

**SURVIVAL OF ACID ADAPTED AND NON-ADAPTED STATIONARY PHASE  
*ESCHERICHIA COLI*O157:H7, *SALMONELLA* TYPHIMURIUM AND  
*LISTERIA MONOCYTOGENES* IN POMEGRANATE JUICE**

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**ABSTRACT**

The purpose of this study was to evaluate the survival of acid adapted and non-adapted stationary phase *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in pomegranate juice at refrigeration and room temperatures. Inoculated juice samples were stored at 4 and 24 ± 2°C. Population of pathogens were enumerated for up to 28 days. Reduction rates, time required for one log reduction, of tested microorganisms were calculated. Enrichment was negative for *E. coli* O157:H7 after a week at all tested conditions. At refrigeration temperature, acid adapted *S. Typhimurium* and *L. monocytogenes* survived one week longer than non-adapted cells. At room temperature, *S. Typhimurium* population decreased up to 7 log CFU/ml in 28 days; *L. monocytogenes* survived less than 12 h. Rates of reduction (days) ranged between 0.06 and 4.29 for all tested pathogens ( $P < 0.05$ ). Tested pathogens can survive in contaminated pomegranate juice until consumption to cause foodborne illness.

**Keywords:** Acid adaptation, survival, pomegranate juice, *Escherichia coli*, *Salmonella*, *Listeria*

**ASİDE ADAPTE VE ADAPTE OLMAYAN DURAĞAN FAZ *ESCHERICHIA COLI* O157:H7, *SALMONELLA* TYPHIMURIUM VE *LISTERIA MONOCYTOGENES*'İN NAR SUYUNDAKİ CANLI KALMA SÜRELERİNİN BELİRLENMESİ**

**ÖZ**

Bu çalışmanın amacı, aside adapte olan ve adapte olmayan durağan faz *Escherichia coli* O157:H7, *Salmonella* Typhimurium ve *Listeria monocytogenes*'in buzdolabı ve oda sıcaklıklarında canlı kalma sürelerini değerlendirmektir. İnoküle edilmiş meyve suyu örnekleri 4 ve 24 ± 2 ° C'de muhafaza edilmiştir. Patojenlerin popülasyonları 28'inci güne kadar sayılmıştır. Popülasyon indirgeme oranları, bir log indirgeme için gereken süre, test edilen mikroorganizmalar için hesaplanmıştır. Bir hafta sonunda *E. coli* O157:H7 için zenginleştirme bütün test edilen koşullarda negatif olarak belirlenmiştir. Buzdolabı sıcaklığında, aside adapte *S. Typhimurium* ve *L. monocytogenes*, adapte olmayan hücrelere göre 1 hafta daha uzun süre hayatta kalmıştır. Oda sıcaklığında, *S. Typhimurium* popülasyonu 28 günde 7 log CFU/ml düşmüştür ama *L. monocytogenes* sadece 12 saat canlı kalabilmiştir. Popülasyon azalma oran aralıkları (gün) bütün test edilen patojenler için 0.06-4.29 aralığındadır ( $P < 0.05$ ). Test edilen patojenler kontamine olmuş nar suyunda gıda kaynaklı hastalığa neden olacak kadar uzun süre hayatta kalabilir.

**Anahtar kelimeler:** Asit adaptasyonu, canlı kalma, nar suyu, *Escherichia coli*, *Salmonella*, *Listeria*

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## INTRODUCTION

Fruit juices are important global commodities with consumer profiles from all age groups. Microorganisms including yeasts, molds and acid tolerant bacteria species can cause spoilage of fruit juices. Fruit juices may harbor foodborne pathogens. Outbreaks of *Cryptosporidium parvum*, shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp. associated with the consumption of unpasteurized fruit juices occurred in the United States (U.S.) between 1995 and 2012 (Centers for Disease Control and Prevention; CDC, 2014; Danyluk et al., 2012; Vojdani et al., 2008). No outbreak of *Listeria* species was implicated for fruit juice related outbreaks; however, *Listeria monocytogenes* is considered as a risk of contamination in fruit juice production due to its ubiquitous nature (FDA, 2004; Vojdani et al., 2008). Pathogens are commonly eliminated by thermal pasteurization in fruit juices to comply with regulations. However, freshly squeezed juices remain as a potential source of foodborne diseases due to no inactivation process prior to consumption.

Pomegranate (*Punica granatum* L.) juice consumption have increased during last decades due to increasing popularity of health promoting potential of fruit and its juice (Türkyılmaz et al., 2013). The several health benefits of pomegranate have been claimed on various health problems including high cholesterol, inflammation, cardiovascular diseases and different type of cancer (Zarfeshany et al., 2014). High anthocyanins content of pomegranate juice provides its characteristic red color that invites consumers' notice as first glimpse on market shelves (Nachay, 2009; Vegara et al., 2013). During thermal pasteurization and storage of juice, bright color of juice turns to a brownish color that is reducing sensory and nutritive properties of juice due to degradation of unstable anthocyanins molecules (Vegara et al., 2013). Brightness and fruity properties of fresh squeezed pomegranate juice attracts the interest of consumers. However, consumption of fresh squeezed juice increases concerns about foodborne pathogens due to risk of contamination.

Pathogens can survive for extended periods of time to cause gastrointestinal diseases in fruit juices (Piotrowski, 2003; Parish and Higgins, 1989; Oyarzabal et al., 2006). Survival of bacterial foodborne pathogen in fruit juice and juice concentrates have been studied at different storage temperatures. Duration of survival varies depending on type and composition of juice, storage temperature, and strains of pathogenic bacterial species (Álvarez-Ordóñez, 2013; Oyarzabal et al., 2006; Parish and Higgins, 1989; Piotrowski, 2003). Some foodborne pathogens, including STEC, *Salmonella* spp., and *L. monocytogenes* have acid adaptation mechanisms that increase their survival and thermal tolerance capability in acidic conditions. Acid adaptation increases time and temperature to inactivate pathogens during pasteurization (Mazzotta, 2001; Ryu and Beuchat, 1998; Sharma et al., 2005; Topalcengiz and Danyluk, 2017).

Several numbers of studies have been published about the survival of pathogens in fruit juices including grape, orange, apple juice and apple cider. Pathogens can survive long enough to cause outbreaks in apple and orange juices. The objective of this study is to determine the survival of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* that are considered a potential cause of outbreak in pomegranate juice. Results reported in here provides information about the risk of outbreak associated with pomegranate juice in case of inadequate pasteurization and the consumption of unpasteurized juice.

## MATERIALS AND METHODS

### Juice

One brand of 100% pasteurized pomegranate juice with a single lot number was bought from a local market. The pH and soluble solid content (°Brix) of pomegranate juice was measured with a pH meter (HACH, HQ30d Portable Multi Meter, CO, USA) and refractometer (Index Instrument, PTR 2a, USA). Fruit juice was stored at -20 °C until use.

### Bacterial strains and growing conditions

One strain of *E. coli* O157:H7 (ZI:10; food isolate), *S. Typhimurium* (ATCC 14028; orange juice outbreaks of 1999) and *L. monocytogenes*

serotype 4b strains (ATCC 19115) were used. Frozen (-80 °C) *E. coli* O157:H7 and *S. Typhimurium* strains were streaked onto tryptic soy agar (TSA; Biolife; Milan, Italy) plates to obtain working cultures. Frozen *L. monocytogenes* was converted to working culture by plating on TSA supplemented with 0.6% yeast extract (TSAY; Biolife; Milan, Italy). All plates were incubated at  $37 \pm 2$  °C for  $24 \pm 2$  h. A colony of active strains were inoculated in 10 ml of tryptic soy broth without glucose (TSBNG; Neogen; Lanchashire, UK) and in TSB containing 1% glucose (Merck KGaA, Darmstadt, Germany: TSBG: 10 g/L) to grow non-adapted and acid-adapted cells, respectively. All media used for *L. monocytogenes* was supplemented with 0.6% yeast extract (Biolife; Milan, Italy) as described in Bacteriological Analytical Method by Food and Drug Administration (Hitchins et al., 2017). Fermentation of glucose in TSBG decreased the pH of media causing the development of acid adaptation ( $P < 0.05$ ) (Table 1) (Buchanan and Edelson, 1996). After incubation at  $37 \pm 2$  °C for  $18 \pm 2$  h, 100 µl of grown cultures were transferred to another sets of TSBNG and TSBG tubes. All tubes were incubated at  $37 \pm 2$  °C for  $18 \pm 2$  h. After incubation, cells were washed three times by centrifugation at  $3030 \times g$  for 10 min (Thermo Scientific, Labofuge 200 Benchtop Centrifuge, Germany) followed by the replacement of supernatant with 10 ml of 1% peptone water (Biolife; Milan, Italy). To prepare inoculum, washed pellets were resuspended in 5 ml pomegranate juice to obtain an initial concentration between  $10^8 - 10^{10}$  CFU/ml.

Table 1. pH values of tryptic soy broth with no glucose (TSB-NG) and tryptic soy broth containing 1% glucose (TSBG) before harvesting grown strains (n=3).

Strain or serotype	Media	pH
<i>E. coli</i> O157:H7 (ZT10)	TSB-NG	$7.02 \pm 0.01a$
	TSBG	$4.82 \pm 0.03b$
<i>S. Typhimurium</i> (ATCC 14028)	TSB-NG	$7.13 \pm 0.02a$
	TSBG	$4.95 \pm 0.04b$
<i>L. monocytogenes</i> (ATCC 19115)	*TSB-NG	$6.63 \pm 0.02a$
	*TSBG	$4.38 \pm 0.03b$

The same letter within each pathogen are not significantly different ( $P > 0.05$ ).

\*Supplemented with 0.6% yeast extract.

### Inoculation of pomegranate juices and enumeration

Fruit juice (10 ml) was dispensed in sterile 15 ml centrifuge tubes (LABSOLUTE®, Germany), aseptically. A separate set of tubes was prepared for each sampling time to minimize the chance of contamination. Tubes were acclimated to storage temperatures ( $4$  and  $24 \pm 2$  °C) 12 hours before inoculation. Inocula (0.1 ml) was added to 10 ml juice and vortexed to achieve a final concentration of  $10^6$  CFU/ml. The tubes were tightly capped and stored at  $4$  and  $24 \pm 2$  °C for 28 days. Population of pathogens from each adaptation and temperature combinations was enumerated at 0, 2, 4, 8, 12, and 24 h and on days 2, 3, 5, 7, 14, 21, and 28. Inoculated fruit juice samples stored at refrigeration and room temperatures were sampled and diluted if necessary for spread plating on TSA for *E. coli* O157:H7 and *S. Typhimurium* and on TSAY for *L. monocytogenes* in duplicate. All plates were incubated at  $37 \pm 2$  °C for  $24 \pm 2$  h for *E. coli* O157:H7 and *S. Typhimurium* and at  $37 \pm 2$  °C for  $48 \pm 2$  h for *L. monocytogenes*. If populations were expected to be below 1.0 log CFU/ml (10 CFU/ml), one ml of undiluted sample was spread plated onto four plates as 250 µl per plate to lower limit of detection. Three repetitions were performed for each temperature and adaptation condition (n=3). Background microbiota of pomegranate juice samples were tested by spreading uninoculated juice samples on TSA.

### Enrichment

When no plate counting ranges were predicted, each replicate of juice samples was enriched. 5 ml of TSB for *E. coli* O157:H7, lactose broth (Merck KGaA, Darmstadt, Germany) for *Salmonella*, and TSB supplemented with 0.6% yeast extract (TSBY) for *L. monocytogenes* was added on remaining juice samples in centrifuge tubes. After incubation at  $37 \pm 1$  °C for  $24 \pm 1$  h, one loopful (10 µl) of enriched samples was streaked onto sorbitol MacConkey agar (SMAC; Biolife; Milan, Italy) for *E. coli* O157:H7, xylose lysine desoxycholate agar (XLD; Biolife; Milan, Italy) for *S. Typhimurium*, and *Listeria* oxford agar (LOX; Liofilchem, Abruzzo, Italy) for *L. monocytogenes*. When typical colonies were

observed, the population of pathogen in each replicate was recorded as 0.3 log CFU/ml (limit of detection).

### Statistical analysis

Log transformed population of tested pathogens were plotted against time. Time required for one log CFU/ml reduction (rates of reduction) were calculated by drawing linear trendlines in survival plots from day zero to first time point that cell population decreased more than 5-log for all microorganisms. Populations of cells for each condition were compared for each time point statistically by Analysis of variance (ANOVA) and Tukey's HSD test. The statistical difference among decline rates were calculated with Analysis of Covariance (ANCOVA). All data was processed using Microsoft Excel, 2013 and JMP 11 software (SAS® Institute Inc., Cary, NC, USA, 2013). The alpha value was set at 0.05.

## RESULTS

No background microbiota was detected in pomegranate juice used in this study. The pH and soluble solid content (corrected °Brix) of pomegranate juice was  $3.23 \pm 0.02$  and 16.1, respectively. Strains grown in TSB without glucose yielded a final pH close to neutral ranging from 6.63 and 7.13 (Table 1). The presence of 1% glucose decreased the pH of growth medium to mild acidic conditions (4.38 – 4.95) where acid adaptation occurs. The growth *L. monocytogenes* in TSBY reduced pH values 0.4 lower compare to *E. coli* O157:H7 and *S. Typhimurium* possible due to the addition of yeast extract to support the growth.

### Survival of *E. coli* O157:H7

The population of non-adapted and acid adapted *E. coli* O157:H7 decreased around 1.4 and 2.6 log CFU/ml after one day of storage at 4°C ( $P < 0.05$ ) (Fig 1A). The populations of acid adapted cell were up to 1.5 log CFU/ml lower than non-adapted cells starting from Day 1 ( $P > 0.05$ ). A gradual decline over 6 log CFU/ml was observed until Day 7 at 4°C. No positive enrichment was detected after Day 5 except for non-adapted cells sampled at Day 21 (Fig 1A). At 24°C, similar survival trends were observed for both non-

adapted and acid adapted cells (Fig 1B). Population of acid adapted *E. coli* O157:H7 was up to 1.0 log CFU/ml higher than non-adapted cells at all sampling times until Day 7. Both non-adapted and acid adapted cell populations depleted at Day 7 at 24°C with the exception of non-adapted cells at Day 14. No significant difference was calculated between non-adapted and acid adapted cells stored at 4 and 24°C after Day 7 ( $P > 0.05$ ). Population of non-adapted and acid adapted *E. coli* O157:H7 was higher in samples stored at 4°C at Day 1, 2 and 3 ( $P < 0.05$ ).

### Survival of *S. Typhimurium*

No increase in *S. Typhimurium* populations occurred at all tested conditions and storage temperatures after incubation of inoculated juice samples. Limited decline (up to 1.0 log CFU/ml) was observed in both non-adapted and acid adapted *S. Typhimurium* population in pomegranate juice samples after 24 h storage at 4°C (Fig 2A). The population of acid adapted *S. Typhimurium* was higher than non-adapted cells at sampling Day 2, 3, 5, 7, and 14 at 4°C ( $P < 0.05$ ). Acid adaptation increased survival of *S. Typhimurium* at 4°C. No positive enrichment was detected starting from Day 14 for non-adapted cells and Day 21 for acid adapted cell. At 24°C, both non-adapted and acid adapted cells showed similar survival trends (Fig 2B). However, population of non-adapted cells fluctuated after Day 7 compare to acid adapted cells with a gradual decrease until Day 21 ( $P < 0.05$ ). The population of non-adapted *S. Typhimurium* was lower than acid adapted cells from 12 h to 21 days of storage at 24°C until Day 14 ( $P < 0.05$ ). Non-adapted and acid adapted *S. Typhimurium* stored at 24°C survived two week and one week longer than cells kept at 4°C, respectively.

### Survival of *L. monocytogenes*

Storage temperature affected the survival of both non-adapted and acid adapted *L. monocytogenes* in pomegranate juice, dramatically. The population of non-adapted and acid adapted *L. monocytogenes* decreased around 2.0 log CFU/ml at 4°C after 24 h storage, but no positive samples were detected at 24°C in samples enumerated at 12 h (Fig 3A). At 4°C, similar but statistically significant survival

trends were observed for both non-adapted and acid adapted cells until Day 14 ( $P < 0.05$ ) (Fig 3B). Acid adaptation extended the survival of cells stored at 4°C for three weeks compare to two weeks of non-adapted survival. A gradual decline of acid adapted *L. monocytogenes* were observed for samples held at 4°C. After storage at 24°C for 12

h, no survived cells were recovered for both non and acid adapted samples. In two hours, *L. monocytogenes* population decreased about 50% compare to initial concentration. The population of non-adapted and acid adapted *L. monocytogenes* stored at 4°C was higher than cells kept at 24°C starting from 2 hours ( $P < 0.05$ ).

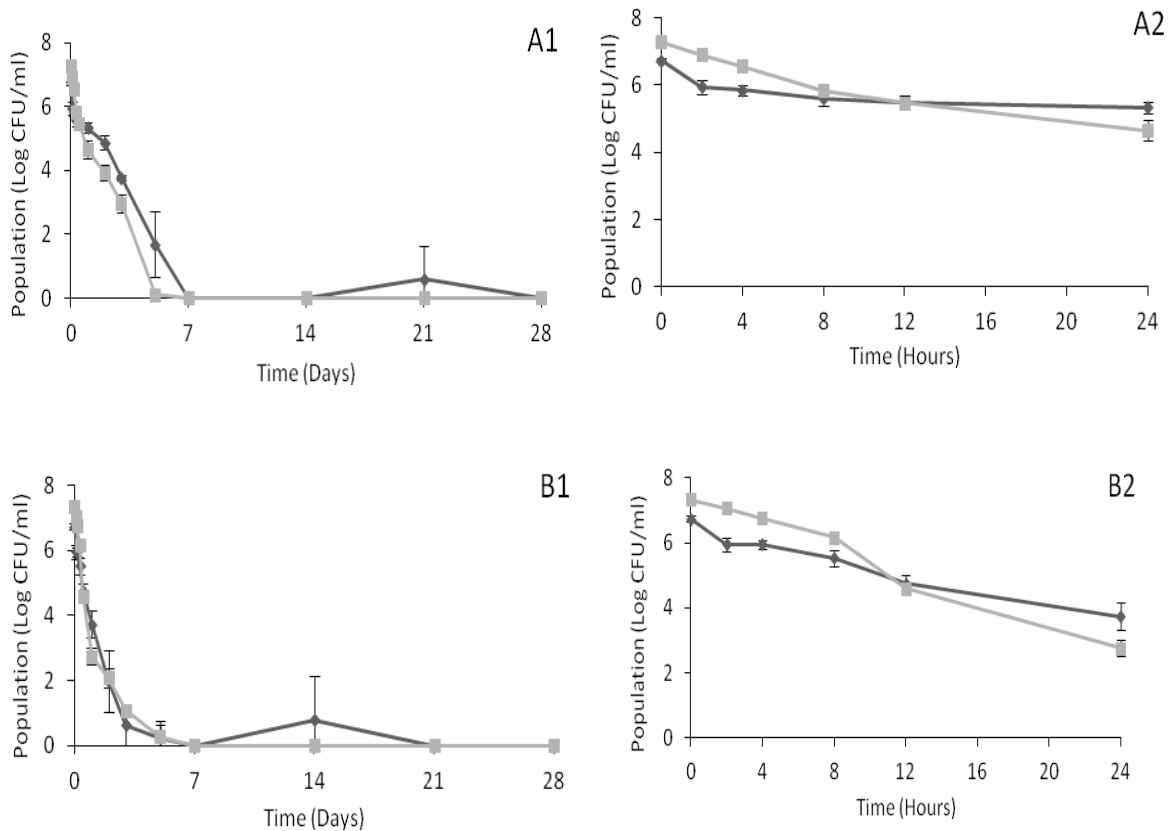
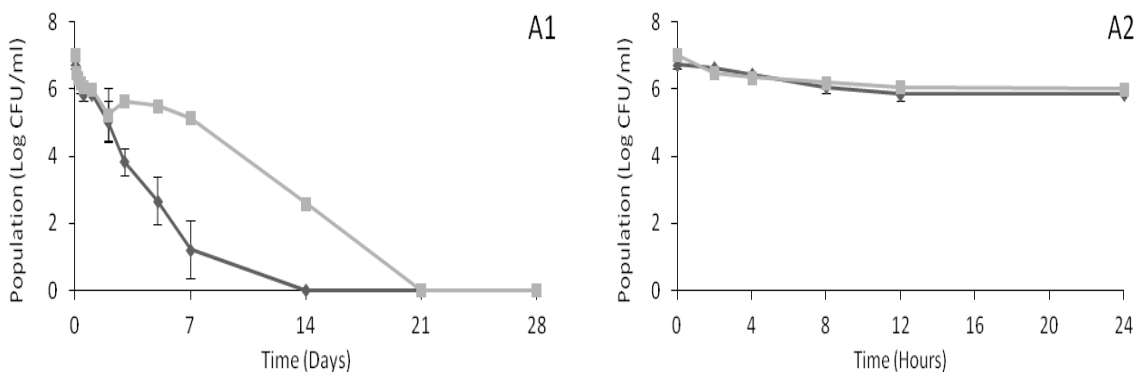


Figure 1. Survival of non-adapted (◆) and acid adapted (■) *E. coli* O157:H7 in pomegranate juice at (A)  $4 \pm 2$  °C and (B)  $24 \pm 2$  °C ( $n=3$ ). (1) All sampling times. (2) Sampling times for first 24 h.



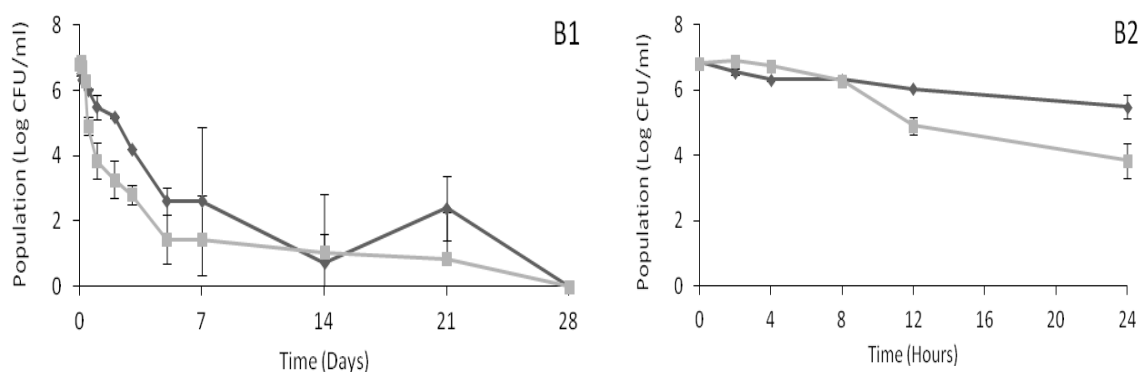


Figure 2. Survival of non-adapted (◆) and acid adapted (■) *Salmonella Typhimurium* in pomegranate juice at (A)  $4 \pm 2$  °C and (B)  $24 \pm 2$  °C (n=3). (1) All sampling times. (2) Sampling times for first 24 h.

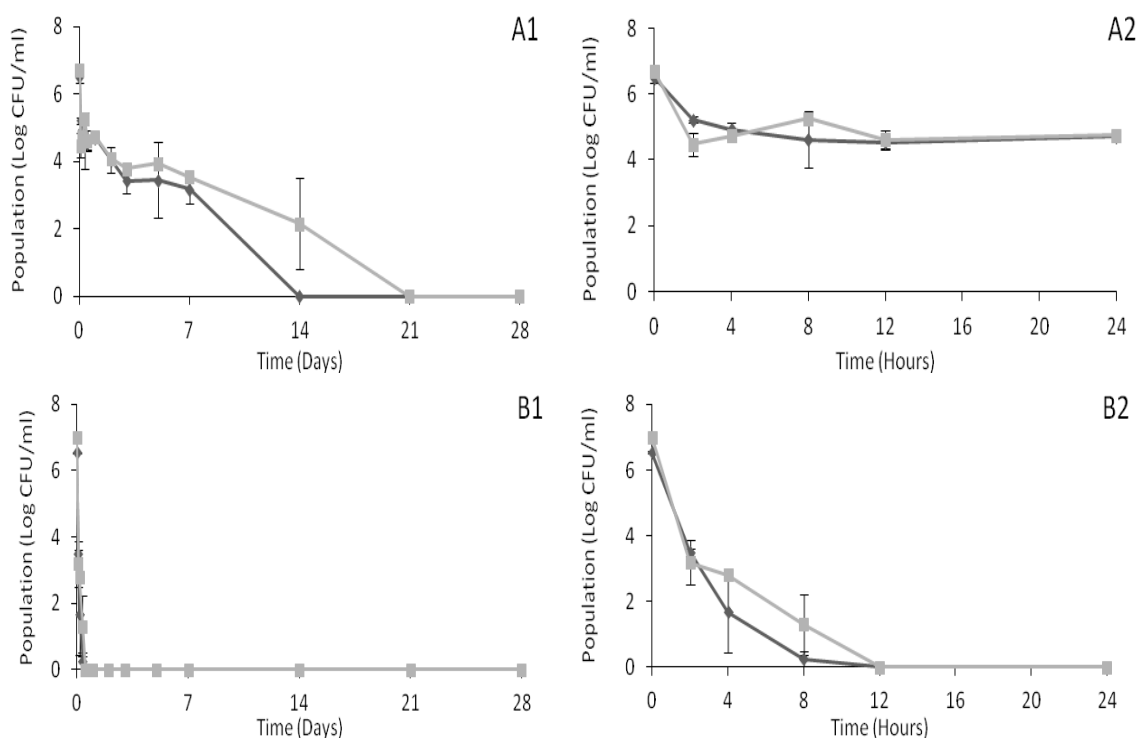


Figure 3. Survival of non-adapted (◆) and acid adapted (■) *Listeria monocytogenes* in pomegranate juice at (A)  $4 \pm 2$  °C and (B)  $24 \pm 2$  °C (n=3). (1) All sampling times. (2) Sampling times for first 24 h.

### Reduction rates of tested strains

All reduction rates are shown with  $R^2$  of linear regression equations in Table 2.  $R^2$  values calculated for linear reduction equations were between 0.79 and 0.99 for all tested pathogens at all conditions. Rate of reductions, time required for one log reduction for each pathogen population, were higher for cells stored at 4°C for all tested microorganisms ( $P < 0.05$ ) except for

non-adapted *S. Typhimurium*. The longest and shortest rate of reduction for *E. coli* O157:H7 were 1.17 days for non-adapted cells stored at 4°C and 0.36 days for acid adapted samples kept at room temperature. *S. Typhimurium* had longer decline rates than *E. coli* O157:H7 at all conditions and *L. monocytogenes* for samples stored at 24°C ( $P < 0.05$ ). Calculated reduction rates of *S. Typhimurium* were 3.43 days for samples stored

at 4°C and 0.91 days for cells held at 24°C ( $P < 0.05$ ). Reduction rates of *L. monocytogenes* were the lowest among all tested pathogens with values less than 2 h for both non-adapted and acid

adapted cells stored at 24 °C. *L. monocytogenes* had the longest reduction rates for samples kept at 4°C with a value of 2.78 days for non-adapted cells and 4.29 days for acid adapted cells ( $P < 0.05$ ).

Table 2. Rates of reduction for each pathogen and temperature obtained from linear decline equations after initial 5-log reduction.

Pathogen	Adaptation	Temperature	Days used in equation	Equation	R <sup>2</sup>	Reduction rates for 1 log (Day)
<i>E. coli</i> O157:H7	Non	4 °C	5	$y = -0.8587x + 6.1813$	0.9569	1.17 <sup>aD</sup>
		24 °C	3	$y = -1.9597x + 6.1442$	0.9714	0.51 <sup>cC</sup>
	Acid	4 °C	5	$y = -1.3131x + 6.622$	0.9639	0.76 <sup>bE</sup>
		24 °C	2	$y = -2.7949x + 6.8821$	0.8625	0.36 <sup>dD</sup>
<i>S. Typhimurium</i>	Non	4 °C	7	$y = -0.7719x + 6.5037$	0.989	1.30 <sup>cD</sup>
		24 °C	14	$y = -0.4459x + 6.1503$	0.9051	2.24 <sup>bA</sup>
	Acid	4 °C	21	$y = -0.2917x + 6.5008$	0.959	3.43 <sup>aB</sup>
		24 °C	5	$y = -1.1015x + 6.2539$	0.8512	0.91 <sup>dB</sup>
<i>L. monocytogenes</i>	Non	4 °C	14	$y = -0.3599x + 5.138$	0.8845	2.78 <sup>bC</sup>
		24 °C	0.33	$y = -17.921x + 5.6075$	0.8857	0.06 <sup>cE</sup>
	Acid	4 °C	21	$y = -0.2333x + 5.0553$	0.8663	4.29 <sup>aA</sup>
		24 °C	0.33	$y = -15.072x + 5.7686$	0.7846	0.07 <sup>cE</sup>

Reduction rates with the same letter within each pathogen are not significantly different ( $P > 0.05$ ).

Reduction rates with the same capitalized letter in within each temperature are not significantly different ( $P > 0.05$ ).

## DISCUSSION

Due to high acidity of fruit juices, food borne pathogens were not considered as a threat to public health until first confirmed outbreak of *E. coli* O157:H7 associated with unpasteurized apple cider (Besser et al., 1993). The U.S. Food and Drug Administration (FDA) requires mandatory 5-log reduction of pertinent microorganisms in fruit juices under HACCP (Hazard Analysis Critical Control Points) rule (FDA, 2001). No outbreak related to the consumption of pasteurized fruit juices have occurred after mandatory HACCP in juice processing and packaging plants, however, unpasteurized fruit juices have been implicated as the source of foodborne outbreaks. Fruit juices can be contaminated by the use of dropped fruit, non-potable water, and the presence of cattle and wildlife in, or close to, the production or processing environment (Harris et al., 2003). Freshly squeezed juices can also be a cause of outbreak due to contaminated utensils and fruit itself or food handlers since pathogens can survive in fruit juice long enough to cause a food borne outbreak.

Various factors may influence the survival of microorganisms in fruit juices including the types of juices, strains of pathogens and storage temperatures. In this study, *E. coli* O157:H7 survived 5 (acid adapted cells) to 21 days (non-adapted cells), but 5 log CFU/ml reduction of *E. coli* O157:H7 took maximum 6 days in pomegranate juice. Similar or contradictory results have been reported about the survival of *E. coli* O157:H7 in various type of fruit juices. The population of *E. coli* O157:H7 declined 4 to 5 log CFU/ml in apple juice at 4 and 21°C after 7 days (Uljas and Ingham, 1998), 5 to 6 log CFU/ml at 4 and 10°C after 21 days in apple cider (Roering et al., 1999), and up to 7 log CFU/ml after 36 days of storage at 4°C and 25°C in apple cider, respectively (Zhao et al., 1993). Also, no positive *E. coli* O157:H7 were detected by Leyer et al. (1995) in apple cider after 28 h storage at 6°C. *S. Typhimurium* survived up to 14 and 21 days at 4 and 24°C in pomegranate juice with 6 to 7 log CFU/ml reduction in population, respectively. In apple juice, *S. Typhimurium* population decreased around 4 logs at 4 and 10°C after 21 days in apple cider (Roering et al., 1999). In contrast, the

population of *S. Typhimurium* dropped around 5 log CFU/ml in 10 days at 4°C and 1 day at 25°C (Álvarez-Ordóñez et al., 2013). Greater and shorter survival of *Salmonella* in apple and orange juice compare to pomegranate juice can be explained different pH and organic acid content and juice compositions.

Population of non-adapted and acid adapted *L. monocytogenes* in pomegranate juice decreased over 6.5 log CFU/ml until Day 14 and 21 at 4°C, respectively. Similar to results in this study, Piotrowski (2003) found that *L. monocytogenes* population decreased over 6.5 log CFU/ml in 12 days in white grape juice, in 14 days in apple cider, in 54 days in orange juice and in 12 hours in red grape juice at 4°C. Results in pomegranate juice is also in contrast to Roering et al. (1999) that reported 5 to 6 log CFU/ml reduction of *L. monocytogenes* in two days in apple cider held at 4°C. No positive sample was detected in any replicate stored at 24°C after 12 h storage. Karabiyikli et al. (2014) observed that decline in the population of *L. monocytogenes* was over 5 log CFU/ml after 3 h storage at 37°C in sour orange juice. This rapid decrease at room and body temperatures may be explained the increased inactivation effect of antimicrobial compounds in pomegranate and sour orange juice at higher temperatures. In general, *L. monocytogenes* in fruit juices is expected survive longer at refrigeration temperatures compare to room temperature similar to *E. coli* and *Salmonella*. However, the population of *L. monocytogenes* decreased up to 2 logs in tomato juice held at 5 and 30°C after 12 days of storage (Diakogiannis et al., 2017). Limited effect of temperature difference on the survival of *L. monocytogenes* in tomato juice is probably due to the higher pH of tomato juice above 4.0.

The addition of 1% glucose decreased the pH of growth medium to mild acidic conditions where strains could develop acid adaptation by fermentation of glucose as previously described (Buchanan and Edelson, 1996; Sharma et al., 2005). Acid adaptation prolonged the survival of *S. Typhimurium* and *L. monocytogenes* at 4°C. Álvarez-Ordóñez et al. (2013) reported that acid adaptation decreased survival of *S. Typhimurium*

at 4 and 10°C, but increased at 25 and 37°C in orange juice. Especially, acid adaptation increased survival ability of *L. monocytogenes* in pomegranate juice dramatically at 4°C ( $P<0.05$ ). No improvement in survival was observed at 24 °C for all tested pathogens after acid adaptation. Thermal response of pathogens increased in fruit juices after acid adaptation and *Salmonella* spp. were reported with the lowest heat resistance in different type of juices at all tested conditions (Mazzotta, 2001; Sharma et al., 2005; Topalcengiz and Danyluk, 2017). In the same studies, shiga toxin-producing *E. coli* (STEC) was determined as the most heat resistant at lower temperatures, but *L. monocytogenes* at higher temperatures. Compare to thermal inactivation studies, *Salmonella* survived longer than *E. coli* O157:H7 and *L. monocytogenes* in pomegranate juice at room and refrigerator temperatures.

The survival of pathogens in pomegranate juice was affected by storage temperatures. Acid adaptation decreased reduction rates for *E. coli* O157:H7; however, acid adapted *Salmonella* and *L. monocytogenes* had longer rates compare to non-adapted cells at both tested temperatures. Except for non-adapted *Salmonella* held at 4°C, all tested non-adapted and acid adapted pathogens stored at 4°C had longer rate of reductions. Especially, reduction rates of *L. monocytogenes* stored at 4°C were up to 70 times longer than cells kept at 24°C. Similar results from previous studies confirms that pathogens survive longer in different fruit juices at near refrigeration storage temperatures (Piotrowski, 2003; Williams et al., 2005; Uljas and Ingham, 1998). Antimicrobial activity of pomegranate fruit has been studied by several researchers. Extracts and compounds purified from different part of pomegranate has antibacterial, antifungal and antiviral activities (Jayaprakasha et al., 2006). The enhanced antimicrobial activity and acidity may be the reason for shorter reduction rates of pathogens stored at higher temperatures.

## CONCLUSION

Pathogens associated with apple and orange juice outbreaks may cause gastrointestinal diseases due to the consumption of pomegranate juice if



pasteurization is performed under required limits. Pasteurization parameters for pomegranate juice should be determined for better understanding of antimicrobial effect. Freshly squeezed pomegranate juice has a potential of contamination since no inactivation process is applied. *E. coli* O157:H7, *S. Typhimurium* and *L. monocytogenes* cannot proliferate in pomegranate juice in refrigeration and room temperatures. However, these pathogens can survive long enough if contamination occurs. Pomegranate fruits can be washed to reduce risk of contamination before freshly squeezed juices are prepared.

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