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Effect of Monolaurin Alone and in Combination with EDTA on Viability of *Escherichia coli* and *Staphylococcus aureus* in Culture Media and Iranian White Cheese

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Abstract

Background: *Escherichia coli* and *Staphylococcus aureus* as important food-borne pathogens are the main concerns of food producers and consumers which create a lot of problems worldwide. The objective of this study was to investigate the inhibitory effect of monolaurin alone and in combination with EDTA on viability of *E. coli* and *S. aureus* in culture media and Iranian white cheese.

Methods: The minimum inhibitory concentration of monolaurin and EDTA was determined by broth microdilution susceptibility test. In the next stage, 10^8 CFU/g of *E. coli* and *S. aureus* and different concentrations of monolaurin and EDTA were added. Samples were maintained at 4 °C for 9 days. Antibacterial effect of monolaurin and EDTA was evaluated on days 0, 1, 3, 5, 7 and 9 by the specific media. Statistical analysis was made using analysis of variance (ANOVA) by SPSS program (16.0) to compare means with a significant difference when $p<0.05$.

Results: Monolaurin alone in both *in vitro* and *in vivo* condition had limited effect on *E. coli* growth but this effect was increased when it was used in combination with EDTA ($p<0.05$). Monolaurin showed strong antibacterial effect on *S. aureus* which was increased significantly when used in combination with EDTA ($p<0.05$). Preservation time had significant effect on antibacterial effect of monolaurin on both pathogenic bacteria ($p<0.05$).

Conclusion: Monolaurin can be used in combination with EDTA to decrease contamination risks and growth of pathogenic bacteria in Iranian white cheese.

Introduction

Iranian white cheese is a soft cheese in which curd is made mainly through the action of chymosin or other milk-clotting enzymes on milk at pH>6.2 (Neyriz-Nagadehi et al., 2012). It is highly susceptible to be infected by pathogenic and spoilage microorganisms due to some characteristics such as the presence of large amounts of nutrients and special manufacturing process (Cao-Hoang et al., 2010).

Food safety is one of the main concerns of food consumers and producers. Although today's hygienic conditions of food processes has improved but these controlling methods don't decrease food-borne diseases (Cao-Hoang et al., 2010). *Escherichia coli* and *Staphylococcus aureus* are

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important agents causing infection by consumption of dairy products especially cheese (Jay, 2000). *S. aureus* is the most common entero-toxigenic species causing food-borne disease and is considered the third most important cause of disease in the world among the reported food-borne illnesses (Jamshidi et al., 2014). Many strains of *E. coli* especially strains belong to enterotoxigenic (ETEC) and enterohaemorrhagic (EHEC) subgroups produce toxins and cause gastroenteritis and diarrhea, and therefore are considered as food-borne pathogens (Amin Zare et al., 2014).

Monolaurin is a non-ionic surfactant which is produced by the reaction of lauric acid and glycerol and has different applications in pharmaceutical, food industry and cosmetics production due to its safety properties and easy metabolized characteristic (Blaszyk and Holley, 1998). It has antiviral and antimicrobial properties in addition to emulsifying characteristic (Ruzicka et al., 2003). Its antibacterial effects on wide range of microorganisms are reported by several researchers such as prevention of exotoxin production by Gram-positive bacteria including *S. aureus*, *β-Hemolytic Streptococci* and *Bacillus anthracis* (Pechous et al., 2004). Even if, monolaurin has useful effects in food preservation; it has limited effect on growth inhibition of Gram-negative bacteria. Gram-negative bacteria has resistant lipopolysaccharide layer but the antimicrobial activity of monolaurin is increased in presence of metal chelators such as Ethylene Diamine Tetra Acetic acid (EDTA) (Kabara, 1984). EDTA may change structure of the outer membrane of Gram-negative bacteria through combination with cationic bridges between lipopolysaccharide layer and peptidoglycan of bacteria which lead to damage of lipopolysaccharide layers resulted in increasing of the cell permeability (Mclay, 2007; Dufoar et al., 2007).

The aim of this experimental study was to investigate the inhibitory effect of monolaurin alone and in combination with EDTA on viability of *E. coli* and *S. aureus* in culture media and Iranian white cheese.

Materials and methods

Chemicals

Monolaurin (lauricidin Inc), EDTA, rennet, calcium chloride and ethanol were all purchased from Sigma chemical Co. St. Louis, Mo., USA.

Bacterial strains

Lyophilized cultures of targeted organisms including a Gram-negative (*E. coli* ATCC 25922) and a Gram-positive (*S. aureus* ATCC 1885) bacterium were obtained from the culture collection of the Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, North-West of Iran.

In vitro analysis of antibacterial effects of Monolaurin alone and in combination with EDTA

Minimal Inhibitory Concentration (MIC) values of antimicrobials were determined based on the broth micro dilution susceptibility test. First, antimicrobial solutions were diluted to the highest concentration (2000 µg/ml) as stock solution (solvents of monolaurin and EDTA were ethanol and distilled water, respectively), and then serial two-fold dilutions were made in a concentration range from 125 to 2000 µg/ml in Brain Heart Infusion Broth (BHIB) media (Sigma chemical Co. St. Louis, Mo., U.S.A). A 96 well microplate was prepared dispensing 160 µl of BHI broth and 20 µl of the inoculums in each well (bacterial suspensions were adjusted to 0.5 McFarland standard turbidity and final inoculums were approximately 10^8 CFU/ml). A 20 µl aliquot from the stock solution of antimicrobial agents was added into the first wells. Then, 20 µl from their serial dilution was transferred into consecutive wells. Positive controls consisted of inoculated BHIB without antimicrobial agent and the negative controls consisted of un-inoculated BHIB along with antimicrobials agents were considered in order to determine sterility. The lowest concentration of monolaurin that inhibited growth of interested bacteria was considered as MIC. To determine the MIC of monolaurin in combination with EDTA, 20 µl of each antimicrobial agent with concentrations of 125, 250, 500, 1000 and 2000 µg/ml, with140 µl of BHIB and 20 µl of inoculums were added into each well. Results were assessed after incubation at 37 °C for 24 h. All experiments were performed in triplicate.

In vivo analysis of antibacterial effects of monolaurine alone and in combination with EDTA

To prepare Iranian white cheese, at first 3 liters milk were heated at 76 °C for 2 min and cooled at room temperature, then 1.2 g calcium chloride were added in container. In the next step, 200 ml aliquot of milk was added in 9 separate sterilized containers. Container number one was considered as control group without monolaurin. Two ml of various concentrations of monolaurin (250, 500, 1000 and 2000 µg/ml) alone were added to containers number two till five and various concentrations of monolaurin (250, 500, 1000 and 2000 µg/ml) along with equal amounts of EDTA were added to containers number six to nine as well. Then, 2 ml of inoculums containing 10^8 CFU/ml bacteria (adjusted by 0.5 McFarland standard turbidity) and 2 ml of rennet were added in containers. After 30 min, the clot of the cheese formed and whey was extracted under pressure. Finally samples were stored at 4 °C and analyzed on days 0, 1, 3, 5, 7 and 9 of storage (Neyriz-Nagadehi et al., 2012). All experiments were performed in triplicate.

In order to samples analysis, 1 g of each specimen was added to sterile tubes containing 9 ml normal saline. After homogenizing, 1 ml of homogenate was inoculated on spe-

cific culture media (Violet Red Bile agar for counting *E. coli* and Baird- Parker agar for counting *S. aureus*). Culture media were incubated at 37 °C for 48 h and then enumeration of bacterial number in ml was carried out.

Statistical analysis

All data were expressed as mean±standard deviations (SD) of three measurements. Statistical analysis of the data was made using the analysis of variance (ANOVA) of the SPSS program, version 16.0. Means with a significant difference (p<0.05) were compared by Duncan's post hoc test.

Results

MIC values of monolaurin against *E. coli* and *S. aureus* were 2000 and 250 µg/ml, respectively and MIC values of monolaurin in combination with EDTA against *E. coli* and *S. aureus* were determined 1000 and 125 µg/ml, respectively.

Tables 1-4 represent changes in *E. coli* and *S. aureus* counts in Iranian white cheese containing monolaurin alone and along with EDTA.

Results of this work showed that higher concentrations of monolaurin had strong effect on reducing the growth rate of *S. aureus* in Iranian white cheese and this effect was increased significantly (p<0.05) in presence of EDTA. Also, its antibacterial effect on growth inhibition of *E. coli* was significantly increased in presence of EDTA as well, while it had no noticeable effect when it was used alone in Iranian white cheese.

Table 1: *E. coli* count (log CFU/g) changes (Mean±SD) in Iranian white cheese samples containing monolaurin during storage at 4 °C

Concentrations of monolaurin (µg/ml)	Storage time (Day)					
	0	1	3	5	7	9
0 (Control)	6.05 ± 0.15^{DE}	8.16 ± 0.1^{BCDE}	8.34 ± 0.2^{BCDE}	9.4 ± 0.1^{BCDE}	10.44 ± 0.1^{BCDE}	10.47 ± 0.2^{BCDE}
250	5.96 ± 0.1	7.11 ± 0.12^{AE}	7.23 ± 0.25^{A}	7.34 ± 0.2^{AE}	$8.38\pm^{A}0.1^{A}$	9.45 ± 0.12^{ADCE}
500	5.9 ± 0.06	7.1 ± 0.15^{AE}	7.33 ± 0.12^{A}	7.38 ± 0.1^{AE}	7.48 ± 0.2^{AE}	8.41 ± 0.1^{ABE}
1000	5.81 ± 0.1^{A}	7.2 ± 0.1^{AE}	7.25 ± 0.15^{A}	7.12 ± 0.22^{AC}	7.32 ± 0.2^{A}	8.34 ± 0.1^{AB}
2000	5.73 ± 0.2^{A}	6.1 ± 0.1^{ABCD}	7.21 ± 0.5^{A}	7.05 ± 0.2^{ABC}	7.12 ± 0.1^{AC}	8.14 ± 0.2^{ABC}

- Letter A in same column showed significant difference with control group (p<0.05)
- Letter B in same column showed significant difference with group containing 250 µg/ml monolaurin (p<0.05)
- Letter C in same column showed significant difference with group containing 500 µg/ml monolaurin (p<0.05)
- Letter D in same column showed significant difference with group containing 1000 µg/ml monolaurin (p<0.05)
- Letter E in same column showed significant difference with group containing 2000 µg/ml monolaurin (p<0.05)

Table 2: *E. coli* count (log CFU/g) changes (Mean±SD) in Iranian white cheese samples containing monolaurin in combination with EDTA during storage at 4 °C

Concentrations of monolaurin and EDTA (µg/ml)	Storage time (Day)					
	0	1	3	5	7	9
0 (Control)	6.05 ± 0.15^{BCDE}	8.16 ± 0.1^{BCDE}	8.34 ± 0.2^{BCDE}	9.4 ± 0.1^{BCD}	10.44 ± 0.1^{BCDE}	10.47 ± 0.2^{BCDE}
250	5.68 ± 0.1^{AE}	5.78 ± 0.22^{AE}	6.93 ± 0.15^{ACDE}	7.04 ± 0.1^{ACD}	7.07 ± 0.12^{ACDE}	8.14 ± 0.06^{ADE}
500	5.38 ± 0.15^{AE}	5.63 ± 0.06^{A}	5.79 ± 0.15^{AB}	6.94 ± 0.2^{AB}	6.94 ± 0.25^{ABDE}	8.06 ± 0.12^{ADE}
1000	5.25 ± 0.21^{A}	5.43 ± 0.06^{A}	5.64 ± 0.1^{AB}	5.78 ± 0.14^{AB}	5.84 ± 0.17^{ABC}	6.82 ± 0.1^{ABC}
2000	5.11 ± 0.25^{AB}	5.28 ± 0.12^{AB}	5.44 ± 0.15^{AB}	5.67 ± 0.1^{AB}	5.88 ± 0.12^{ABC}	6.13 ± 0.25^{ABC}

- Letter A in same column showed significant difference with control group (p<0.05)
- Letter B in same column showed significant difference with group containing 250 µg/ml monolaurin and EDTA (p<0.05)
- Letter C in same column showed significant difference with group containing 500 µg/ml monolaurin and EDTA (p<0.05)
- Letter D in same column showed significant difference with group containing 1000 µg/ml monolaurin and EDTA (p<0.05)
- Letter E in same column showed significant difference with group containing 2000 µg/ml monolaurin and EDTA (p<0.05)

Table 3: *S. aureus* count (log CFU/g) changes (Mean±SD) in Iranian white cheese samples containing monolaurin during storage at 4 °C

Concentrations of monolaurin (µg/ml)	Storage time (Day)					
	0	1	3	5	7	9
0 (Control)	5.92 ± 0.15^{DE}	7.13 ± 0.1^{BCDE}	8.33 ± 0.2^{BCDE}	9.35 ± 0.1^{BCDE}	10.36 ± 0.1^{BCDE}	11.34 ± 0.1^{BCDE}
250	5.57 ± 0.1	5.93 ± 0.25^{ADE}	6.97 ± 0.2^{ADE}	8.07 ± 0.1^{ABCDE}	9.13 ± 0.12^{ACDE}	10.2 ± 0.2^{ACDE}
500	5.55 ± 0.15	5.75 ± 0.06^{AD}	6.8 ± 0.12^{AD}	6.85 ± 0.2^{AB}	7.95 ± 0.15^{ABDE}	9.08 ± 0.25^{ABDE}
1000	5.32 ± 0.2^{A}	5.54 ± 0.06^{ABC}	5.61 ± 0.2^{ABC}	6.75 ± 0.1^{AB}	6.87 ± 0.06^{ABC}	7.96 ± 0.15^{ABC}
2000	5.1 ± 0.1^{A}	5.51 ± 0.2^{ABC}	6.28 ± 0.25^{ABCD}	6.6 ± 0.15^{AB}	6.81 ± 0.08^{A}	7.85 ± 0.2^{ABC}

- Letter A in same column showed significant difference with control group ($p<0.05$)
- Letter B in same column showed significant difference with group containing 250 µg/ml monolaurin ($p<0.05$)
- Letter C in same column showed significant difference with group containing 500 µg/ml monolaurin ($p<0.05$)
- Letter D in same column showed significant difference with group containing 1000 µg/ml monolaurin ($p<0.05$)
- Letter E in same column showed significant difference with group containing 2000 µg/ml monolaurin ($p<0.05$)

Table 4: *S. aureus* count (log CFU/g) changes (Mean±SD) in Iranian white cheese samples containing monolaurin in combination with EDTA during storage at 4 °C

Concentrations of monolaurin and EDTA (µg/ml)	Storage time (Day)					
	0	1	3	5	7	9
0 (Control)	5.92 ± 0.15^{DE}	7.13 ± 0.1^{BCDE}	8.33 ± 0.2^{BCDE}	9.35 ± 0.1^{BCDE}	10.36 ± 0.1^{BCDE}	11.34 ± 0.1^{BCDE}
250	5.94 ± 0.06	5.86 ± 0.1^{ACE}	6.92 ± 0.12^{ACDE}	6.69 ± 0.25^{ACDE}	8.1 ± 0.17^{ACDE}	8.15 ± 0.2^{ACDE}
500	5.89 ± 0.12	5.32 ± 0.15^{ABD}	5.53 ± 0.15^{ABE}	5.23 ± 0.1^{ABE}	6.92 ± 0.06^{ABDE}	7.11 ± 0.12^{ABDE}
1000	5.78 ± 0.25^{A}	5.61 ± 0.2^{ABC}	5.57 ± 0.1^{ABE}	5.05 ± 0.15^{ABE}	5.27 ± 0.17^{ABCE}	5 ± 0.2^{ABCE}
2000	5.62 ± 0.2^{A}	5.3 ± 0.1^{ABC}	4.92 ± 0.2^{ABCD}	4.78 ± 0.1^{ABCD}	4.63 ± 0.15^{ABCD}	4.34 ± 0.2^{ABCD}

- Letter A in same column showed significant difference with control group ($p<0.05$)
- Letter B in same column showed significant difference with group containing 250 µg/ml monolaurin and EDTA ($p<0.05$)
- Letter C in same column showed significant difference with group containing 500 µg/ml monolaurin and EDTA ($p<0.05$)
- Letter D in same column showed significant difference with group containing 1000 µg/ml monolaurin and EDTA ($p<0.05$)
- Letter E in same column showed significant difference with group containing 2000 µg/ml monolaurin and EDTA ($p<0.05$)

Discussion

The results obtained from this study indicated that monolaurin manifested strong activity on growth inhibition of *S. aureus* but did not inhibited growth of *E. coli*. However, the use of monolaurin in combination with EDTA had appropriate antibacterial effect against *E. coli.* These results are in agreement with the findings of other researchers (Bautista et al., 1993; Branen and Davidson, 2004; Oh and Marshal, 1993).

Razavi Rohani and Griffiths (1994) showed that monolaurin had effect on all of the Gram-positive bacteria such as *S. aureus* and *Listeria monocytogens,* but the effect of monolaurin on Gram-negative bacteria such as *E. coli* and *Salmonella typhimurium* were only observed when it was used in combination with EDTA. Branen and Davidson (2004) revealed that using EDTA could increase antibacterial effect of monolaurin as EDTA and monolaurin interacted additively against targeted microorganisms. In another study, Bautista et al. (1993) reported that MIC of monolaurin on Gram-positive bacteria is variable from 8 µg/ml for *Lactococcus lactis* to 96 µg/ml for *L. monocytogens* and it is not capable to prevent the growth of Gram-negative tested bacteria at concentrations less than 3170 µg/ml.

Antimicrobial effect of monolaurin was increased significantly when equal amount of EDTA was added to the medium. Mechanisms of antimicrobial action of monolaurin which is a lipophilic component are excretion of interacellular proteins, destroying outer membrane or cytoplasmic membrane, inhibiting synthesis of macromolecules and denaturation of proteins and DNA (Kabara, 1984). It has been proven that components such as monolaurin that cause ion transportation through the cell membrane are more effective against Gram-positive bacteria than Gram-negative (Hansen et al., 2001).

The results obtained from this study showed that using EDTA in combination with monolaurin cause appropriate effect on growth prevention of *E. coli*. In general, chelating agents such as EDTA can increase cell permeability because of their effects on outer membrane of the bacterial cells. Therefore, efficient effect of monolaurin against Gram-negative bacteria can be observed (Marounek et al., 2003). Referring to results of this study, concentration of monolaurin that inhibited growth of *S. aureus* in cheese was variable from 250 to 2000 µg/ml. As reported by several investigators, effect of antimicrobial agents in food system is lower than *in vitro* condition because of

combination of antimicrobials with food components (Devlieghere et al., 2004; Petrou et al., 2012). The results showed that monolaurin only in combination with EDTA could inhibit growth of *E. coli* in Iranian white cheese.

Conclusion

Regarding to the results of this study, monolaurin is a natural component with efficient antibacterial effect and EDTA can enhance its antibacterial effect if it is used with in combination with monolaurin in culture media and in Iranian white cheese. Therefore, they can be used in Iranian white cheese to increase its shelf life.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Amin Zare M., Razavi Rohani S.M., Raeisi M., Javadi Hosseini S.H., Hashemi M. (2014). Antibacterial effects of monolaurin, sorbic acid and potassium sorbate on *Staphylococcus aureus* and *Escherichia coli*. *Journal of Food Quality and Hazards Control*. 1: 52-55.

Bautista D.A., Durisin M.D., Razavi Rohani S.M., Hill A.R., Griffiths M.W. (1993). Extending the shelf life of cottage cheese using monolaurin. *Food Research International*. 26: 203-208.

Blaszyk M., Holley R.A. (1998). Intraction of monolaurin, eugenol and sodium citrate on growth of common meat spoilage and pathogenic organisms. *International Journal of Food Microbiology*. 39: 175-183.

Branen J.K, Davidson P.M. (2004) Enhancement of nisin, lysozime, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin. *International Journal of Food Microbiology*. 90: 63-74.

Cao-Hoang L., Chaine A., Gregoire L., Wache Y. (2010). Potential of nisin incorporated sodium caseinate films to control *Listeria* in artificially contaminated cheese. *Food Microbiology*. 27: 940-944.

Devlieghere F., Vermeulen A., Debevere J. (2004). Chitosan: antimicrobial activity, inter actions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology*. 21: 703–714.

Dufoar M., Manson J.M., Bremer P.J., Dufoar J.P., Cook G.M., Simmonds R.S. (2007). Characterization of monolaurin resistance in *Entrococcus faecalis*. *Applied Environmental Microbiology*. 9: 5507-5515.

Hansen L.T., Austin J.W., Gill T.A. (2001). Antibacterial effect of protamine in combination with EDTA and refrigeration. *International Journal of Food Microbiology*. 66: 149-161.

Jamshidi A., Khanzadi S., Azizi M., Aziz zadeh M., Hashemi M. (2014). Modeling the growth of *Staphylococcus aureus* as affected by black zira (*Bunium persicum*) essential oil, temperature, pH and inoculum levels. *Veterinary Research Forum*. 5(2): 107-114.

Jay M.J. (2000). Modern food microbiology. 2nd edition. Chapman and Hall, New York. pp: 114-118.

Kabara J.J. (1984). Inhibition of *Staphylococcus aureus* in a model agar meat system by monolaurin. *Food Safety*. 4: 13-25.

Marounek M., Skrivanova E., Rada V. (2003). Susceptibility of *Escherichia coli* to c2-c18 fatty acids. *Folia Microbiologica*. 48:731-735.

Mclay J.K. (2007). The effect of monolaurin and lactoperoxidase on *E. coli* O_{157}:H_7. *Food Microbiology*. 73: 11-19.

Neyriz-Nagadehi M., Razavi-Rohani S.M., Karim G., Zeynali A. (2012). Effects of monolaurin and lactic acid bacteria starter culture on growth of vegetative cells of *Bacillus cereus* in Iranian white fresh cheese. *Iranian Journal of Veterinary Science and Technology*. 4 (1): 75-84.

Oh D.H., Marshal D.L. (1993). Antimicrobial activity of ethanol, glycerol monolaurate or lactic acid against *Listeria monocytogenes*. *International Journal of Food Microbiology*. 20: 239-246.

Pechous R., ledola N., Wilkinson B.J., Jayaswal R.K. (2004). Regulation of the expression of cell wall stress stimuli on member gene *msrA1* in methicillin susceptible or resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*. 48: 3057-3063.

Petrou S., Tsiraki M., Giatrakou V., Savvidis I.N. (2012). Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. *International Journal of Food Microbiology*. 156: 264-271.

Razavi Rohani S.M., Griffiths M.W. (1994). The effect of mono and polyglecerol laurate on spoilage and pathogenic bacteria associated with food. *Food Safety*. 14: 131-151.

Ruzicka J., Velclova K., Janis R., Krejci J. (2003). Antimicrobial effects of 1-monoacylglycerols prepared by catalytic reaction of glycerol with fatty acids. *European Food Research Technology*. 217(4): 329-331.