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Full Length Research Paper

The survival of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in black mulberry (*Morus nigra*) juice

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Black mulberry juice gains popularity based on the high antioxidant content of it. However, acid adapted pathogens in fresh fruit juice are strong threats for food safety. The survival and growth patterns of *Escherichia coli* O157:H7 (932) and *Salmonella* Typhimurium (NRRL B 4420) in black mulberry (*Morus nigra*) juice were determined in this study. The mulberry juices were differently concentrated (100, 50, 10 and 1%) and have different pH values (3.70, 4.34, 4.80, 4.89 and 7.00) were used as test medium. Acid adapted and also non acid adapted *E. coli* O157:H7 and *S.* Typhimurium were used as test microorganisms. All the juice samples were inoculated (6 log-cfu/ml) with the test cultures separately and incubated at two different temperatures (4 and 37°C) during 7 days. The survival and growth pattern of the test cultures were periodically determined during incubation by enumeration. It was detected that the test results of all the test microorganisms were similar at 37°C. However, *S.* Typhimurium was more resistant than *E. coli* O157:H7 at 4°C. None of the microorganisms (acid adapted and non acid adapted) were detected at the end of 7 days incubation. The results were generally correlated with the concentration of the black mulberry juice as well as the incubation temperature and time.

Key words: Black mulberry, juice, *Escherichia coli* O157:H7, *Salmonella* Typhimurium, survival pattern.

INTRODUCTION

Fresh fruits, vegetables and also their juices are very important for a healthy diet. The main reason for increasing demand on fresh fruit juices and/or vegetable juices is their positive contribution to the health. However, it should be considered that they are also very important as causative agents for several food borne illnesses (Parish, 1997). The fresh fruits and fruit juices can be contaminated with pathogenic microorganisms during growing (in the fields or orchards), harvesting, postharvest handling, processing and distribution (Beuchat, 1996; Buck et al, 2003). It was recently reported that juices -especially unpasteurized ones- are potential sources of bacterial pathogens such as *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes* (Bunning et al, 1986; Zhuang and Beuchat, 1995; Parish, 1998; Ryu and Beuchat, 1998; Anonymous, 2000a).

There are several reports about food-borne outbreaks related with consumption of fruits or fruit products. In particular, illness has been associated with apple cider (Parish, 2000), unpasteurized orange juice (Parish, 1997), unpasteurized apple juice (Anonymous, 2000a),

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carrot juice (Anonymous, 2000a), watermelon juice (Anonymous, 2000a), mamey juice (Anonymous, 2000b), raspberries (Herwaldt and Ackers, 1997) and frozen strawberries (Niu et al., 1995).

Nowadays, modified atmosphere packaging, gamma irradiation, ozone treatment, heat, steam, hot water or acidification with organic acids are common applications for killing pathogens in fresh or processed foods (Beuchat, 1996). However, many reports have been documented about *Salmonella* spp. and *E. coli* O157:H7 being more tolerant to acidic conditions than previously believed (Beuchat, 1996; Parish, 2000). Acid adaptation and increasing resistance to acidic conditions have been observed for various organisms including *Salmonella* (Goverd et al., 1979; Foster, 1991) and *E. coli* (Foster, 1991; Miller and Kaspar, 1994). The ability to survive under extreme acidic conditions of human stomach, greatly contributes to the virulence of all the enteric human pathogens (Itoh et al., 1998; Lin et al., 1995).

E. coli O157:H7 is one of the major food-borne pathogens, causes diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (Doyle and Schoeni, 1984; Besser et al., 1993). Various acidic foods such as apple cider (Besser et al., 1993), mayonnaise (Weagant et al., 1994) and yoghurt (Morgan et al., 1993) have been implicated in the outbreaks of food-borne disease caused by *E. coli* O157:H7. This pathogenic microorganism can also survive for extended storage time at low pH values and at low temperatures. Zhao et al. (1993) reported that *E. coli* O157:H7 survived for up to 31 days at 8°C in unpasteurized apple cider (pH 3.6 to 4.0).

According to World Health Organization Collaborating Centre for Reference and Research on *Salmonella* (Popoff and Le Minor, 1997), *Salmonella enterica* and *Salmonella bongori* currently include 2356 and 19 serovars, respectively. Optimum pH range of Salmonella spp. is similar to that of *E. coli* O157:H7 (Doyle and Cliver, 1990). It was reported that many food-borne outbreaks related with consuming acidic foods, such as mamey juice, apple cider, orange juice, grape juice and lemonade contaminated with *Salmonella* spp. (Anonymous, 2000a; Anonymous, 2000b).

Black mulberry fruit has intense color and flavor and is usually consumed as fresh fruit or fruit juice. Its juice is one of the most popular drinks in Turkey. The black mulberry fruit and its juice are both commonly used as flavoring and/or coloring additive for some foods, such as ice cream and cakes. Although, there are several studies on the antioxidant content of the mulberry (Arabshahi-Delouee and Urooj, 2007; Butt et al., 2008; Aramwit et al., 2010; Pérez-Gregorio et al., 2011), microbiological studies are very limited. Non-processed mulberry juice generally assumed as a safety food cause of the acidic environment. However, it may include serious risk for food-borne diseases when it contaminated by acid adapted pathogen microorganisms. This study was aimed to investigate the survival and growth pattern of acid-adapted and non-acid-adapted *E. coli* O157:H7 and *Salmonella* Typhimurium in black mulberry juice.

MATERIALS AND METHODS

Test cultures

E. coli O157:H7 (932) and *S.* Typhimurium (NRRL B 4420) were used as test organisms in the experiments. Stock cultures were kept at 4°C on slants of Tryptone Soya Agar (TSA, pH 7.3±0.2, Oxoid, CM0131). Tryptone Soya Broth (TSB, pH 7.3±0.2, Oxoid, Basingstoke, Hampshire, England) was used to activate the cultures. Both cultures were separately grown in TSB at 37°C for 18 to 24 h, the population was approximately 9.0 log-cfu/ml at the end of this incubation period. The cultures were serially diluted with 0.1% sterile peptone water (PW, pH 6.3±0.2, Difco, Detroit, MI, USA) to have an initial flora approximately 7.0 log-cfu/ml for the inoculation. The procedure described by Tsai and Ingham (1997) was applied to adapt the cells to acid environment. The cultures separately grown at 37°C for 18 to 24 h in TSB (pH 7.3±0.2) were centrifuged at 5000 rpm for 20 min. The supernatant was discarded and the cell pellets were washed with 0.1% sterile PW. Then these cell pellets were suspended in 10 ml of TSB (pH 5.0±0.2) and then incubated at 37°C for 4 h. The adaptation of the cells was checked out by plating on TSA (pH 5.0±0.2). The cells were adapted to the environment pH 4.0±0.2 by the same procedure described above. The acid-adapted cultures were prepared for the inoculation by the same method described for non acid adapted cells.

Preparation of black mulberry juice

Black mulberry (*Morus nigra*) fruits were purchased from a local producer in Tire, Izmir city, Turkey. The fruits were homogenized for 2 min in a stomacher (Stomacher Lab-Blender 400, Seward Medical, London, UK) just for having the juice of it. The liquid part was filtered aseptically by a sterilized tea filter cloth, and juice was collected in a sterilized glass bottle and it was used as undiluted (100%) sample in the experiments. Also, different concentrations of the juice were prepared with sterilized distilled water (50, 10 and 1%). The pH values of all the juice samples (diluted and undiluted) were measured.

It was aimed to observe the survival and growth pattern of test organisms in juices at original pH values (pH after dilution) and also at neutral pH (pH 7.00) value. So, the juice samples were prepared as mentioned above in two parallels. The pH values of one of the serials were adjusted aseptically with 1 N NaOH. After the dilution and pH adjustment, all the juice samples were dispensed in 18 ml quantities into sterile Erlenmeyer flask and were autoclaved at 110°C for 10 min to inactivate the naturally existing bacterial population. Aerobic mesophilic bacteria count results showed there is no existing flora after heat treatment.

In order to detect the effect of heat treatment on the antimicrobial activity of black mulberry juice a few pre-experiments were designed. Non heat treated (100%) juice samples and heat treated juice samples (100%) were both inoculated with test organisms separately and the count results were compared. Therefore, there is no differences between the results of both heat treated and non heat treated juices ($P<0.05$), it was decided to heat treat all the juices before the analyses to avoid the presence of natural microflora. On the other hand, heat treatment processing could cause decreasing of the pH value as a result of evaporation and concentration. The pH values of the juices were detected before

Table 1. Count results of *E. coli* O157:H7 in black mulberry juice during incubation at 37°C.

Concentration (%)	pH	0 h		1 Day		2 Days		7 Days	
		NAA[a]	AA[b]	NAA	AA	NAA	AA	NAA	AA
100	7.00	6.16 (0.14)[c]	5.93 (0.03)	8.40 (0.51)	8.65 (0.02)	8.60 (0.04)	8.42 (0.03)	ND[d]	ND
50	7.00	6.12 (0.04)	5.96 (0.02)	8.39 (0.43)	8.60 (0.02)	8.75 (0.09)	8.64 (0.02)	ND	ND
10	7.00	6.13 (0.10)	6.02 (0.01)	8.10 (0.20)	8.26 (0.02)	8.31 (0.14)	8.44 (0.05)	ND	ND
1	7.00	6.21 (0.02)	6.01 (0.01)	7.93 (0.03)	7.81 (0.04)	7.83 (0.26)	7.77 (0.02)	ND	ND
100	3.70	6.03 (0.09)	5.91 (0.01)	3.35 (0.41)	3.80 (0.04)	ND	1.81 (0.10)	ND	ND
50	4.34	6.07 (0.11)	5.97 (0.03)	4.15 (0.23)	4.64 (0.09)	1.76 (0.68)	2.53 (0.21)	ND	ND
10	4.80	6.09 (0.09)	5.99 (0.01)	5.32 (0.15)	5.69 (0.03)	3.36 (0.10)	3.75 (0.04)	ND	ND
1	4.89	6.09 (0.10)	5.95 (0.03)	4.87 (0.36)	4.83 (0.01)	4.19 (0.39)	4.52 (0.05)	ND	ND

[a] Non acid adapted *E. coli* O157:H7 (log-cfu/ml); [b] Acid-adapted *E. coli* O157:H7 (log-cfu/ml); [c] Mean (n=3) with standard deviation in parentheses. [d] Not detected.

and after the heat treatment. The variation of the pH values before and after heat treatment was negligible (P<0.05).

Inoculation of the samples

In order to achieve an initial population of approximately 6.0 log-cfu/ml, 2 ml of culture (7.0 log-cfu/ml) were inoculated into 18 ml of black mulberry juice sample. This procedure was applied with *S.* Typhimurium and *E. coli* O157:H7 separately for the juice samples: i) neutralized and 100, 50, 10 and 1% concentrated; ii) unneutralized, 100, 50, 10, 1%. Inoculated juice samples were prepared in two parallel series to detect the impact of the incubation temperature on the survival pattern of the test cultures. One of them was incubated 4°C for a week and the other one at 37°C during the same period.

Control of the survival of the test microorganisms

The samples were prepared described above and 1 ml of inoculum from each of the inoculated juice samples was taken at the beginning and 1st, 2nd and 7th days of the incubation. It was serially diluted with 0.1% sterile PW, and surface plated on TSA and the results were recorded as log-cfu/ml of juice. It is known that bacterial cells could be injured at low pH and low storage temperature (4°C) and it could cause inaccurate count results. Also, injured cells may not be enumerated by selective media unless they are first allowed to repair (Ray and Adams, 1984; Willshaw et al., 1994; Silk and Donelly, 1997; Kang and Fung, 2000).

In order to see whether there is an adverse effect of low pH and low incubation temperature on the recovery of non acid adapted test organisms, counts were performed on both non-selective and selective media in pre-experiments. Bismuth Sulphite Agar (BSA, pH 7.6±0.2, Oxoid CM 201) were used as selective media for *S.* Typhimurium. Presumptive colonies were confirmed by using biochemical tests [Triple Sugar Iron Agar (TSI, pH 7.4±0.2, Oxoid-CM0277) and Lysine Iron Agar (LI, pH 6.7±0.2, Oxoid-CM0381) reactions] and serological tests (FDA-BAM, 2007). Enumeration of *E. coli* was performed by most probable number technique. Lauryl Sulphite Tryptose Broth (LSTB, pH 6.8±0.2, Oxoid-CM451) tubes were incubated at 37°C for 24 to 48 h and after incubation period the tubes produced gas, were inoculated to *E. coli* Broths (EC, pH 6.9±0.2, Difco Laboratories, Detroit MI, 48232-7058 USA, 231430) and incubated at 44.5°C for 24 to 48 h. Gas producing tubes were inoculated to Tryptone Water (TW, pH 7.5±0.2, Oxoid-CM0087) to confirm indol production (FDA-BAM, 2002). The results of the non-selective and selective media were compared and no significant difference were found (P<0.05). Consequently, in further experiments, only non-selective media were used.

The presences of test organisms were also assessed in uninoculated juice samples at the beginning of each experiment. The juice samples were serially diluted with 0.1% sterile PW and surface-plated on appropriate selective media. Also, the presences of microorganisms in juice samples after heat treatment were controlled by the same method.

Chemical analyses

The pH values of the uninoculated juice samples were determined by digital pH meter (NEL-Mod 821). Titratable acidity on the basis of citric acid was determined using the method described in Turkish Standard 1125 (Anonymous, 1972). Sugars (total and reducing) were determined using the Lane-Eynon method (Egan et al., 1981).

Statistical analysis

The mean values and standard deviation were calculated and regression multiple tests (SPSS 13.0 Windows Pocket Program; SPSS Inc., Chicago, USA) were analyzed.

RESULTS AND DISCUSSION

E. coli O157:H7 and *S.* Typhimurium could not be detected in the natural microflora of black mulberry juice samples. The mean values of total sugar content and reducing sugar content of undiluted juice samples before inoculation were 13.7 and 12,5% (m/v), respectively. The mean value of titratable acidity of the juice was 1.80% (v/v, citric acid) and the mean value of pH before heat treatment was 3.72. After heat treatment, the mean values of the pH of the juice samples were found as 3.70, 4.34, 4.8 and 4.89 for 100, 50, 10 and 1% concentrated juices, respectively.

Table 1 shows that the viable population of non acid adapted and acid adapted *E. coli* O157:H7 cells in

Table 2. Count results of *E. coli* O157:H7 in black mulberry juice during incubation at 4°C.

Concentration (%)	pH	0 h		1 Day		2 Days		7 Days	
		NAA[a]	AA[b]	NAA	AA	NAA	AA	NAA	AA
100	7.00	6.15(0.11)[c]	5.94 (0.01)	6.02 (0.05)	5.44 (0.11)	5.93 (0.09)	5.37 (0.14)	5.78 (0.16)	5.72 (0.13)
50	7.00	6.16(0.05)	5.99 (0.01)	6.06 (0.11)	5.62 (0.04)	6.02 (0.11)	5.53 (0.05)	5.76 (0.35)	5.44 (0.05)
10	7.00	6.12(0.03)	6.02 (0.02)	5.98 (0.13)	5.76 (0.03)	5.98 (0.05)	5.71 (0.02)	5.70 (0.08)	5.52 (0.4)
1	7.00	6.15(0.08)	6.03 (0.03)	6.05 (0.06)	5.76 (0.02)	6.10 (0.08)	5.92 (0.02)	5.90 (0.02)	5.78 (0.2)
100	3.70	6.09(0.14)	5.93 (0.02)	5.95 (0.09)	5.85 (0.11)	5.92 (0.05)	5.89 (0.08)	5.58 (0.49)	6.05 (0.02)
50	4.34	6.09(0.09)	5.98 (0.01)	5.98 (0.09)	5.93 (0.04)	5.99 (0.04)	5.92 (0.05)	5.94 (0.03)	5.99 (0.2)
10	4.80	6.18(0.03)	6.00 (0.03)	6.04 (0.09)	6.09 (0.05)	6.14 (0.15)	6.29 (0.01)	6.08 (0.13)	6.05 (0.3)
1	4.89	6.11(0.08)	6.00 (0.01)	5.81 (0.63)	6.12 (0.02)	6.10 (0.02)	6.26 (0.01)	6.04 (0.07)	6.06 (0.01)

[a] Non acid adapted *E. coli* O157:H7 (log-cfu/ml); [b] Acid-adapted *E. coli* O157:H7 (log-cfu/ml); [c] Mean (n=3) with standard deviation in parentheses.

neutralized juice samples (for all concentrations) increased about 2 log units in 2 days at 37°C, whereas no viable cells were detected in undiluted (100%) and unneutralized (pH 3.7) juice sample at the end of 2 days incubation at the same temperature. The reduction in the numbers of viable cells in unneutralized juice samples in 2 days were found as approximately 2, 3 and 4 log-cfu/ml for 1% (pH 4.89), 10% (pH 4.80) and 50% (pH 4.34) concentrations, respectively. Significant decreases were evident correlated with the pH of the juice. Acid-adapted *E. coli* O157:H7 cells were consistently more resistant than non-acid-adapted cells in all concentrations at 37°C (100%, pH 3.70; 50%, pH 4.34; 10%, pH 4.80 and 1%, pH 4.89). No viable *E. coli* O157:H7 cells were detected after 7 days incubation in all concentrations of juice samples.

Incubation at 4°C dramatically enhanced the survival of *E. coli* O157:H7, regardless of acid adaptation (Table 2). There was no remarkable decrease in the number of non-acid-adapted and acid-adapted *E. coli* O157:H7 cells in all concentration of juice samples incubated at 4°C up to 7 days. It was construed as a result of metabolic activity of the microorganisms treated at different temperatures. The metabolic activity is at the highest level at the optimum growing temperature (37°C); so they grow fast and also they draw back fast. However, at low temperatures (4°C), these microorganisms hold their metabolic activity at the minimum level and they could resist to improper conditions for longer time periods. As a result, at low incubation temperatures the cells could survive longer.

The results of the survival pattern of acid adapted and refrigerated *E. coli* O157:H7 cells in black mulberry juice are consistent with the reports of several researchers. Hsin-Yi and Chou (2001) reported that acid adaptation enhanced the survival of *E. coli* O157:H7 (ATCC 43895) in mango and asparagus juice at 25°C, but at 7°C there was no significant difference. Tsai and Ingham (1997) reported that adaptation to acid and low temperature enhanced the survival of *E. coli* O157:H7 and *Salmonella* strains in ketchup. Raghubeer et al. (1994), Weagant et al. (1994), Miller and Kaspar (1994), Leyer et al. (1995) and Degirmenci et al. (2012) also detected similar results and concluded that the adaptation to acidic environment and incubation at low temperature built up the survival of *E. coli* O157:H7.

It was detected that the survival and growth pattern of *S.* Typhimurium was similar to *E. coli* O157:H7 in black mulberry juice during storage at 4 and 37°C (Table 3 and 4). No viable *S.* Typhimurium cells were detected at the end of 2 days incubation in undiluted (pH 3.70) juice samples at 37°C. The growth of *S.* Typhimurium in 1% (pH 4.89) and 10% (pH 4.80) concentrated mulberry juice was inhibited over 7 days at 37°C, although there was no decrease in the number of cells after 2 days of incubation. Acid adaptation significantly ($P< 0.05$) enhanced the survival of *S.* Typhimurium in all concentrations of juice samples at original pH values at 37°C (Table 3). The differences between acid-adapted and non-acid-adapted cells were ranged from 0.30 log-cfu/ml (10%, at 1 day) to 1.96 log-cfu/ml (100%, at 2 days). Also incubation at 4°C significantly enhanced the survival of *S.* Typhimurium (Table 4). At this temperature there was no remarkable decrease in the number of non acid adapted *S.* Typhimurium cells in neutralized juice samples after 7 days. However, viable population of non acid adapted *S.* Typhimurium decreased slightly during incubation in unneutralized juice samples. It was detected a remarkable reduction only at end of the 7 days incubation in unneutralized juice samples.

S. Typhimurium which was acid adapted and incubated at 4°C in black mulberry juice has a survival pattern similar with the studies that were reported elsewhere. *S.* Typhimurium was adapted to acid by exposure to hydrochloric acid at pH 5.8 for one to two doublings. Acid-adapted cells had increased resistance to inactivation by organic acids commonly present in cheese, including lactic, propionic, and acetic acids. Acid-adapted cells survived better than non-adapted cells during milk fermentation by a lactic acid culture. Acid-

Table 3. Count results of *Salmonella* Typhimurium in black mulberry juice during incubation at 37°C.

Concentration (%)	pH	0 h		1 Day		2 Days		7 Days	
		NAA[a]	AA[b]	NAA	AA	NAA	AA	NAA	AA
100	7.00	6.36 (0.05)[c]	6.23 (0.04)	8.98 (0.01)	8.76 (0.06)	8.68 (0.41)	8.90 (0.02)	ND	ND
50	7.00	6.41 (0.07)	6.26 (0.02)	8.96 (0.18)	8.91 (0.01)	8.64 (0.49)	8.35 (0.05)	ND	ND
10	7.00	6.33 (0.07)	6.24 (0.01)	8.62 (0.17)	8.81 (0.01)	8.37 (0.09)	8.57 (0.06)	ND	ND
1	7.00	6.22 (0.13)	6.26 (0.01)	7.96 (0.23)	7.79 (0.02)	6.72 (0.36)	7.02 (0.10)	ND	ND
100	3.70	6.27 (0.06)	6.16 (0.04)	3.25 (0.97)	3.94 (0.02)	ND[d]	1.96 (0.08)	ND	ND
50	4.34	6.35 (0.06)	6.14 (0.00)	5.19 (0.50)	5.71 (0.08)	3.52 (0.31)	4.16 (0.01)	ND	ND
10	4.80	6.41 (0.10)	6.21 (0.02)	7.42 (0.12)	7.72 (0.03)	7.15 (0.24)	7.75 (0.15)	ND	ND
1	4.89	6.31 (0.14)	6.23 (0.01)	7.00 (0.09)	7.47 (0.03)	6.35 (0.08)	6.83 (0.10)	ND	ND

[a] Non acid adapted *Salmonella* Typhimurium (log-cfu/ml); [b] Acid-adapted *Salmonella* Typhimurium (log-cfu/ml); [c] Mean (n=3) with standard deviation in parentheses; [d] Not detected.

Table 4. Count results of *Salmonella* Typhimurium in black mulberry juice during incubation at 4°C.

Concentration (%)	pH	0 h		1 Day		2 Days		7 Days	
		NAA[a]	AA[b]	NAA	AA	NAA	AA	NAA	AA
100	7.00	6.31 (0.04)[c]	6.23 (0.04)	6.31 (0.01)	6.09 (0.04)	6.20 (0.05)	6.02 (0.01)	5.84 (0.26)	5.54 (0.40)
50	7.00	6.38 (0.11)	6.22 (0.02)	6.28 (0.08)	6.07 (0.06)	6.29 (0.08)	6.04 (0.01)	5.93 (0.01)	5.30 (0.11)
10	7.00	6.29 (0.05)	6.24 (0.01)	6.12 (0.04)	6.10 (0.05)	6.20 (0.04)	6.10 (0.02)	5.90 (0.01)	5.86 (0.03)
1	7.00	6.21 (0.14)	6.25 (0.00)	5.79 (0.06)	5.99 (0.04)	5.69 (0.04)	5.82 (0.03)	5.95 (0.01)	5.63 (0.10)
100	3.70	6.25 (0.05)	6.28 (0.05)	5.97 (0.02)	6.11 (0.04)	5.57 (0.06)	6.05 (0.03)	4.86 (0.00)	5.88 (0.04)
50	4.34	6.34 (0.07)	6.25 (0.05)	6.17 (0.16)	6.07 (0.03)	6.25 (0.10)	6.01 (0.04)	5.02 (0.05)	5.60 (0.06)
10	4.80	6.35 (0.10)	6.20 (0.03)	6.30 (0.01)	6.14 (0.02)	6.12 (0.06)	6.11 (0.02)	5.34 (0.01)	6.07 (0.05)
1	4.89	6.27 (0.14)	6.23 (0.00)	6.29 (0.04)	6.16 (0.02)	6.11 (0.11)	6.08 (0.01)	5.48 (0.11)	6.02 (0.04)

[a] Non acid adapted *Salmonella* Typhimurium (log-cfu/ml); [b] Acid-adapted *Salmonella* Typhimurium (log-cfu/ml); [c] Mean (n=3) with standard deviation in parentheses.

adapted cells also showed enhanced survival over a period of two months in cheddar, Swiss, and mozzarella cheeses kept at 5°C. These observations support the theory that acid adaptation is an important survival mechanism enabling *Salmonella* spp. to persist in fermented dairy products and possibly other acidic food products (Leyer and Johnson, 1992). Tsai and Ingham (1997) examined the effects of adaptation to acid and storage temperature on the survival of a nonpathogenic *E. coli* strain, three strains of *E. coli* O157:H7, and three strains of *Salmonella* spp. in ketchup, mustard, and sweet pickle relish. Stationary-phase cells were adapted to acidic conditions in pH 5.0 Trypticase soy broth for 4 h at 37°C. Samples inoculated with individual strains were stored at 5 and 23°C. Acid adaptation enhanced the survival of all three *Salmonella* strains (ATCC 6962, 13311, and 25957) and all three *E. coli* O157:H7 strains (ATCC 43889, 43894, and 43895), with the magnitude of the effect depending on the strain and storage temperature. Cells of all tested strains survived longer at 5°C than at 23°C.

The survival of three-strain mixtures of *S.* Typhimurium DT104, *L. monocytogenes*, and *E. coli* O157:H7 in pasteurized and unpasteurized preservative-free apple cider (pH 3.3 to 3.5) during storage at 4 and 10°C for up to 21 days. *S.* Typhimurium DT104 populations decreased by <4.5 log cfu/ml during 14 days storage at 4 and 10°C in pasteurized cider, and by ≥5.5 log cfu/ml during 14 days in unpasteurized cider stored at these temperatures. However, after 7 days at 4°C, the *S.* Typhimurium populations had decreased by only about 2.5 log cfu/ml in both pasteurized and unpasteurized cider (Roering et al., 1999). Survival of *S.* Typhimurium and *E. coli* O157:H7 was studied by Ingham et al. (2000). Three strain mixtures of *S.* Typhimurium and *E. coli* O157:H7 were inoculated separately 23% model brine with or without added pasteurized whey (2%) and as a combined inoculum into the commercial brines. The model brines were incubated at 8 and 15°C for 28 days, and the commercial brines at 4 and 13°C for 35 days. Populations of both pathogens in the model brine + whey decreased slowly over 28 days (1.0 to 2.0 log cfu/ml) with greater survival at 8°C than at 15°C. Both *S.* Typhimurium and *E. coli* O157:H7 survived significantly better at 4°C than at 13°C in two of the commercial brines. The survival of each pathogen in the commercial brines at 13°C was significantly influenced by brine pH. Degirmenci et al. (2012) were investigated the survival and growth patterns of *E. coli* O157:H7, S. Typhimurium and *L. monocytogenes* in various concentrations of

black carrot juice were incubation during the period at 4 and 37°C for 7 days. Incubating at low temperature (4°C) enhanced the survival of test microorganisms.

Recent outbreaks related with *E. coli* O157:H7, *S.* Typhimurium involving fruit juice consumption have raised concerns about the safety of fresh fruit juices (Beuchat, 1996; Anonymous, 2000a; Parish, 2000). In this frame, the results of this study about survival of these test pathogens in black mulberry juice could be beneficial. Based on the data obtained from the present study, it is important to prevent the contamination of these pathogens in acidic drinks especially held at 4°C because of limited shelf life of this product.

It was concluded that acid adaptation increased the acid tolerance of *E. coli* O157:H7 and *S.* Typhimurium at 4 and 37°C for all concentration of unneutralized mulberry juice samples (pH 3.70 to 4.89). However, low temperature and neutralization enhanced the survival of test organisms. Black mulberry contain organic acids, tannin, high amounts of pigments and anthocyanins (Baytop, 1984; Elmaci, 1998). All these compounds exhibit antimicrobial properties (Niu et al., 1995; Anonymous, 1998). It could explain the dramatic decrease in numbers of inoculated bacteria at the end of 7 days incubation. However, this conclusion obviously needs further investigation on the antimicrobial constituents of the product.

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