{Aa} ± 0.06
4	6.89^{Aa} ± 0.04	6.83^{Aa} ± 0.03	6.85^{Aa} ± 0.10	6.92^{Aa} ± 0.05	7.13^{Aa} ± 0.05
5	6.83^{Aa} ± 0.03	6.80^{Aa} ± 0.01	6.79^{Aa} ± 0.08	6.73^{Aa} ± 0.13	7.08^{Aa} ± 0.04
24	5.01^{Bb} ± 0.47	5.46^{Bb} ± 1.00	5.46^{Bb} ± 1.00	6.41^{Aa} ± 0.11	3.26^{Bc} ± 0.11
48	2.24^{Cb} ± 0.29	2.32^{Cb} ± 0.72	2.33^{Cb} ± 0.14	1.95^{Bb} ± 0.10	<1.00^{Cc} ± 0.00

Values are the average of 2 replicates.
*A, B, C (↓); $^{a, b, c\,(\rightarrow)}$: The difference between averages having the same letters is not statistically significant ($P > 0.01$).

Table 2. The survival level of acetic acid-adapted and nontreated *E. coli* O157:H7 in pH 3.5 TSB (log CFU/mL).

Incubation (min)	Acid-adapted period (h)				Nontreated
	1	2	3	4	
0	6.99^{Aa} ± 0.01	6.95^{Aa} ± 0.10	7.02^{Aa} ± 0.02	7.15^{Aa} ± 0.00	7.00^{Aa} ± 0.00
30	6.46^{Bab} ± 0.23	6.41^{Bb} ± 0.03	6.66^{Ba} ± 0.07	6.25^{Bb} ± 0.02	3.91^{Bc} ± 0.03
60	<1.00^{Ca} ± 0.00	<1.00^{Ca} ± 0.00	<1.00^{Ca} ± 0.00	<1.00^{Ca} ± 0.00	<1.00^{Ca} ± 0.00
90	<1.00^{Ca} ± 0.00	<1.00^{Ca} ± 0.00	<1.00^{Ca} ± 0.00	<1.00^{Ca} ± 0.00	<1.00^{Ca} ± 0.00
120	<1.00^{Ca} ± 0.00	<1.00^{Ca} ± 0.00	<1.00^{Ca} ± 0.00	<1.00^{Ca} ± 0.00	<1.00^{Ca} ± 0.00

Values are the average of 2 replicates.
*A, B, C (↓); $^{a, b, c\,(\rightarrow)}$: The difference between averages having the same letters is not statistically significant ($P > 0.01$).

shown in Table 3. After 120 min of incubation, the highest acid resistance was detected in the *E. coli* O157:H7 cells exposed to adaptation for 3 and 4 h ($P < 0.01$).

Table 4 presents the results regarding the survival level at pH 3.5 of acid-adapted and nontreated *E. coli* O157:H7 in TSB with pH set to 5.5 by citric acid. In the adaptation trials performed using citric acid, it was seen that the difference between the average of the trial groups was statistically insignificant and the application of citric acid did not result in any increase in resistance. Interestingly, the nontreated cells were not affected at a pH of 3.5 set using citric acid.

In the adaptation trial using acetic acid, the highest acid resistance was seen in the 3-h adaptation samples, and in the lactic acid trials in the 3- and 4-h adaptation samples. In the adaptation trials with HCl, it was detected that the duration of the adaptation period did not increase the resistance.

3.2. The numbers of acid-adapted and nontreated *E. coli* O157:H7 in sucuk

The survival of *E. coli* O157:H7 in the sucuk samples and the effects of acid adaptation were examined. For this purpose, the samples were inoculated with acid-adapted *E. coli* O157:H7 cells for 3 h in TSB of pH 5.5 with lactic acid and nontreated cells. The survival levels of acid-adapted and nontreated *E. coli* O157:H7 during the sucuk ripening and storage period are shown in Table 5.

The numbers of acid-adapted *E. coli* O157:H7 decreased to <2.00 log CFU/g on the 30th day and the nontreated *E. coli* O157:H7 decreased to the same level on the 8th day. The acid-adapted *E. coli* O157:H7 cells maintained their survival for a longer time when compared to nontreated *E. coli* O157:H7 cells ($P < 0.01$). There was a significant increase in the survival rate of *E. coli* O157:H7 in sucuk through acid adaptation ($P < 0.01$).

3.3. The number of LAB and *Staphylococcus* in sucuk

The difference between the LAB numbers determined in the sucuk samples inoculated with acid-adapted and nontreated *E. coli* O157:H7 cells was found to be statistically insignificant. The difference in the number of *Staphylococcus* spp. detected in the sucuk samples into which acid-adapted and nontreated *E. coli* O157:H7 cells were inoculated was found to be statistically insignificant (Table 6).

Table 3. The survival level of lactic acid-adapted and nontreated *E. coli* O157:H7 in pH 3.5 TSB (log CFU/mL).

Incubation (min)	Acid-adapted period (h)				Nontreated
	1	2	3	4	
0	$7.01^{Aa} \pm 0.03$	$7.03^{Aa} \pm 0.05$	$7.04^{Aa} \pm 0.11$	$7.06^{Aa} \pm 0.06$	$7.08^{Aa} \pm 0.08$
30	$6.93^{Aa} \pm 0.07$	$6.97^{Aa} \pm 0.00$	$6.94^{Aa} \pm 0.03$	$6.94^{Aa} \pm 0.04$	$6.89^{Aa} \pm 0.10$
60	$5.39^{Bb} \pm 0.25$	$6.38^{Aa} \pm 0.12$	$6.68^{Aa} \pm 0.23$	$6.80^{Aa} \pm 0.06$	$5.31^{Bb} \pm 0.77$
90	$4.17^{Cb} \pm 0.25$	$5.30^{Ba} \pm 0.33$	$5.84^{Ba} \pm 0.39$	$5.81^{Ba} \pm 0.04$	$2.74^{Cc} \pm 0.26$
120	$<1.00^{Dc} \pm 0.00$	$3.19^{Cb} \pm 0.33$	$4.65^{Ca} \pm 0.54$	$4.34^{Ca} \pm 0.04$	$<1.00^{Dc} \pm 0.00$

Values are the average of 2 replicates.
*A, B, C (↓); $^{a, b, c (\rightarrow)}$: The difference between averages having the same letters is not statistically significant ($P > 0.01$).

Table 4. The survival level of citric acid-adapted and nontreated *E. coli* O157:H7 in pH 3.5 TSB (log CFU/mL).

Incubation (min)	Acid-adapted period (h)				Nontreated
	1	2	3	4	
0	$7.06^{Aa} \pm 0.02$	$7.08^{Aa} \pm 0.05$	$7.06^{Aa} \pm 0.02$	$7.08^{Aa} \pm 0.03$	$7.10^{Aa} \pm 0.05$
30	$7.00^{Aa} \pm 0.06$	$7.06^{Aa} \pm 0.02$	$7.04^{Aa} \pm 0.03$	$7.06^{Aa} \pm 0.02$	$7.04^{Aa} \pm 0.04$
60	$6.96^{Aa} \pm 0.04$	$7.02^{Aa} \pm 0.03$	$6.96^{Aa} \pm 0.08$	$7.00^{Aa} \pm 0.01$	$7.01^{Aa} \pm 0.03$
90	$6.94^{Aa} \pm 0.08$	$6.99^{Aa} \pm 0.02$	$6.95^{Aa} \pm 0.09$	$6.99^{Aa} \pm 0.02$	$6.97^{Aa} \pm 0.04$
120	$6.89^{Aa} \pm 0.03$	$6.96^{Aa} \pm 0.04$	$6.86^{Aa} \pm 0.04$	$6.91^{Aa} \pm 0.01$	$6.86^{Aa} \pm 0.06$

Values are the average of 2 replicates.
*A, B, C (↓); $^{a, b, c (\rightarrow)}$: The difference between averages having the same letters is not statistically significant ($P > 0.01$).

Table 5. The survival level of *E. coli* O157:H7 cells during ripening and storage periods at 4 °C of the sucuk samples (log CFU/mL).

Days	Acid-adapted	Nontreated
H0*	5.80 ± 0.08	5.53 ± 0.05
Ripening		
0	5.37 ± 0.07	4.64 ± 0.22
1	4.82 ± 0.06	4.37 ± 0.07
2	4.31 ± 0.05	3.89 ± 0.15
3	4.15 ± 0.27	3.68 ± 0.02
4	4.03 ± 0.33	3.51 ± 0.07
6	3.11 ± 0.21	2.30 ± 0.20
Storage		
8	2.72 ± 0.12	<2.00 ± 0.00
15	2.50 ± 0.50	<2.00 ± 0.00
30	<2.00 ± 0.00	<2.00 ± 0.00

Acid-adapted cells were obtained by keeping them in TSB (pH 5.5) acidified with lactic acid for 3 h.
H0*: Keeping the sucuk batter at 4 °C overnight.
Values are the average of 2 replicates.

3.4. Change in the pH and humidity levels during ripening and storage

It was detected that as the number of LAB increased, the pH decreased, and this interaction was found statistically significant ($P < 0.01$). Changes in the pH values of the sucuk samples containing acid-adapted and nontreated *E. coli* O157:H7 were observed during ripening and storage (Table 7). It was seen that the acid-adapted *E. coli* O157:H7 proved resistant to pH changes occurring in fermentation and that it could survive for at least 15 days.

The differences between the humidity levels detected in the sucuk samples with acid-adapted and nontreated *E. coli* O157:H7 cells were found to be statistically insignificant (Table 7). It was determined that there was a positive interaction between the humidity level and the pH value; as the former decreased, so did the latter ($P < 0.05$). Furthermore, as the humidity level decreased, it was found that the survival level of the *E. coli* O157:H7 cells also decreased ($P < 0.01$).

4. Discussion

Acid-tolerant pathogenic bacteria can survive for a long time in acidic food. Furthermore, acid is one of the most important defense mechanisms of the body. In particular, the acidic medium of the stomach has a significant role in protecting the body against bacteria. Studies have shown

Table 6. The number of LAB and *Staphylococcus* spp. in the sucuk samples inoculated with *E. coli* O157:H7 cells (log CFU/mL).

Days	Acid-adapted		Nontreated	
	LAB	*Staph.* spp.	LAB	*Staph.* spp.
H0*	6.48 ± 0.05	6.43 ± 0.16	6.45 ± 0.02	6.33 ± 0.12
Ripening				
0	6.65 ± 0.08	6.39 ± 0.05	6.52 ± 0.03	6.29 ± 0.01
1	9.25 ± 0.07	6.69 ± 0.07	9.14 ± 0.09	6.17 ± 0.43
2	9.42 ± 0.01	6.09 ± 0.05	9.31 ± 0.11	6.28 ± 0.20
3	9.35 ± 0.06	6.16 ± 0.01	9.36 ± 0.02	6.12 ± 0.12
4	9.19 ± 0.01	6.09 ± 0.05	9.20 ± 0.16	5.98 ± 0.13
6	9.21 ± 0.13	6.69 ± 1.09	9.32 ± 0.11	6.08 ± 0.04
Storage				
8	9.22 ± 0.04	6.90 ± 0.09	9.12 ± 0.08	7.15 ± 0.07
15	8.75 ± 0.03	6.21 ± 0.21	8.79 ± 0.19	7.24 ± 0.36
30	8.83 ± 0.09	5.75 ± 0.40	8.57 ± 0.07	6.15 ± 1.33

Acid-adapted cells were obtained by keeping them in TSB (pH 5.5) acidified with lactic acid for 3 h.
H0*: Keeping the sucuk batter at 4 °C overnight.
Values are the average of 2 replicates.

Table 7. pH values and humidity levels (%) of the sucuk samples inoculated with *E. coli* O157:H7 cells.

Days	Acid-adapted		Nontreated	
	pH	Moisture %	pH	Moisture %
H0*	5.78 ± 0.03	59.19 ± 0.57	5.75 ± 0.01	59.22 ± 1.47
Ripening				
0	5.80 ± 0.07	57.15 ± 0.50	5.75 ± 0.03	58.62 ± 1.93
1	4.65 ± 0.01	56.11 ± 1.06	4.62 ± 0.02	58.51 ± 1.01
2	4.42 ± 0.06	54.35 ± 1.76	4.39 ± 0.04	54.00 ± 3.30
3	4.56 ± 0.04	50.21 ± 3.56	4.59 ± 0.11	50.47 ± 1.14
4	4.54 ± 0.05	48.20 ± 0.52	4.52 ± 0.02	49.19 ± 7.39
6	4.48 ± 0.01	43.01 ± 3.65	4.44 ± 0.07	38.49 ± 2.22
Storage				
8	4.61 ± 0.01	39.82 ± 1.83	4.59 ± 0.11	35.88 ± 1.03
15	4.74 ± 0.03	35.37 ± 1.27	4.60 ± 0.03	31.04 ± 0.04
30	4.76 ± 0.06	34.39 ± 0.01	4.72 ± 0.13	30.06 ± 0.02

Acid-adapted cells were obtained by keeping them in TSB (pH 5.5) acidified with lactic acid for 3 h.
H0*: Keeping the sucuk batter at 4 °C overnight.
Values are the average of 2 replicates.

that *E. coli* O157:H7 adapts and gains resistance to acid when subjected to a medium of pH 4.5–5.5. ATR increases the acid resistance of *E. coli* O157:H7, allowing it to survive both in acidic and fermented food (1,7).

Many researchers found that acid adaptation of *E. coli* O157:H7 varied according to the duration of the adaptation period. It was stated by Cheng et al. (19) that after being exposed to moderate acidic conditions (pH 5.0) the resistance of *E. coli* O157:H7 to strong acid stress (pH 3.0) varied according to adaptation period, and the highest acid tolerance was obtained after the bacteria had undergone a 4-h adaptation period. Tosun and Aktuğ Gönül (23) reported an increase in the acid tolerance to pH 3.0 of the acid-adapted *E. coli* O157:H7 and the highest acid tolerance was obtained after a 2-h adaptation period at pH 4.5.

The current study has also shown that the HCl adaptation of *E. coli* O157:H7 increased the acid tolerance; however, the adaptation time did not result in a significant difference (Table 1). The dissimilar results obtained from the current study can be explained by the acid tolerance of the bacteria. There are studies showing that a decrease in the pH increased the acid resistance of *E. coli* O157:H7. Cheng et al. (19) reported that the acid adaptation of *E. coli* O157:H7 subjected to mild acidic conditions (pH 5.0) varied depending on the adaptation time, and as the pH increased (pH 4.0–5.0), the difference between the adaptation times was reduced. Öztürk (24) found that the resistance of *E. coli* O157:H7 to pH 3.0 varied with different adaptation times; however, there was no significant difference between the adaptation times for pH 3.5.

The inhibitory effect of hydrochloric, acetic, lactic, malic, and citric acids on *E. coli* O157:H7 has been investigated. It was determined that the highest and lowest inhibitory effects were obtained from the lactic acid and HCl treatment, respectively, with different sensitivity to acetic, malic, and citric acid among strains (25). In the study carried out by Ryu et al. (26) to detect the effect of different acids on *E. coli* O157:H7, the bacteriostatic effect was determined to be in the sequence of acetic > lactic > citric acid. In the current study, it was found that acetic, lactic, and citric acids were more effective than HCl and the bactericidal effect of the organic acids was determined to be in the order of acetic > lactic > citric acid.

Leyer et al. (7) detected that the resistance of acid-adapted *E. coli* O157:H7 to lactic acid was increased by culturing at pH 5.0 over a period of 1–2 generations. It was determined by Goodson and Rowbury (27) that the survival of 3 different strains of acid-adapted *E. coli* at pH 5.0 increased in a culture medium of lactic, benzoic, propionic, sorbic, and acetic acids with the pH set to 3.5.

The development characteristics of *E. coli* O157:H7 acid-shocked, acid-adapted, and control cells in TSB acidified with lactic acid and acetic acid were examined by Ryu and Beuchat (6). In the acetic acid trials (pH 3.4), these three cell types proved to have the same behavior. In the lactic acid trials (pH 3.9), it was detected that the numbers of acid-adapted and acid-shocked cells were higher than that of the control cells, and the acid-adapted cells were more tolerant of acidic conditions than acid-shocked cells.

As explained above, the ATR depends on the variety of acid, pH, and cell type. In this current research, we detected that the acid adaptation of *E. coli* O157:H7 varied according to the variety of acid used and the adaptation period. While the tolerance of *E. coli* O157:H7 to acetic and lactic acid increased, there was no increase in its tolerance to citric acid.

In this research, it was determined that the survival rate of *E. coli* O157:H7 in sucuk increased through acid adaptation. The same result was obtained in other studies of various food systems and it has been detected that acid adaptation increases acid resistance. Leyer et al. (7) reported that *E. coli* O157:H7 has an acid adaptation mechanism and the survival rate of the bacteria increases in fermented sucuk and similar products, sliced dried salami, and cider by this mechanism. Weagant et al. (5) drew similar conclusions after examining the survival rate of acid-adapted and nontreated *E. coli* O157:H7 in ketchup and mayonnaise.

Contrary to this research, in a study conducted by Riordan et al. (28) it was detected that acid adaptation did not result in any increase in the survival rate of *E. coli* O157:H7 in pepperoni, another fermented meat product. Dlamini and Buys (13) claimed that a significant difference did not exist between survival levels of acid-adapted and nontreated *E. coli* O157:H7 cells in amasi, also a traditional fermented dairy product, but that in commercial amasi the nontreated cells had a higher survival rate.

Dlamini and Buys (13) also reported that fermented products inoculated with acid-adapted *E. coli* O157:H7 cells showed no significant difference in the number of LAB. Similarly, the results of the current study have shown no significant difference in the number of LAB. As the number of LAB increased, the pH decreased, and acid-adapted *E. coli* was resistant to changes in pH during fermentation and could survive at a certain level.

In this research, it was determined that *E. coli* O157:H7 increases its acid toleration after exposure to moderately acidic (pH 5.5) conditions and it can survive for a long time (at least 2 h) in highly acidic medium (pH 3.5), which without the adaption process would have killed the bacteria. In addition, it is understood that the type of acid and the duration of the adaptation period have an important effect on acid adaptation. In the sucuk samples the survival level of *E. coli* O157:H7 with acid adaptation in sucuk (pH of ~4.8) increased. The acid-adapted *E. coli* O157:H7 cells maintained their survival in sucuk for 30 days. The existence of pathogenic bacteria in acidic foods such as sucuk, which has traditionally been considered as safe, is of great importance for both the food industry and human health.

References

1. Parry-Hanson AA, Jooste PJ, Buys EM. Relative gene expression in acid-adapted *Escherichia coli* O157:H7 during lactoperoxidase and lactic acid challenge in Tryptone Soy Broth. Microbiol Res 2010; 165: 546–556.
2. Rekow CL, Brashears MM, Brooks JC, Loneragan GH, Gragg SE, Miller MF. Implementation of targeted interventions to control *Escherichia coli* O157:H7 in a commercial abattoir. Meat Sci 2011; 87: 361–365.
3. Yoon Y, Mukherjee A, Belk KE, Scanga JA, Smith GC, Sofos JN. Effect of tenderizers combined with organic acids on *Escherichia coli* O157:H7 thermal resistance in non-intact beef. Int J Food Microbiol 2009; 133: 78–85.
4. Sofos JN, Geornaras I. Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of *Escherichia coli* O157:H7 in nonintact, and *Listeria monocytogenes* in ready-to-eat, meat products. Meat Sci 2010; 86: 2–14.
5. Weagant SD, Bryant JL, Bark DH. Survival of *Escherichia coli* O157 H7 in mayonnaise and mayonnaise-based sauces at room and refrigerated temperatures. J Food Protect 1994; 57: 629–631.
6. Ryu JH, Beuchat LR. Influence of acid tolerance responses on survival, growth, and thermal cross-protection of *Escherichia coli* O157:H7 in acidified media and fruit juices. Int J Food Microbiol 1998; 45: 185–193.
7. Leyer GJ, Wang L, Johnson EA. Acid adaptation of *Escherichia coli* O157:H7 increases survival in acidic foods. Appl Environ Microbiol 1995; 3752–3755.
8. Castanie-Comet Cornet MP, Cam K, Bastiat B, Cros A, Bordes P, Gutierrez C. Acid stress response in *Escherichia coli*: mechanism of regulation of *gadA* transcription by RcsB and GadE. Nucleic Acids Res 2010; 38: 3546–3554.
9. Alegre I, Abadias M, Anguera M, Usall, J, Vinas. I. Fate of *Escherichia coli* O157:H7, *Salmonella* and *Listeria innocua* on minimally-processed peaches under different storage conditions. Food Microbiol 2010; 27: 862–868.
10. Krin E, Danchin A, Soutourina O. Decrypting the H-NS-dependent regulatory cascade of acid stress resistance in *Escherichia coli*. BMC Microbiol 2010; 10: 273.

11. Singh M, Mullins HR, Simpson SM, Dickson JS. Effect of acid adaptation on thermal tolerance of *Escherichia coli* O157:H7 and *Salmonella enterica* in meat serum. J Food Safety 2010; 30: 111–123.

12. Senouci-Rezkallah K, Schmitt P, Jobin MP. Amino acids improve acid tolerance and internal pH maintenance in *Bacillus cereus* ATCC14579 strain. Food Microbiol 2011; 28: 364–372.

13. Dlamini BC, Buys EM. Adaptation of *Escherichia coli* O157:H7 to acid in traditional and commercial goat milk amasi. Food Microbiol 2009; 26: 58–64.

14. Toldra F, Reig M. Sausages. In: Hui YH, Chandan C, Clark S, editors. Handbook of Food Products Manufacturing: Health, Meat, Milk, Poultry, Seafood, and Vegetables. Hoboken, NJ, USA: Wiley & Sons; 2007. pp. 251–264.

15. Stolzenbach S, Lindahl G, Lundstrom K, Chen G, Byrne DV. Perceptual masking of boar taint in Swedish fermented sausages. Meat Sci 2009; 81: 580–588.

16. Ravyts F, Sten L, Goemaere O, Paelinck H, Vuyst, L, Leroy F. The application of staphylococci with flavour-generating potential is affected by acidification in fermented dry sausages. Food Microbiol 2010; 27: 945–954.

17. Aro JM, Nyam-Osor P, Tsuji K, Shimad K, Fukushima M, Sekikawa M. The effect of starter cultures on proteolytic changes and amino acid content in fermented sausages. Food Chem 2010; 119: 279–285.

18. Apaydin G, Ceylan ZK, Kaya M. The behavior of *E. coli* O157:H7 in sucuk. J Food Process Pres 2009; 33: 827–836.

19. Cheng HY, Yu RC, Chou CC. Increased acid tolerance of *Escherichia coli* O157:H7 as affected by acid adaptation time and conditions of acid challenge. Food Res Int 2003; 36: 49–56.

20. Soyer A, Ertas AH, Uzumcuoglu U. Effect of processing conditions on the quality of naturally fermented Turkish sausages (sucuks). Meat Sci 2005; 69: 135–141.

21. Harrigan WF. Laboratory Methods in Food Microbiology. 3rd ed. San Diego, CA, USA: Academic Press; 1998.

22. AOAC. Official Methods of Analysis. Washington, DC, USA: Association of Official Analytical Chemists; 2000.

23. Tosun H, Aktuğ Gönül Ş. The effect of acid adaptation conditions on acid tolerance response of *Escherichia coli* O157:H7. Turk J Biol 2005; 29: 197–202.

24. Öztürk F. Determination of the survival of *Escherichia coli* O157:H7 and *Listeria monocyto*genes adapted to acid and salt stress conditions in Turkish sucuk. PhD, Ankara University, Ankara, Turkey, 2010.

25. Buchanan RL, Edelson RL. pH-dependent stationary-phase acid resistance response of enterohaemorrhagic *Escherichia coli* in the presence of various acidulants. J Food Protect 1999; 62: 211–218.

26. Ryu JH, Deng Y, Beuchat LR. Behavior of acid-adapted and unadapted *Escherichia coli* O157:H7 when exposed to reduced pH achieved with various organic acids. J Food Protect 1999; 62: 451–455.

27. Goodson M, Rowbury RJ. Habituation to normally lethal acidity by prior growth of *Escherichia coli* at a sub-lethal acid pH value. Lett Appl Microbiol 1989; 8: 77–79.

28. Riordan DCR, Duffy G, Sheridan JJ, Whiting RC, Blair JS, McDowell DA. Effects of acid adaptation, product pH, and heating on survival of *Escherichia coli* O157:H7 in pepperoni. Appl Environ Microbiol 2000; 66: 1726–1729.