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CAN ACID ADAPTATION OF *Listeria monocytogenes* INCREASE SURVIVAL IN SUCUK (A TURKISH DRY-FERMENTED SAUSAGE)?

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ABSTRACT

In this research, acid resistance levels of *Listeria monocytogenes* have been examined under the conditions of acid adaptations. In addition, the effect of acid adaptation on the survival of *L. monocytogenes* in sucuk have also been determined. *L. monocytogenes* were adapted to pH 4.5 for the periods of 1, 2, 3 and 4 hours. The survival of *L. monocytogenes* that were adapted to acid have been ascertained at pH 2.5, 3.0 and 3.5 respectively. It has been found that HCl acid adaptations at pH 3.5 have resulted in no increase in the survival of *L. monocytogenes*. A three-hour adaptation process has led to an increase in survival level at pH 2.5 while 1, 2, 3 or 4-hour adaptation processes lead to an increase in survival level at pH 3.0. However, it was found that the survival level of *L. monocytogenes* in sucuk did not increase as a result of acid adaptation procedure. Acid adapted pathogens have many risks for food safety and human health. These pathogens maintain their viability in acidic foods and cause foodborne diseases. Therefore, understanding the mechanisms of acid adaptation of pathogens will help to create more effective food safety systems and will play a role in the prevention of foodborne diseases.

Keywords: Acid adaptation, Inorganic acids, HCl, *Listeria monocytogenes,* Sucuk

Introduction

Listeria monocytogenes is a Gram-positive, facultative anaerobe, non-spore, rod-like bacterial species that causes sporadic or epidemic infections in humans and animals (Ferrari *et al*., 2017; Mikš-Krajnik *et al*., 2017; *Suo et al*., 2018). Contamination occurs by the consumption of infected foodstuffs (Bergholz *et al*., 2018). Although it is rarely seen in healthy individuals, it is an important pathogen for individuals with weak immune system, newborns, elderly and pregnant women. It causes gastroenteritis, septicemia, meningitis, meningoencephalitis, also miscarriages in pregnant women and stillbirths (Drevets and Bronze, 2008; Giaouris et al., 2014; Calvo *et al*., 2016; No *et al*., 2016; Bergholz *et al*., 2018).

L. monocytogenes is an important pathogen in terms of public health, which can spread widely in the environment, develop at refrigerator temperature, maintain its viability even under adverse conditions such as refrigeration, freezing, heating and drying processes (Cacace *et al*., 2010; Hingston *et al*., 2017; Omac *et al*., 2018). It can grow at a temperature range of 1-45 °C, low pH and high salinity (10% NaCl) (Drevets and Bronze, 2008; Gahan and Hill, 2014; Omori *et al*., 2017; Santos *et al*., 2018). The low pH in acidic foods plays an important role in terms of microbial stability and food safety. However, recent studies have shown that *L. monocytogenes* have an increased survival level in foods with low pH due to its acid tolerance mechanism (Gahan *et al*., 1996; Koutsoumanis *et al*., 2003; Chung *et al*., 2018).

The acid tolerance response (ATR) mainly forms as a result of the exposure of certain pathogenic bacteria to moderately acidic (pH 5.0-6.0) environments for a certain period of time, resulting in resistance to high acidic environments by synthesizing acid shock proteins controlled by specific genes (Lou and Yousef, 1997; Giaouris *et al*., 2014; Park *et al*., 2016; Ijabadeniyi and Mnyandu, 2017; Kapetanakou et al., 2017; Santos et al., 2018). It is known that the resistance of *L. monocytogenes* to acidic pH depends on the composition of the growing environment, bacterial strain, the phase of bacterial growth and the acid type used (Phan-Thanh *et al*., 2000; Cataldo *et al*., 2007). Acid-tolerant pathogenic bacteria are of importance in terms of both food industry and human health (Leyer *et al*., 1995; Gahan *et al*., 1996; Cheng *et al*., 2003; Öztürk and Halkman, 2015; Omori *et al*., 2017; Guariglia-Oropeza *et al*., 2018).

The aim of this study was to obtain the acid-tolerated *L. monocytogenes* cells which cause food poisoning and to determine the tolerance of these cells to strong acidic conditions. It was also aimed to investigate the effect of this acid adaptation on the survival level of *L. monocytogenes* in sucuk (A Turkish Dry-Fermented Sausage).

Materials and Methods

Bacterial Cultures

The *L. monocytogenes* ATCC 7644 strain used in the trial was obtained from Ankara University Food Engineering Department's culture collection.

Preparation of Acid-Adapted Listeria monocytogenes Cells

The cells of 15-hour stationary phase of *L. monocytogenes* cells were used in the trials. Accordingly, 5 µL of active *L. monocytogenes* was transferred to 50 mL TSB and incubated for 15 hours at 37 °C. At the end of this period, 9 mL cultures were placed in centrifuge cells and centrifuged at 5000 rpm (Hettich EBA 12; Germany) for 10 minutes. Cell pellets suspended using physiological saline water (PSW) were washed two times more by centrifugation. The pH values of the obtained cell pellets were adjusted to 4.5 using 6 N HCl (37 %; Merck) solution and left to incubate for 1, 2, 3 or 4 hours in 10 mL TSB. As a result, acid-adapted cells were obtained. For the preparation of the nontreated cells, the cell pellet was suspended in 10 mL TSB medium at pH 7.0 (Cheng *et al*., 2003).

Acid Tolerance of L. monocytogenes

To determine the resistance of *L. monocytogenes* cells to strongly acidic conditions, 0.5 mL of acid-adapted and non-treated cells were inoculated in 50 mL TSB with 2.5, 3.0 or 3.5 using HCl and incubated at 37 °C (Cheng *et al*., 2003). At the 0th, 1st, 2nd, 3rd, 4th, and 5th hours of incubation, samples were inoculated in Tryptic Soy Agar (TSA, Merck) by spread plate method, incubated at 37 °C and bacterial count was determined as CFU/mL.

Production of Sucuk

Beef and tail fat (25 %) were chopped and minced through a 3-mm-diameter plate. The minced meat was irradiated at 25 kGy at Turkish Atomic Energy Authority Sarayköy Nuclear Research and Training Center and kept at -20 ± 2 °C until use. The minced meat was thawed the night before the production

of sucuk, and 1.6 % NaCl, 1.2 % garlic, 0.5 % sucrose, 0.5 % bitter red pepper, 0.6 % sweet pepper, 0.6 % black pepper, 0.8 % cumin, 0.04 % $NaNO_3$ and 0.01 % $NaNO_2$ were added (Soyer et al., 2005). Commercial starter culture (*Staphylococcus carnosus*, *Staphylococcus xylosus* ve *Lactobacillus curvatus*) was homogeneously added to the mix using a mixer. Following this stage, two experimental groups were formed. Acid-tolerated *L. monocytogenes* (10^5 CFU/g) was added to the sucuk batter of the first experimental group, while non-acid-tolerated *L. monocytogenes* (10^5 CFU/g) was added to the sucuk batter of the second experimental group (control group). The prepared sucuk batter was kept in the refrigerator (at 4 °C) overnight and the additives were allowed to diffuse to the meat. Sucuk batter was filled in artificial casings in 50-60 g portions using a manual meat mincer. The sucuk samples were ripened in a conditioner room where temperature and moisture can be adjusted automatically at 85-90 % for 3 days at 22 °C, at 80-85 % for 3 days at 22 °C and at 65-70 % for 3 days at 20 °C (Soyer *et al*., 2005) sequentially. Following the ripening process, the sucuk samples were stored at 4 °C.

Bacteriological Analyses of Sucuk

For the bacteriological analyses of sucuk samples, 10 g sample was transferred into stomacher bags containing 90 mL Maximum Recovery Diluent (MRD, Merck) and homogenized in the stomacher (Seward Stomacher®400 Circulator; England) at 235 rpm for 1 minute. Inoculations from appropriate dilutions prepared using 9 mL MRD were carried out using selective media by spread plate method. PALCAM Agar (Merck) was used for the *L. monocytogenes*, and incubated at 37 °C for 48 hours. 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 (Harrigan 1998).

Physical and Chemical Analysis of Sucuk

For pH determination, 100 mL pure water was added to 10 g sucuk sample and homogenized. The pH of the mixture was measured using an Inolab (level 2) pH meter (AOAC 2000). In order to determine the dry matter, approximately 5 g sucuk sample was weighed and dried at 105 °C until a constant weight was obtained (AOAC 2000).

Statistical Analysis

In terms of the studied characteristics (different pH and different adaptation times), the findings were analyzed by repeated measurement ANOVA in factorial order. DUNCAN test was used to determine the different groups. In the variance analysis, CMSTAT package program was used while SPSS 15 package program was used for the DUNCAN tests.

Results and Discussion

Acid Adaptation

The survival rate of *L. monocytogenes* ATCC 7644 strain with acquired tolerance to at pH 4.5 using HCl to pH 2.5 varied depending on the adaptation time. Bacterial counts in the acid-adapted group exhibited a faster decrease trend compared to those in the other nontreated group. It was found that, at the 4th and 5th hour of the incubation, the group with the highest level of tolerance to acid was the experimental group which was adapted to acid for 3 hours. Acid adaptation performed at pH 4.5 for 1, 2, 3 or 4 hours using HCl increased the resistance of *L. monocytogenes* to pH 3.0 ($P<0.05$). Different adaptation times did not have any different effects on the increase in resistance (Table 1, 2 and 3). Acid adaptation performed at pH 4.5 for 1, 2, 3 or 4 hours using HCl caused no increase in the resistance of *L. monocytogene*s to pH 3.5.

The studies conducted in recent years focused on food pathogens such as *L. monocytogenes* have revealed that these bacteria have mechanisms that enable them to adapt to acidic environments (Leyer *et al*., 1995; O'driscoll *et al*., 1996). Koutsoumanis and Sofos (2004), in their study on the survival levels of *E. coli* O157:H7, *L. monocytogenes* and *S. typhimurium* subjected to pH values ranging between 4.0 and 6.0, it was found that, pathogens were protected from lethal acidic conditions with acid adaptation procedure and the acid tolerance varied depending on the species and pH. At pH values between 5.0 and 6.0, acid resistance of *L. monocytogenes* increased, and the highest resistance was determined at pH 5.5. Phan-Thanh *et al*. (2000) adapted the *L. monocytogenes* LO28 strain to HCl for a couple of hours at pH 5.5 before the acid stress. The researchers found that the highest tolerance to pH 3.7 in groups where adaptation was carried out for 2 and 3 hours. The researchers have also reported that acid tolerance decreased when adaptation period was extended to 24 hours. Giaouris *et al*. (2014) have reported an increase was determined in the resistance to lethal acidic (pH 2) conditions of the *L. monocytogenes* Scott A strain, which was acid-

adapted in TSB containing glucose. Koutsoumanis *et al*. (2003) have reported that the acid tolerance of *L. monocytogenes* which was acid-adapted in TSB containing glucose at pH values 5.0, 5.5 and 6.0 for 90 minutes increased, however acid adaptation procedures carried out at pH values 4.0, 4.5 and 7.0 did not cause any increase in acid tolerance. In the present study, acid adaptation performed at pH 4.5 for 1, 2, 3 or 4 hours caused increase in the resistance of *L. monocytogenes* to pH 2.5 and pH 3.0. the highest increase in the survival level at pH 2.5 was determined in the experimental group adapted to acid for 3 hours.

Table 1. The survival level of HCI (pH 4.5) adapted and nontreated *L. monocytogenes* in pH 2.5 (log CFU/mL)

Incubation (h)	Acid-adapted period (h)				Nontreated
	1	2	3	4	
0	$7.02A^{a}\pm0.06$	$7.00A^{a}\pm0.04$	$7.02A^{a}\pm0.14$	$7.05A^{a}\pm0.22$	$7.06A^{a}\pm0.21$
1	$6.21B^{a}\pm0.06$	$6.23B^{a}\pm0.05$	$6.27B^{a}\pm0.07$	$6.10B^{a}\pm0.02$	$5.60B^{a}\pm0.06$
2	$5.78B^{a}\pm0.38$	$5.98B^{a}\pm0.28$	$5.91B^{a}\pm0.17$	$5.95B^{a}\pm0.10$	$4.48C^{b}\pm0.33$
3	$4.03C^{b}\pm0.26$	$4.65C^{ab}\pm0.00$	$5.30C^{a}\pm0.44$	$4.71C^{ab}\pm0.78$	$2.33D^{c}\pm0.10$
4	$3.42D^{bc}\pm0.24$	$3.00D^{c}\pm0.00$	$5.09C^{a}\pm0.44$	$4.08D^{b}\pm0.25$	$<1.00E^{d}$
5	$2.08E^{b}\pm0.25$	$1.95E^{b}\pm0.00$	$3.42D^{a}\pm0.43$	$<1.00E^{c}$	$<1.00E^{c}$

A, B, C (↓); a, b, c (→): The difference between averages having the same letters is not statistically significant (P>0.05).

Table 2. The survival level of HCI (pH 4.5) adapted and nontreated *L. monocytogenes* in pH 3.0 (log CFU/mL)

Incubation (h)	Acid-adapted period (h)				Nontreated
	1	2	3	4	
0	$7.41A^{a}\pm0.05$	$7.23A^{ab}\pm0.19$	$7.06A^{ab}\pm0.10$	$6.93A^{b}\pm0.35$	$7.28A^{ab}\pm0.08$
1	$6.47B^{a}\pm0.13$	$6.32B^{ab}\pm0.02$	$6.28B^{ab}\pm0.00$	$6.06B^{b}\pm0.10$	$6.25B^{ab}\pm0.21$
2	$6.25B^{a}\pm0.02$	$6.23BC^{a}\pm0.05$	$6.22B^{a}\pm0.04$	$6.02B^{ab}\pm0.07$	$5.67C^{b}\pm0.06$
3	$6.19BC^{a}\pm0.01$	$6.15BC^{a}\pm0.04$	$6.16B^{a}\pm0.05$	$5.82B^{a}\pm0.11$	$5.18D^{b}\pm0.03$
4	$6.10BC^{a}\pm0.02$	$6.06BC^{a}\pm0.02$	$6.12B^{a}\pm0.04$	$5.76BC^{a}\pm0.14$	$4.56E^{b}\pm0.15$
5	$5.87C^{a}\pm0.12$	$5.92C^{a}\pm0.04$	$6.02B^{a}\pm0.02$	$5.45C^{a}\pm0.15$	$4.17F^{c}\pm0.38$

A, B, C (↓); a, b, c (→): The difference between averages having the same letters is not statistically significant (P>0.05).

Table 3. The survival level of HCI (pH 4.5) adapted and nontreated *L. monocytogenes* in pH 3.5 (log CFU/mL)

Incubation (h)	Acid-adapted period (h)				Nontreated
	1 saat	2 saat	3 saat	4 saat	
0	7.40	7.21	7.16	6.64	7.31
1	7.25	6.82	6.75	6.35	7.17
2	7.02	6.55	6.27	6.29	7.01
3	6.71	6.40	6.10	6.00	6.74
4	6.25	6.32	6.06	5.85	6.32
5	5.83	6.16	5.43	5.67	6.06
Ort.	6.74A±0.18	6.57A±0.11	6.29A±0.18	6.13A±0.10	6.77A±0.14

A, B, C (↓); a, b, c (→): The difference between averages having the same letters is not statistically significant (P>0.05).

It was stated that the resistance of *L. monocytogenes* to low pH varied depending on the bacterial strain (Phan-Thanh *et al*., 2000). Vialette *et al*. (2003) have reported that the adaptation ability of clinical isolates of *L. monocytogenes* to adverse conditions such as acid and osmotic stress was higher than those isolated from foods. Berk *et al*. (2005) have reported that *S. typhimurium* strains isolated from humans had a higher acid tolerance compared to those of isolated from foods. In addition, numerous studies have reported that the development phase was also effective on acid resistance. In the present study, in which stationary phase cells were used, acid adaptation procedure carried out using HCl at pH 4.5 for 1, 2, 3 or 4 hours lead to an increase in the acid tolerance of *L. monocytogenes* to pH 2.5 and 3.0 whereas it did not cause any increase in survival levels at pH 3.5. Lee *et al*. (1994), on their study on *S. typhimurium*, have reported that the tolerance of stationary phase cells to pH 3.0 were 1000 times higher than those of the logarithmic phase cells. O'Driscoll *et al*. (1996) have reached similar results, reporting that the stationary phase cells of *L. monocytogenes* were naturally resistant to pH changes (pH 3.5 however, for logarithmic phase cells to survive at pH 3.5, they should be acid-adapted to pH 5.5 environment and acid tolerance response should be induced. Similarly, Lou and Yousef (1997) have reported that, as a result of one-hour adaptation at pH 4.5 and 5.0, acid tolerance of logarithmic phase cells of *L. monocytogenes* Scott A strain to pH 3.5 increased.

***L. monocytogenes* Count in Sucuk**

After 3 hours of incubation in a TSB medium adjusted to pH 4.5 using HCl, the acid-adapted *L. monocytogenes* were added to the sucuk batter. *L. monocytogenes* counts in sucuk samples during the ripening and storage period are given in Table 4.

Acid-adapted and nontreated *L. monocytogenes* counts added to sucuk batter decreased during the ripening and storage periods and determined to be 2.89 log CFU/g in the experimental group and <2.00 log CFU/g in the control group at the 40th day of the storage period. However, no significant difference was found between the experimental groups ($P>0.05$). It was determined that the survival rate of *L. monocytogenes* did not increase with acid adaptation in sucuk samples. Similar results were determined by Calicioglu et al. (2002). In their study, beefs inoculated with acid-adapted *L. monocytogenes* strains (LM101, LM103, N7143, N7144, TB2000) were marinated with different solutions and dried at 60 °C for 10 hours. As a result of the study, it has been reported that acid adaptation did not cause an increase in the survival level in *L. monocytogenes*. Gahan *et al*. (1996) determined that the survival levels of lactic acid-adapted *L. monocytogenes* increased in yoghurt and cottage cheese containing lactic acid, orange juice containing citric acid and salad sauce containing acetic acid. However, in foods with higher pH, such as mozzarella cheese and low-fat cheddar cheese, acid adaptation did not increase the survival level. Unlike the results reported in the studies mentioned above, Francis and O'Beirne (2001) have reported that survival level of *L. monocytogenes* (ATCC 19114) which was acid-adapted for 1 hour at TSB medium at pH 5.5 using lactic acid increased in vegetables packaged under modified atmospheric conditions. In another study, it has been stated that the survival level of *L. monocytogenes* Scott A strains acid adapted using TSYB increased in non-neutralized berry juices (pH 3.70-4.89) (Karabiyikli *et al.*, 2017). In the present study, it was determined that the acid tolerance of the *L. monocytogenes* ATCC 7644 increased with acid adaptation in the experiments performed under *in vitro* conditions. However, in the trials performed in sucuk samples, no increase was observed in the survival level values in acid-adapted cells It was thought that this might be due to the combined effect of protective factors including starter culture, low water activity, low pH, nitrite and sodium chloride in fermented sausages (Erol *et al*. 1999; Lindqvist and Lindblad 2009; Kaya and Gökalp, 2004; Kara and Akkaya 2010). In the studies conducted in different food systems, it was determined that the acid tolerance varied depending on the composition of the media. In the study conducted by Gahan *et al*. (1996), acid-adapted *L. monocytogenes* and acid-resistant mutant strains showed higher survival rates in commercial yoghurt and home cheese produced under laboratory conditions. The acid-resistant mutant showed higher resistance during the ripening of hard cheeses such as cheddar cheese, and a high number of cells were recovered after 70 days of ripening. Chung *et al*. (2018) have reported that, with the acid adaptation, the survival rate of *L. monocytogenes* (ATCC 19111, 19115 and 9117) increased in commercial fruit juices, however it was eliminated by adding carvacrol and thymol to the composition of the media. Tchuenchieu (2016) has reported that the acid types used in acidification of fruit juices was effective in the inactivation of *L. monocytogenes* 56 LY cells adapted to citric acid. Malic and hydrochloric acid added fruit juices

were found to have higher inactivation rates compared to citric acid.

LAB and Staphylococcus count in Sucuk

LAB and *Staphylococcus* spp. counts determined in sucuk samples during the ripening and storage are given in Table 5. *L. curvatus* was added to sucuk batter as the starter culture at 6.48-6.45 log CFU/g. LAB counts increased with the onset of fermentation and reached 9.25 log CFU/g in the experimental group and 9.14 log CFU/g in the control group on the 1st day of fermentation ($P>0.05$). After the 8th day, which was the onset of the storage period, LAB counts started to decrease, and determined to be 8.75 and 8.79 log CFU/g in the experimental and the control groups, respectively. It was determined that pH decreased as the LAB count increased, and this interaction was found to be statistically significant ($P<0.05$).

The use of starter culture on *L. monocytogenes* in fermented sucuk is known to be effective. Kaya and Gökalp (2004) showed that the use of starter culture inhibited the development of *L. monocytogenes*. The number of *L. monocytogenes* in sucuk produced without using starter culture increased by 10^3 log CFU/g on the 3rd day of ripening period. In sucuk produced using starter culture, it was stated that in the first three days, LAB number reached 10^9 log CFU/g, pH value decreased below 5.0 and *L. monocytogenes* could not develop. Porto-Fett *et al.* (2008) reported that, fermentation and drying stage of fermented semi-dry sucuk, when pH 5.3 and 4.8, the number of *L. monocytogenes* was decrease 0.07-0.74 log CFU/g. In this study, the number of *L. monocytogenes* continued to decrease from the beginning of ripening period. Erol *et al.* (1999), with the addition of starter cultures producing bacteriocin, the number of *L. monocytogenes* at 10^5 CFU/g decreased to 0.03 EMS/g at the end of the ripening period (14 days), this value decreased 2.4 EMS/g in sucuk samples containing *L. curvatus* strain which not produce bacteriocin. In this study, using the same starter culture, the number of *L. monocytogenes*, which was 5.89-5.69 log CFU/g at the start of fermentation, reached 3.53-2.97 log CFU/g on the 15th day.

Staphylococcus spp. counts determined in sucuk samples during the ripening and storage are given in Table 5. 6.43 log CFU/g and 6.33 log CFU/g of *Staphylococcus* spp. as starter culture were added to the sucuk dough of the experimental and control group, respectively. The difference between the *Staphylococcus* spp. numbers determined in the experimental and control groups was not statistically significant ($P>0.05$). Ensoy (2004) reported that the most commonly used species in fermented meat products in the family of *Micrococcaceae* were *S. carnosus* and *S. xylosus*. It is stated that these starter cultures are used to improve the flavor and color characteristics of the product.

Table 4. The survival level of *L. monocytoges* during ripening and storage periods at 4 °C of the sucuk samples (log CFU/mL)

Days	Acid-adapted	Nontreated
H0*	5.89	5.69
Ripening		
0	5.87	5.14
1	5.78	5.13
2	5.57	4.96
3	5.51	4.94
4	5.43	4.69
6	4.76	4.13
Storage		
8	4.44	3.66
15	3.53	2.97
30	3.37	2.70
40	2.89	<2.00

H0*: Keeping the sucuk batter at 4 °C overnight. Values are the average of 2 replicates. No significant difference was found between the experimental groups ($P>0.05$).

Table 5. The number of LAB and *Staphylococcus* spp. in the sucuk samples inoculated with *L. monocytogenes* cells (log CFU/mL)

	Acid-adapted		Nontreated	
Days	LAB	*Staph.* spp.	LAB	*Staph.*spp.
HO*	6.48	6.43	6.45	6.33
Ripening				
0	6.65	6.39	6.52	6.29
1	9.25	6.69	9.14	6.17
2	9.42	6.09	9.31	6.28
3	9.35	6.16	9.36	6.12
4	9.19	6.09	9.20	5.98
6	9.21	6.69	9.32	6.08
Storage				
8	9.22	6.90	9.12	7.15
15	8.75	6.21	8.79	7.24
30	8.83	5.75	8.57	6.15
40	8.47	5.91	8.33	6.19

H0*: Keeping the sucuk batter at 4 °C overnight. Values are the average of 2 replicates. No significant difference was found between the experimental groups (P>0.05).

The Changes in pH and Moisture Level in Sucuk

In the experimental and control groups, moisture values, which were initially 59.19% and 59.22%, showed a rapid decrease especially during the drying period. (Table 6). On the 8th day, moisture value was determined to be 39.82% in the experimental group and 35.88% in the control group. On the 40th day, moisture value was determined to be 33.78% in the experimental group and 29.42% in the control group. The difference between the moisture levels of the experimental and control groups was statistically not significant (P>0.05). It was determined that there was a positive interaction between pH value and moisture level, the moisture decreased with the decrease in pH (P<0.05). It was seen that the survival level of *L. monocytogenes* decreased as the moisture level decreased (P<0.05). Kaya and Gökalp (2004) reported that while the moisture content of the sucuk produced by using starter culture was between 59.38% and 60.11% at the beginning of ripening period, this value was between 36.72- 37.34% on the 12th day of ripening period. Dalmış ve Soyer (2008) stated that, the moisture content of starter cultured sucuk was 60.12% at the beginning of ripening period this value was decreased to 39.5% on the 9th day of ripening period. In this study, while moisture value was measured 59.19-59.22% in beginning of ripening period; this value was measured 39.82-35.88% on the 8th day of ripening period.

It was observed that the pH of the experimental group decreased to 5.78 in the sucuk batter and while it decreased to 5.75 in the sucuk batter in the control group with the onset of fermentation. Changes were determined in pH values during the ripening and storage periods, however no significant difference was found between the experimental groups (Table 6). Similar to this study, the decrease in pH due to lactic acid bacteria which increased during the fermentation process was also determined by many researchers. Hampikyan and Uğur (2007) stated that the initial pH value in fermented sucuk was 5.87-5.90 and the pH reached 4.72-4.82 on the 30th day of the ripening. According to Yıldız-Turp and Serdaroğlu (2008), the pH value of fermented sucuk with an initial pH of 5.49-5.59 reached 4.60-4.82 on the 12th day of ripening period. In the study conducted by Erkmen (2009), it was stated that in the sucuk produced using starter culture, during the fermentation, pH decreased rapidly and reached the lowest level on day 3 (pH 4.82-4.92).

Table 6. pH values and moisture levels (%) of the sucuk samples inoculated with *L. monocytogenes*

	Acid-adapted		Nontreated	
Days	pH	% Moisture	pH	% Moisture
HO*	5.78	59.19	5.75	59.22
Ripening				
0	5.80	57.15	5.75	58.62
1	4.65	56.11	4.62	58.51
2	4.42	54.35	4.39	54.00
3	4.56	50.21	4.59	50.47
4	4.54	48.20	4.52	49.19
6	4.48	43.01	4.44	38.49
Storage				
8	4.61	39.82	4.59	35.88
15	4.74	35.37	4.60	31.04
30	4.76	34.39	4.72	30.06
40	4.74	33.78	4.80	29.42

H0*: Keeping the sucuk batter at 4 °C overnight. Values are the average of 2 replicates. No significant difference was found between the experimental groups (P>0.05).

Conclusion

In this study, it was determined that *L. monocytogenes* ATCC 6644 was adapted to acid by exposure to moderately acidic (pH 4.5) conditions and can survive at certain levels in highly acidic environments which are lethal for the bacteria without acid adaptation. However, acid adaptation did not cause an increase in the *L. monocytogenes* counts in sucuk. Acid adapted pathogens, such as *L. monocytogenes*, pose a risk to food safety and human health. These pathogens taken through the food have resistance to gastric acidity. The virulence of these pathogens increases and the infective doses decrease. They also gain resistance to other environmental stresses such as high temperature, salinity, cold storage and freezing-thawing. Therefore, it is necessary to reconsider the preservation methods used in the food industry.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

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