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Antibacterial Effects of Monolaurin, Sorbic Acid and Potassium Sorbate on *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Background: Food borne pathogens are of the main concerns of food producers and consumers and *Escherichia coli* and *Staphylococcus aureus* create a lot of problems worldwide. The aim of this study was to evaluate antibacterial effects of monolaurin, sorbic acid and potassium sorbate on *S. aureus* and *E. coli* at different pH values and NaCl concentrations.
Methods: Micro-well dilution assay was used to determine antimicrobial potency of monolaurin, sorbic acid and potassium sorbate. First, stock solutions of each antimicrobial compounds were prepared and then two-fold dilution method was used to obtain final concentrations of tested antimicrobials. A 96 well microplate was inoculated with different concentrations of antimicrobials and bacterial inoculums (final inoculums was approximately 5×10^5 CFU/ml). After incubation, growth of *E. coli* and *S. aureus* were evaluated. Statistical analysis was made by the analysis of variance using SPSS software, version 16.0.
Results: The MICs of monolaurin, sorbic acid and potassium sorbate were respectively >4000, >5000, >10000 µg/ml for *E. coli*, and 128, 1250 and 2500 µg/ml for *S. aureus*. The results showed that all of these compounds had considerable effect on *S. aureus* while *E. coli* was less sensitive. It should be noted that, monolaurin had strong antimicrobial effect on *E. coli* when used in combination with ethylene diamine tetra acetic acid.
Conclusion: According to the results of this study, monolaurin and sorbates can be used effectively as food preservative and growth inhibitor of these food borne pathogens. Using NaCl and/or lower pH values may fortify their bacteriostatic effects.

Introduction

Food can contain a variety of microorganisms such as bacteria, yeasts and moulds which have been reported as the causal agents of food borne diseases and/or food spoilage (Betts et al., 1999). *Staphylococcus* spp., especially *Staphylococcus aureus* is one of the most common causes of food related diseases throughout the world (Boughattas and Salehi, 2014; Hennekinne et al., 2012). Many types of food (milk, dairy products, chicken, and meat) are associated with *S. aureus* related diseases (Chao et al., 2007). Nowadays, several strains of *Escherichia coli* are considered as food borne pathogens and the strains belongs to the subgroups of enteropathogenic and enterotoxigenic *E. coli* produce gastroenteritis and mild or severe diarrhea when contaminated food or water is ingested (Jay et al., 2005). Also, shigatoxigenic *E. coli*, including *E. coli* $_{O157:H7}$, produce heat resistant toxins which are not inactivated by pasteurization treatment (Rasooly and Do, 2010).

Due to the known side effects of chemical and synthetic

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antimicrobial agents and emerging bacterial resistance to current antibiotics, a widespread trend in the world is the movement towards ‘‘natural’’ food products. So, there is an increasing interest in the food industry for using antimicrobial preservatives that are perceived as more ‘‘natural’’ (Jay et al., 2005).

Monolaurin (glycerol monolaurate) is a Generally Regarded As Safe (GRAS) emulsifier which has been used as the most effective antimicrobial substance among fatty acid derivatives (Jay et al., 2005; Trotter and Marshal, 2003). Monolaurin and other mono esters fatty acids are the lipophilic substances and the inhibitory effect of monolaurin is due to interference with cytoplasmic membrane of microorganisms (Ruzicka et al., 2003). Ethylene Diamine Tetra Acetic acid (EDTA) as a chelating agent has been widely used in a variety of food products to prevent oxidation and other deteriorative reactions catalyzed by metal ions (Branen and Davison, 2004; Kopermsub et al., 2011). Sorbic acid and most of its water-soluble salts (known as sorbates) are active against yeasts and moulds, as well as many bacteria. Several bacterial species including *E. coli* and *S. aureus* have been inhibited by sorbate (Koodie and Dhople, 2001). In addition to food-grade antimicrobials, intrinsic factors of the food, such as pH value and NaCl concentration can have a significant controlling effect on bacteria (Doores, 1993).

The aim of the present study was to determine antimicrobial effects of monolaurin, sorbic acid and potassium sorbate against selected Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) food borne bacteria at different pH values and NaCl concentrations.

Materials and methods

Antimicrobial materials

To prepare monolaurin and EDTA stock solutions, monolaurin (Lauricidin, Galena, IL) was dissolved in 95% (w/v) ethanol (Sigma-Aldrich) and Disodium EDTA (Fisher, Fairlawn, NJ) was dissolved in distilled water and autoclaved at 121 $^{\circ}$C. Sorbic acid ($C_6H_8O_2$) and potassium sorbate ($C_6H_7KO_2$) obtained from Sigma-Aldrich Co. Monolaurin stock solutions were prepared before each experiment and sterilized using a 0.45 µm pore-size membrane filter.

Microorganisms

Escherichia coli$_{O157:H7}$ ATCC 43895 and *Staphylococcus aureus* ATCC 6538 were obtained from the culture collection of food hygiene department of Urmia university, Urmia, Iran. All cultures were maintained on Brain Heart Infusion Agar (BHIA; Oxoid, UK) slants at 41 ºC. An overnight before use, cultures were transferred to Brain Heart Infusion Broth (BHIB; Oxoid, UK) and incubated at 37 ºC to obtain a working culture. Suspensions of the bacteria were made in sterile water and adjusted to 0.5-unit of the McFarland scale (1-2×10^8 CFU/ml).

Antimicrobial susceptibility testing

Minimal Inhibitory Concentration (MIC) of antimicrobials against bacterial strains was determined based on the micro-well dilution method (Basti et al., 2007; Millet et al., 2006). Concentrations of each antimicrobial were as follows (µg/ml): monolaurin, 125, 250, 500, 1000, 2000, 4000 (for *E. coli*$_{O157:H7}$) and 8, 16, 32, 64, 128 (for *S. aureus*); EDTA, 125, 250, 500, 1000, 2000, 4000 (for *E. coli*$_{O157:H7}$) and 8, 16, 32, 64, 128 (for *S. aureus*); sorbic acid, 78.1, 156.25, 312.5, 625, 1250, 2500, 5000; potassium sorbate, 78.1, 156.25, 312.5, 625, 1250, 2500, 5000, 10000. Appropriate quantities of antimicrobial stock solutions were added to BHIB to reach final concentrations using two-fold dilution method. Micro titer plates with 300 µl wells were used in this study. For each mixture, 190 µl of each antimicrobial and 10 µl of inoculums diluted in BHIB were used (final inoculum was approximately 5×10^5 CFU/ml). Each analysis was performed in triplicate. Positive controls was inoculated in BHIB containing ethanol without antimicrobial and negative controls was uninoculated BHIB in order to determine sterility. The pH of mixture was adjusted to 5, 6 and 7 using HCl or NaOH. Also the effective NaCl concentration for antimicrobials inactivation of bacterial strains was determined by 3% and 6% NaCl. MIC values were determined by incubating the plate at 37 $^{\circ}$C for 24 h. After incubation, the broth was visually observed for turbidity. Turbid broths were scored as positive (+) for growth, whereas non-turbid broth was scored as negative (-) for no growth. The lowest concentration of antimicrobial that prevented growth was considered as the MIC of antimicrobials in BHIB for each strain.

Statistical analysis

Statistical analysis of the data was made using the analysis of variance (ANOVA) of the SPSS software, version 16.0. All experiments were carried out in triplicate.

Results

According to the results, MIC values were varied, depending on the kind of preservative and type of microorganism tested. MICs for monolaurin alone and in combination with EDTA, sorbic acid and potassium sorbate are given in Table 1. Monolaurin alone inhibited *S. aureus* at a level of 128 µg/ml but did not inhibit *E. coli*. The strongest antibacterial activity against *S. aureus* was manifested by combination of monolaurin and EDTA (32 µg/ml). Monolaurin and EDTA acted synergistically against both bacteria. Sorbic acid and potassium sorbate inhibited *S. aureus* at levels of

1250 and 2500, respectively. However, no inhibitory effect was observed on *E. coli* at maximum concentrations. As shown in Table 2, antimicrobial effects of the examined compounds had a reverse relative with the pH level ($p<0.05$). Also increase in NaCl concentration, leads to decrease ($p<0.05$) of MICs for all antimicrobials (Table 3).

Table 1: MICs (µg/ml) at 24 h for monolaurin, monolaurin+EDTA, sorbic acid and potassium sorbate in BHIB

Strain	MIC (µg/ml)[a]			
	Monolaurin	**Monolaurin+EDTA**	**Sorbic acid**	**Potassium sorbate**
S. aureus	128	32	1250	2500
E. coli	>4000	2000	>5000	>10000

[a]MICs are concentrations where the average from three replicate wells were not greater than uninoculated controls

Table 2: MICs (µg/ml) at 24 h for monolaurin, sorbic acid and potassium sorbate at different levels of pH in BHIB

Strain	pH	MIC (µg/ml)[a]		
		Monolaurin	**Sorbic acid**	**Potassium sorbate**
S. aureus	5	16	156.25	156.5
	6	64	312.5	625
	7	128	1250	2500
E. coli	5	500	312.5	625
	6	2000	1250	1250
	7	>4000	>5000	>10000

[a]MICs are concentrations where the average from three replicate wells were not greater than uninoculated controls

Table 3: MICs (µg/ml) at 24 h for monolaurin, sorbic acid and potassium sorbate with different levels of NaCl in BHIB

Strain	NaCl (%)	MIC (µg/ml)[a]		
		Monolaurin	**Sorbic acid**	**Potassium sorbate**
S. aureus	3	64	1250	2500
	6	32	625	312.5
E. coli	3	2000	2500	5000
	6	500	625	1250

[a]MICs are concentrations where the average from three replicate wells was not greater than uninoculated controls

Discussion

Food borne diseases occur frequently all over the world and *E. coli* and *S. aureus* are among the most important causes of these diseases (Dinges et al., 2000). Different natural and chemical antimicrobials have been used to inhibit food borne microorganism's growth. As observed in Table 1, monolaurin in combination with EDTA had the strongest effect on tested bacteria ($p<0.05$), followed by sorbic acid and potassium sorbate.

Monolaurin destroy cell structure by its lipophilic characteristics (Branen and Davidson, 2004; Delamare et al., 2007). According to our results, Gram positive tested bacterium (*S. aureus*) was more sensitive to all tested antimicrobials. In Gram-positive bacteria, peptidoglycan forms the outer membrane of the cell but in Gram-negative bacteria the cell structure is different and peptidoglycan layer lies between plasma membrane and lipopolysaccharide outer membrane; therefore antimicrobials cannot pass through outer layer of Gram-negative bacteria easily (Branen and Davidson, 2004). As described in the result section, EDTA in combination with monolaurin, fortified monolaurins antibacterial effect and affected both Gram-positive and negative bacteria. Results obtained from the current study match with the results of previous researches demonstrating that combination of EDTA with monolaurin and other antimicrobials enhance bactericidal effect of tested antimicrobials (Hansen et al., 2001; Oh and Marshal, 1994; Razavi Rohani and Griffiths, 1994). Previously, it has been shown that the antimicrobial spectrum and activity of monolaurin can be increased in the presence of organic acids (Oh and Marshall, 1994), EDTA and NaCl (Razavi Rohani and Griffiths, 1994). *S. aureus* was more sensitive to ascorbic acid and potassium sorbate than *E. coli*. This property is related to difference in cell structure of these two bacteria. The use of intrinsic factors such as pH values and NaCl concentrations in combination with a GRAS antimicrobial agent may increase the antimicrobial activity and spectrum of the agent through synergism (Lieberman et al., 2006). Oh and Marshall (1994) determined that combination of low pH and high temperature increases the antimicrobial effect of monolaurin against *Listeria monocytogenes*; a Gram-positive psychrotrophic bacterium. In this study, three different pH values were considered and results showed that antimicrobials had the strongest effect in the lowest pH level ($p<0.05$). It is known that antimicrobials are more effective in lower pH values because of cellular changes and damages that occur in these conditions (Oh and Marshal, 1994). Monolaurin, sorbic acid and potassium sorbate had stronger antimicrobial activity in the presence of 6% NaCl in comparison with 3% concentration. Our results are in agreement with previous results that showed antimicrobial activity of chemical and natural antimicrobials greatly increased in presence of NaCl (Lieberman et al., 2006; Oh and Marshal, 1994; Razavi Rohani and Griffiths, 1994).

Conclusion

In conclusion, monolaurin, sorbic acid and potassium sorbate can be used for inhibiting the growth of these food borne pathogens and consequently for food preservation. Also, their antimicrobial effects could be enhanced in the presence of NaCl and low pH.

Conflicts of interest

The authors declare no conflicts of interest.

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