The Effects of Monolaurin on Ecoli O157:H7 Growth in Dairy Food Products

Esghjoo S*, Mahdavi S, Daustani M, Ahmadi R, Razavi SM, and Neyriz M

Abstract—By increasing of public interest in natural foods, manufacturers are urged to develop preservative systems based on natural compounds. This study was exerted to determine the effects of monolaurin, also known as glycerol monolaurate, on E.coli O157:H7 growth in dairy food products [Iranian cheese].

In this laboratory experimental study 1ml of E.coli containing 10⁶ CFU/ml was inoculated to 1000ml milk. Cheeses were divided to control, 250, 500, 1000, 1500 and 2000 ppm monolaurin containing groups. Bacteria growth was measured by spectrophotometry method 1, 5, 10 and 14 days after processing cheese. Data were analyzed using one-way ANOVA and student’s t-test. Bacteria number in cheeses containing 250 and 500 ppm of monolaurin was not significantly different from bacteria number in control group, while bacteria number was significantly lower in cheeses containing 1000, 1500, and 2000 ppm of monolaurin compared with control cheese (P<0.001). Our findings clearly indicate that monolaurin has significant inhibitory effect on Escherichia coli growth in dairy products.

Keywords— Monolaurin, Escherichia coli O157: H7, Iranian white cheese.

I. INTRODUCTION

FOODBORNE illnesses are a significant problem and a major public health concern [1]. There is a growing consumer demand for natural methods of controlling micro-organisms that may be associated with food. A large range of natural antimicrobials has been reported as useful, including enzyme systems such as lysozyme [2], ethylene diaminetetraacetic acid [EDTA] [3], organic acids (lactic, acetic, etc.) and essential oils from plants [4]. Fatty acids and their esters serve both as flavouring and emulsifying agents but are also potential antimicrobials [5]. Monolaurin, a food grade glycerol monoester of lauric acid, has been reported to have the greatest antimicrobial activity of all of the monoglycerides [6]. However, its inhibitory properties have not been fully investigated. E. coli O157:H7 is one of many E. coli organisms that contain genes encoding one or more toxins similar in structure and function to Shiga toxin. E. coli O157:H7 is the most frequently isolated diarrheagenic type of E. coli isolated in North America today [7]. Enterohemorrhagic Escherichia coli O157:H7 was first identified as a human pathogen in 1982 [8] and since 1982 has been implicated in numerous outbreaks of hemorrhagic colitis and the life-threatening hemolytic uremic syndrome [9]. Multiple factors contribute to the pathogenicity of this serotype; these factors include the production of Shiga toxin type 1 and/or type 2, the eae genes, and a 60-MDa plasmid encoding adhesins and hemolysins [10]. Most outbreaks of E.coli O157:H7 infection have been linked to foods of bovine origin, such as undercooked ground beef and dairy products [11]. The purpose of the present study was to determine the effects of monolaurin on Escherichia O157:H7 growth in Iranian white cheese.

II. MATERIAL AND METHODS

A. Materials

Monolaurin was purchased from Med-chem Laboratories of USA. Escherichia coli O157-H7 was purchased from Food Hygiene and Quality Control Group– Faculty of Veterinary Medicine, University of Tehran (granted by Dr Dariush Khashabi, Austrian Agency for Health and Food Safety - Veterinary Medicine Institute). Chy-Max Rennet was purchased from Chy Hansen Company of Denmark, with 100% Chymosin and 162 2080 IMCU/g power. Lyophilized Starter and DVS163, produced by Chr Hansen Company of Denmark, was used in our study as starter.

B. Protocol of study

Cheeses were divided to control, 250, 500, 1000, 1500 and 2000 ppm of Monolaurin containing groups. Escherichia coli O157:H7 was kept in nutrient blood broth and were stored in refrigeration temperatures and re-cultured during the respite periods of 3-4 weeks in order to maintain its activity. In order to achieve single colonies from suspensions, bacteria were streak cultured in nutrient agar. Three single colonies were taken from bacteria and cultured in culture tube containing 5 ml of nutrient broth and incubated at 35 degrees centigrade for 18 hours. Simultaneously, in order to achieve approximate number of bacteria in the 18-hour culture, dilutions of 10 to 10⁻⁷ times were provided and 0.1 ml of 10⁻⁵, 10⁻⁶ and 10⁻⁷ dilutions were double streak cultured. Plates were incubated at 35 degrees centigrade for 24 hours. Then, the plates with 25-250 colonies (CFU) were selected for numeration. Approximate numbers of Escherichia coli O157:H7 in the 18-hour broth was 109 CFU/ml. Various quantities of the 18-hour cultures of bacteria

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were transferred to cuvettes containing 1ml of nutrient broth. Then, the cuvettes containing 106 CFU/ml were selected for production of cheese to be inoculated to milk.

In order to make cheese, pasteurized cow milk with 2.5% fat, 8.95 solid non-fat SNF4, and pH=6.7 was used and in order to control the pasteurization, alkaline phosphatase test was used. Escherichia coli O157:H7 and lyophilized starter were added to milk. Various concentrations of monolaurin were added to milk and mixed well using a magnetic mixer. Rennet was also added to the mixture. The milk was kept at 35 degrees centigrade for 55 minutes. After forming the clots, they were cut into cubes. The clots were then squeezed for 30-45 minutes under gradual pressure of a weight of 2 kg. Finally, the number of bacteria was determined at zero, first, 5th, 10th and 14th days after production of cheese to be inoculated to milk.

**Statistical Analysis**

All values are presented as mean±SEM. Statistical significance was evaluated by one-way analysis of variance (ANOVA) and student’s t-test using SPSS 19. Significance was measured using Turkey’s significant for the exact P values and significant differences are noted in the results. Differences with P<0.05 were considered significant.

III. Results

Table I represents the effects of various concentrations of monolaurin on E coli O157:H7 growth in various days after making the cheese.

### Table I

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<th>Monolaurin (ppm)</th>
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The data are indicated as mean ± SEM. P values are expressed in comparison with control group. (*) indicates significant difference. Common letters indicate non-significant difference. Subscripted letters in English indicate the comparison effect of monolaurin various concentrations on logarithmic average of Escherichia coli O157:H7 during fixed day of keeping the cheese (row comparison). Top letters in English indicate comparison of fixed concentration of monolaurin on logarithmic average of Escherichia coli O157:H7 during various days of keeping the cheese (column comparison).

Bacteria number in cheeses containing 250 and 500 ppm of monolaurin was not significantly different from control group (p>0.05), while bacteria number in cheeses containing 1000, 1500 and 2000 ppm of monolaurin was significantly lower compared to control group (p<0.05). The most effective concentrations of monolaurin were 1500-2000 ppm. Moreover, results of Tukey HSD test revealed that there is no significant difference between logarithmic average of Escherichia coli O157:H7 on the 1st, 5th and 10th days after making the cheeses (p>0.05), while a significant difference was observed between the 14th day after cheeses making compared to 1st, 5th and 10th days after making the cheeses (p<0.05).

IV. Discussion

The results indicated the potential inhibitory effect of monolaurin on bacterial growth in dairy products. Monolaurin is a natural substance composed of monoglycerides and fatty acids with potential antimicrobial properties [12]. Other studies...
also champion the use of certain lipids as antimicrobials [12] – [14]. The investigations have shown that monolaurin is effective in blocking or delaying production of exotoxins by pathogenic gram-positive bacteria [15] and inhibits the synthesis of most staphylococcal and other exoproteins at the level of transcription [16]. Monolaurin also inhibits signal transduction pathways and, thereby, the expression of virulence factors including protein A, alpha-hemolysin, B-lactamase, and toxic shock syndrome toxin 1 in S. aureus and the induction of vancomycin resistance in Enterococcus faecalis [17] – [18]. Concerning gram-negative organisms, the failure of monolaurin against E. coli was expected, because monolaurin is known to kill primarily gram-positive organisms [12]. The antimicrobial potential of monolaurin could be of great importance for two reasons. First, many antimicrobial agents produced by the pharmaceutical industry have been associated with serious side effects that limit their long-term use. Second, the potential for creating microorganisms that are resistant to antimicrobial agents is a major concern. Hence, the accepted practice is to encourage the use of antimicrobial agents only when necessary to treat infections, thus precluding their prophylactic use under many circumstances. On the other hand, natural products, many of which can be used for long periods, might be less likely to produce side effects.

V. CONCLUSION

We have shown that monolaurin has antibacterial activity against Escherichia coli O157:H7 in produced cheese. Thus, use of monolaurin as natural antibacterial substance may be a proper replacement for chemical preservatives.

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