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Antibacterial Effect of Malic Acid Against *Listeria monocytogenes*, *Salmonella enteritidis* and *Escherichia coli* in Mango, Pineapple and Papaya Juices

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ABSTRACT

Consumption of un-pasteurized fruit juices has increased in recent years due to their freshness and other attractive characters. Contamination of those by pathogenic microorganisms is considered as one of the major problems. Organic acid treatments, such as malic acid treatment, are used to control these contaminations. Aim of this study was to evaluate the antimicrobial activity of malic acid against *Listeria monocytogenes*, *Salmonella enteritidis* and *Escherichia coli* in Mango, Pineapple and papaya juices. Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of malic acid against those bacteria were evaluated for all tested fruit juices which were stored at 5, 20 and 35°C. Antibacterial effect of malic acid was observed against all tested bacteria under all tested conditions. The antibacterial activity was depended upon the type of bacteria, type of fruit juice and storage conditions. Antibacterial effect was higher in high temperature storage than low temperature storage. High concentration of malic acid was needed for Papaya juice compared to other fruit juices to reduce microbial population by a same amount. *E. coli* was found to be more resistant to antimicrobial activity of malic acid compared to the other two bacteria. According to the results of this study, *E. coli* can be recommended as a test bacterium in testing organic acids to be used as food preservatives. Further, these tests should be carried out in refrigerated temperature to find out more effective organic acids, as the lowest antibacterial activity of malic acid was observed at 5°C in this study.

Key words: Antibacterial effect, malic acid, fruit juice, pathogenic microorganisms

INTRODUCTION

Due to the promotions on their characteristics such as freshness, high vitamins content and low caloric contribution, the consumption of un-pasteurized fruit juices has increased in recent years (Raybaudi-Massilia *et al*., 2009). However, although these fruit juices are considered as important component of healthy diet, there is a risk of food borne microbial infections which is associated with the consumption of those. Number of food borne disease outbreaks caused by different food borne microbial pathogens, associated with un-pasteurized fruit juice, have been reported (Harris *et al*., 2003; Ingham *et al*., 2006; Raybaudi-Massilia *et al*., 2006). As such, application of treatments which are capable of reducing these pathogenic microorganisms, in to standard limits, is essential in the fruit juice production.

Many treatments are available which are capable of reducing such microbial pathogens. Out of those, treatments which have the minimal effect to the organoleptic and nutritional properties of the fruit juice are mostly preferred. Due to their preservative, antioxidant, flavoring and acidifying properties and low cost, naturally available organic acids are used to treat food borne microbial pathogens in fruit juice industry. Malic acid, a dicarboxylic acid which is found in many sour or tart taste foods, is one of such organic acid which can be used to treat food borne pathogens (Shirzadeh and Kazemi, 2011; Nahar *et al*., 2011; Anvoh *et al*., 2009; Kossah *et al*., 2009; Samappito and Butkhup, 2008). Malic acid affects food borne microbial pathogens by lowering the pH value (Beuchat and Golden, 1989) or by causing significant damage to the cytoplasm of those microorganism cells as they can diffuse across the cell membrane (Eswaranandam *et al*., 2004).

Listeria monocytogenes, *Salmonella enteritidis* and *Escherichia coli* are three major bacterial pathogens which can be contaminated with food and beverages. *Listeria monocytogenes* is considered as an important cause of human food and water borne infections. It is an opportunistic intracellular pathogen causes the disease called listeriosis (Liu, 2006). *Salmonella* contaminate with food and water mainly by contaminated egg, meat and poultry products and cause the disease called salmonellosis (Wang *et al*., 1996; Nowak *et al*., 2007). Many non-pathogenic serogroups of *Escherichia coli* are present in the intestinal tract of warm blooded animals as its normal micro flora. However, some serogroups, such as enterohemorrhagic *Escherichia coli* O157:H7, are pathogenic and cause severe diarrhea and fever (Stender *et al*., 2001). As there is a possibility to contaminate un-pasteurized fruit drinks with these bacterial pathogens, study on possible contamination prevention methods for those products is important.

The present study was conducted to find out the ability of using malic acid to control bacterial pathogens, *Listeria monocytogenes, Salmonella enteritidis* and *Escherichia coli* in juices of Mango, Pineapple and papaya fruits.

MATERIALS AND METHODS

Fruit juice preparation and addition of malic acid: Mango, Pineapple and Papaya which were at ripening stage were purchased from local market. Each fruit was washed, peeled, cut into pieces and blended to obtain fruit juice. Then, obtained fruit juice were centrifuge at 12500 rpm for 15 min at 4°C and fruit juice supernatant was bottled and autoclaved at 121°C for 15 min and microorganism free fruit juice was obtained. Then, malic acid added fruit juice solutions were prepared in a laminar flow cabinet to maintain the aseptic conditions. Ten solutions of 100 mL for all three fruit juice were prepared in 150 mL polypropylene containers which were added with 0, 0.2, 0.4, 0.6, 0.8, 1, 1.5, 2%, 2.5 and 3% of sterile malic acid.

Preparation of bacterial cultures and inoculation: *Listeria monocytogenes, Salmonella enteritidis* and *Escherichia coli* which were maintained in Tryptone Soy Agar (TSA) slants at refrigerated conditions were used for the study. Those were cultured using Tryptone Soy Broth Yeast Extract medium (TSBYE) which contains 30 g of tryptone soy broth powder with dextrose, 6 g of yeast extracts and 1 L of water. Cultures were incubated 6 h at 36°C to exponential growth phase and serially diluted (10^{-1}-10^{-10}) in sterile distilled water for enumeration. Bacteria were enumerated using Tryptone Soy Agar (TSA) plates at 37°C overnight and bacterial concentration was estimated by calculating the average number of colonies on plates containing 30 to 300 colonies. Then those bacterial cultures were adjusted to the concentrations of 10^8 cfu mL^{-10} using sterile distilled water. One milliliter of each of those bacterial cultures was individually added to the prepared each fruit juice solutions.

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC): Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of malic acid against *Listeria monocytogenes, Salmonella enteritidis* and

Escherichia coli in Mango, Pineapple and Papaya fruit juices were determined using the broth dilution method proposed by Davidson and Paris (1989). In this, the prepared all fruit juice solutions were subjected to three treatments culturing at 5°C for 24 h, culturing at 20°C for 24 h and culturing at 35°C for 24 h. After these treatments, 1 mL of each solution were taken, serially diluted, added to sterile Petri plates and molten and cooled TSA medium were added to enumerate viable bacteria. Simultaneously 500 µL of each solution were added to 4.5 mL of TSB medium to reconfirm the cellular death as explained by Raybaudi-Massilia *et al*. (2009). Then those plates and tubes were incubated at 35°C for 24 h.

Statistical analysis: Microbial counts of each and every treatment were statistically analyzed individually by statgraphics plus v.5.1 software. Multifactor analysis of variance with posterior multiple range test was used.

RESULTS AND DISCUSSION

Minimal inhibitory concentration (MIC): Minimal Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial compound which inhibits the visible growth of microorganisms after overnight incubation. As such, the minimum maic acid concentration which inhibits the growth of tested bacteria over its initial concentration (10^8 cfu mL^{-10}) was considered as the minimal inhibitory concentration in this study.

As per the results of the experiment carried out at 5°C temperature, no microbial count was higher than 10^8 CFU mL^{-10} at 0 or 24 h for *Listeria monocytogenes* (Table 1), *Salmonella enteritidis* (Table 2) and *Escherichia coli* (Table 3) in all tested fruit juices. The

Table 1: Effect of malic acid on *Listeria monocytogenes* in different experimental conditions

		Survival population in juice ($\log_{10}$ cfu mL^{-10})								
		Mango			Pineapple			Papaya		
Storage time (h)	Malic acid (%)	5°C	20°C	35°C	5°C	20°C	35°C	5°C	20°C	35°C
0	0	6.88±0.04	7.28±0.05	7.15±0.05	6.75±0.06	6.68±0.06	6.75±0.05	7.35±0.05	7.21±0.08	7.37±0.05
	0.2	6.23±0.03	6.20±0.04	6.11±0.04	6.18±0.04	5.80±0.05	5.71±0.03	6.78±0.02	6.60±0.03	6.51±0.04
	0.4	5.36±0.06	5.22±0.04	5.00±0.06	4.96±0.04	5.12±0.04	4.90±0.06	5.96±0.04	5.42±0.05	5.20±0.03
	0.6	4.20±0.02	3.22±0.03	3.42±0.07	3.40±0.03	2.32±0.06	2.63±0.03	5.12±0.03	3.79±0.03	3.48±0.02
	0.8	2.89±0.03	1.97±0.04	1.33±0.06	2.62±0.05	ND	ND	4.72±0.05	3.27±0.05	2.53±0.03
	1.0	ND	ND	ND	ND	ND	ND	3.35±0.06	1.23±0.07	ND
	1.5	ND	ND	ND	ND	ND	ND	2.02±0.03	ND	ND
	2.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	2.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	0	6.65±0.05	6.45±0.07	6.49±0.03	6.55±0.03	6.41±0.04	6.59±0.03	6.75±0.07	8.45±0.07	8.49±0.03
	0.2	5.93±0.03	2.33±0.03	1.24±0.03	3.93±0.03	1.43±0.05	1.13±0.04	5.63±0.03	6.03±0.05	3.24±0.04
	0.4	1.86±0.03	ND	ND	1.26±0.05	ND	ND	3.96±0.06	4.25±0.03	1.26±0.03
	0.6	ND	ND	ND	ND	ND	ND	2.36±0.06	2.03±0.05	ND
	0.8	ND	ND	ND	ND	ND	ND	1.22±0.04	ND	ND
	1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
	2.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	2.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3.0	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: Bacteria was not detected

Table 2: Effect of malic acid on *Salmonella enteritidis* in different experimental conditions

		Survival population in juice (log_{10} CFU mL^{-10})								
		Mango			Pineapple			Papaya		
Storage time (h)	Malic acid (%)	5°C	20°C	35°C	5°C	20°C	35°C	5°C	20°C	35°C
0	0	7.28±0.03	7.48±0.03	7.45±0.04	6.98±0.04	7.08±0.03	6.95±0.06	7.46±0.04	7.45±0.07	7.44±0.03
	0.2	6.83±0.05	6.80±0.03	6.73±0.04	6.42±0.02	6.21±0.03	6.54±0.02	6.87±0.03	6.68±0.05	6.53±0.03
	0.4	5.66±0.03	5.64±0.02	5.63±0.02	5.13±0.03	4.88±0.07	4.91±0.07	5.86±0.03	5.51±0.04	5.31±0.05
	0.6	4.67±0.03	4.26±0.07	3.57±0.03	4.10±0.04	3.62±0.06	3.56±0.05	5.42±0.04	3.84±0.04	3.68±0.06
	0.8	3.89±0.06	2.97±0.05	2.03±0.04	3.33±0.04	1.89±0.04	1.77±0.04	4.80±0.06	3.30±0.07	2.93±0.06
	1.0	2.57±0.03	1.07±0.06	ND	1.43±0.05	ND	ND	3.78±0.05	1.46±0.03	1.10±0.04
	1.5	1.67±0.05	ND	ND	ND	ND	ND	2.57±0.02	ND	ND
	2.0	ND	ND	ND	ND	ND	ND	1.08±0.04	ND	ND
	2.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	0	6.85±0.05	7.45±0.05	7.46±0.03	5.85±0.06	5.73±0.03	5.59±0.04	7.52±0.04	8.88±0.05	9.43±0.07
	0.2	6.29±0.07	5.33±0.03	3.24±0.06	4.93±0.04	4.04±0.07	2.84±0.05	5.88±0.05	6.21±0.03	4.44±0.03
	0.4	3.85±0.03	2.17±0.04	1.43±0.04	2.26±0.02	1.94±0.04	1.31±0.03	4.11±0.02	4.34±0.03	2.66±0.06
	0.6	1.42±0.04	ND	ND	ND	ND	ND	3.30±0.04	2.38±0.06	1.18±0.05
	0.8	ND	ND	ND	ND	ND	ND	2.24±0.03	ND	ND
	1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
	2.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	2.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3.0	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: Bacteria was not detected

maximum value recorded was 7.52±0.04 log_{10} cfu mL^{-10} for *Salmonella enteritidis* inoculated papaya juice without malic acid at 24 h. There was no microbial growth, over its initial level, observed even in the control samples which were without malic acid. Hence, establishment of MICs for those conditions were not necessary. However, reduction of microbial counts was observed with the increase of malic acid concentration in all treatments for *Listeria monocytogenes* (Table 1), *Salmonella enteritidis* (Table 2) and *Escherichia coli* (Table 3). This indicates the antibacterial effect of malic acid against all tested bacteria. Results of this study were in agreement with the similar studies carried out by other authors for other fruit juices (Miller and Kasper, 1994; Yuste and Fung, 2002; Ceylan *et al.*, 2004; Raybaudi-Massilia *et al.*, 2009).

Results of the experiment carried out at 20 and 35°C temperatures also shown no microbial growth at 0 or 24 h for *Listeria monocytogenes* (Table 1), *Salmonella enteritidis* (Table 2) and *Escherichia coli* (Table 3) in Mango and Pineapple juices. The maximum value recorded was 7.48±0.03 log_{10} cfu mL^{-10} for *Salmonella enteritidis* inoculated Mango juice without malic acid at 20°C at 0 h and for *Escherichia coli* inoculated Mango juice without malic acid at 35°C at 0 h. As microbial growth was not observed even in control samples which were without malic acid, establishment of MICs were not necessary in these conditions as well. However, as observed before, decrease in microbial counts was observed in all treatments with the increase of maic acid concentration. These results are inline with the results obtained by other authors in researches carried out in similar conditions for different fruit juices (Zhao *et al.*, 1993; Yuste and Fung 2002; Ceylan *et al.*, 2004; Raybaudi-Massilia *et al.*, 2006, 2009).

Table 3: Effect of malic acid on *E. coli* in different experimental conditions

		Survival population in juice (log_{10} cfu mL^{-10})								
		Mango			Pineapple			Papaya		
Storage time (h)	Malic acid (%)	5°C	20°C	35°C	5°C	20°C	35°C	5°C	20°C	35°C
0	0	7.31±0.07	7.28±0.07	7.48±0.03	7.22±0.06	7.38±0.04	7.14±0.08	7.34±0.05	7.33±0.02	7.39±0.08
	0.2	6.88±0.06	6.82±0.03	6.78±0.08	6.99±0.03	6.74±0.02	6.55±0.03	7.08±0.04	7.23±0.02	7.33±0.04
	0.4	6.73±0.02	6.69±0.05	6.64±0.03	6.76±0.08	6.56±0.04	6.61±0.07	6.87±0.05	7.09±0.07	7.10±0.03
	0.6	6.75±0.03	6.57±0.02	6.59±0.07	6.79±0.06	6.48±0.05	6.35±0.03	6.88±0.04	6.82±0.01	6.72±0.03
	0.8	6.89±0.08	6.61±0.08	6.35±0.03	6.72±0.03	6.33±0.06	6.21±0.08	6.75±0.02	6.85±0.02	6.43±0.07
	1.0	6.77±0.07	6.30±0.03	6.41±0.06	6.77±0.04	6.38±0.05	6.13±0.03	6.69±0.04	6.73±0.06	6.38±0.01
	1.5	6.62±0.06	5.85±0.05	5.73±0.04	6.53±0.06	5.63±0.04	5.47±0.01	6.71±0.05	6.54±0.06	6.17±0.03
	2.0	6.63±0.06	4.53±0.02	4.22±0.07	5.50±0.02	4.37±0.01	4.23±0.03	6.66±0.03	5.73±0.03	5.43±0.03
	2.5	4.38±0.03	3.08±0.02	3.12±0.01	4.08±0.04	2.22±0.05	2.32±0.06	5.82±0.06	5.28±0.04	4.77±0.06
	3.0	2.87±0.03	1.66±0.03	1.37±0.02	1.98±0.04	1.36±0.04	ND	3.41±0.05	3.19±0.04	2.71±0.05
24	0	6.81±0.06	6.92±0.02	7.12±0.01	6.70±0.08	6.83±0.07	6.99±0.03	7.00±0.04	9.72±0.06	9.98±0.04
	0.2	6.63±0.04	6.55±0.04	6.47±0.02	6.54±0.05	6.32±0.06	6.16±0.09	6.81±0.09	8.21±0.01	8.14±0.03
	0.4	6.15±0.03	5.87±0.04	5.66±0.07	6.10±0.05	5.43±0.03	5.28±0.03	6.43±0.08	6.37±0.03	6.09±0.09
	0.6	5.94±0.02	5.22±0.03	5.01±0.06	5.48±0.03	5.00±0.04	4.80±0.03	5.99±0.04	5.45±0.07	5.36±0.08
	0.8	5.88±0.08	4.76±0.04	4.48±0.03	5.06±0.03	4.51±0.02	4.33±0.04	5.62±0.05	4.66±0.07	4.57±0.04
	1.0	5.28±0.04	3.35±0.06	3.11±0.04	4.55±0.07	3.22±0.08	2.87±0.05	4.85±0.06	3.53±0.05	3.65±0.04
	1.5	4.93±0.03	2.24±0.01	1.86±0.03	2.61±0.06	ND	ND	3.34±0.05	2.88±0.04	2.47±0.05
	2.0	2.23±0.04	ND	ND	ND	ND	ND	1.33±0.05	ND	ND
	2.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3.0	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: Bacteria was not detected

At 20 and 35°C temperatures, no growth was observed for all tested bacteria in Papaya juice at 0 h. However, at 24 h, all tested bacteria were shown to be grown in some Papaya samples. At 0% malic acid, *Listeria monocytogenes* count at 20 and 35°C were 8.44±0.07 and 8.49±0.03 log_{10} CFU mL^{-10}, respectively. *Salmonella enteritidis* count at 20 and 35°C were 8.88±0.05 and 9.43±0.07 log_{10} CFU mL^{-10}, respectively. *Escherichia coli* count at 20 and 35°C were 9.72±0.06 and 9.98±0.04 log_{10} CFU mL^{-10}, respectively. At 0.2% malic acid, *Escherichia coli* count at 20 and 35°C were 8.21±0.01 and 8.14±0.03 log_{10} CFU mL^{-10}, respectively. Hence MICs could be calculated for all tested bacteria after 24 h storage at 20 and 35°C in Papaya juice. MICs for *Listeria monocytogenes* and *Salmonella enteritidis* were found to be 0.2 (Table 1, 2). MIC for *E. coli* was found to be 0.4.

One of the factors that effect to the microbial growth in fruit juices is pH. Minimum reported pH level which growth of microorganism used in this study was recorded are pH 4.4 for *Listeria monocytogenes*, pH 3.99 for *Salmonella enteritidis* and pH 4.00 for *E. coli* O157:H7 (D'Aoust *et al*., 2001; Lou and Yousef, 1999; Meng *et al*., 2001). The initial pH of Mango, Pineapple and Papaya juice used in this study were 4.21, 3.43 and 5.41, respectively. Hence, pH of the used juices can be identified as one of the reasons for the above results of the present study and the results of previous studies. Moreover, clear inhibition of the tested three microbes by maic acid was observed in all experimental conditions. This inhibition was shown to be increased with the increase of maic acid concentration. Hence, not only the pH of juice but also inhibition effect of maic acid has caused for above results.

Minimal bactericidal concentration (MBC): Bactericidal action of malic acid against *Listeria monocytogenes*, *Salmonella enteritidis* and *E. coli* which were inoculated into Mango, Pineapple and Papaya juice were studied. Malic acid was shown to be effective in reducing the studied bacterial populations in to undetectable level in those juices. Minimal Bactericidal Concentration (MBC) of an antibacterial compound is the lowest concentration that prevents the growth of bacteria after subculture onto antibacterial compound free media. Hence, the bactericidal action was confirmed by re-culturing the samples which bacterial populations were in undetectable level. This re-culturing was done in TSB medium at 35°C for 24 h. The Bactericidal action of malic acid against the studied microorganisms was found to be depended on kind of microorganism, kind of juice, storage temperature, storage time and malic acid concentration.

Effect of the kind of bacteria on bactericidal action: As per the results of the present study, compared to *E. coli*, other two tested bacteria were more sensitive to the bactericidal action of malic acid. MBCs of malic acid after storage at 5°C for 24 h in Mango, Pineapple and Papaya Juices were 2.5, 2.0 and 2.5 for *E. coli* (Table 3), 0.6, 0.6 and 1.0 for *Listeria monocytogenes* (Table 1) and 0.8, 0.6 and 1.0 for *Salmonella enteritidis* (Table 2), respectively. Similar results were obtained for those juices which were stored for 24 h at 20 and 35°C as well. These results are according to the results reported by other authors for similar studies (Miller and Kasper, 1994; Arnold and Kasper, 1995; Benjamin and Dutta, 1995; Lin *et al*., 1996; Raybaudi-Massilia *et al*., 2009). Reason for these results could be the differences in membrane structures and acid tolerance of these bacteria. As reported by Nikaido (1996, 2003), the resistance mechanisms of Gram negative bacteria are more complicated than Gram positive bacteria due to the differences in their cell wall composition. This can explain the difference in malic acid tolerance of *Listeria monocytogenes* (Gram positive) and *E. coli* (Gram negative).

Effect of the type of fruit juice on bactericidal action: As previously explain by Raybaudi-Massilia *et al*. (2009), bactericidal action of malic acid is depends upon the initial pH of the fruit juices. In the present study, when consider the three tested fruit juices, a clear difference in bactericidal action were observed with respect to their initial pH values. Papaya juice, which had the lowest initial pH, showed the lowest bactericidal effect in all treatments (Table 1-3). At 0 h, highest MBC values for *Listeria monocytogenes* in Mango, Pineapple and Papaya juices were 1.0, 0.8 and 2.0, respectively. Similar results were shown for other bacteria at 0 h and for all bacteria at 24 h as well. Further, bacterial growth, from its initial concentration 10^8 CFU mL^{-10}, was only observed in Papaya juice. Hence, initial pH of fruit juice should be considered as an important factor when organic acids are used to control microorganisms in fruit juices.

Effect of malic acid concentration on bactericidal action: Malic acid has been identified as an organic acid which has low lipid solubility (Leo *et al*., 1971). As such, it has low ability to enter into bacterial cells through their cell walls as these cell walls are impermeable to polar compounds (Lucke, 2003). However, some other authors argue that the entry of organic acid to cells is depended on their molecular weight. As explained by Eswaranandam *et al*. (2004), malic acid has high ability to enter into bacterial cells as it is a smaller molecule compared to some other organic acids. Raybaudi-Massilia *et al*. (2009) has observed entry of malic acid into bacterial cells and damage their cytoplasm by Transmission Electron Microscopy (TEM). In the present study, in general, significant inhibition and bactericidal effects of malic acid were observed against all tested bacteria in all tested fruit juices. Those effects of malic acid were shown to be increased with the increase of the maic acid concentration (Table 1-3).

Effect of storage time on bactericidal action: Storage time had significant effect on bactericidal action of malic acid against all tested bacteria in all tested fruit juices. The concentrations of mailc acid needed to reduce the bacterial population in to an undetectable level after 24 h storage were lower than that of just after inoculation of bacteria in to fruit juices (Table 1-3). As an example, MBC of *Listeria monocytogenes* in Pineapple juice at 0 and 24 h were 0.8 and 0.4 (Table 1). All other treatments were also shown similar results. Malic acid needs time to enter into bacterial cells trough cell membranes and damage those. This is the reason for higher antibacterial action of malic acid at 24 than 0 h.

Effect of storage temperature on bactericidal action: As per the results of the present study, after storage at 5°C for 24 h, MBCs of Malic acid for *Listeria monocytogenes* were 0.6, 0.6 and 1.0 for Mango, Pineapple and Papaya juices, respectively. Those values after storage at 20°C for 24 h were 0.4, 0.4 and 0.8, respectively. Those values after storage at 35°C for 24 h were 0.4, 0.4 and 0.6, respectively (Table 1). As per these results, a clear effect of storage temperature on MBC of malic acid against *Listeria monocytogenes* was observed in this study. Similar results were obtained for other two tested bacteria as well. At low storage temperatures, high malic acid concentration was needed to control bacteria in to undetectable level. At low temperatures phospholipids in bacterial cell membranes are closely packed in to rigid structure but in high temperatures those are arrange in less ordered manner. As explained by Aronsson and Ronner (2001), this could be the reason for better bactericidal action of malic acid in high temperatures. In high temperatures bacterial cell membranes could favor the entry of maic acid in to the cells.

CONCLUSION

No bacterial growth was observed in control fruit juice samples which were without addition of malic acid, except Papaya juice stored at 20 and 35°C for 24 h. However, with the increase of malic acid concentration, the detected bacterial numbers were decreased at all conditions. This indicates the inhibition effect of malic acid to the bacterial growth. Population of all tested bacteria could be reduced to undetectable limit by addition of maic acid. Minimum Bactericidal Concentrations (MBCs) were established for all tested bacteria. The inhibition and bactericidal action of malic acid was depended upon the type of fruit juice, type of bacteria, storage type and storage temperature. Out of the three tested bacteria *E. coli* was found to be the most tolerant against malic acid. Hence, it can be used as a test organism in testing organic acids to use as fruit juice preservatives. Further studies should be carried out to evaluate the effect of malic acid to the sensory properties of fruit juices.

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