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Study the effects of Mint and Zataria multiflora on the survival of the *Escherichia coli* O157:H7 on Lighvan cheese throughout ripening

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Research Article

Abstract

Background: Vegetable essential oils and its components have anti-bacterial known effects. The aim of this study was to evaluate different concentrations of essential oils of thyme and mint on survival rate of *Escherichia coli* O157:H7 in traditional Lighvan cheese during ripening.

Methods and findings: Essential oils were extracted using Kelevenger apparatus. To determine the essential oil concentration in terms of organoleptic, sensory evaluation was conducted by a test panel. Cheese samples prepared with the help of local manufacturers based in the village of Lighvan in triplicates, including zero and 100 and 200 ppm from essential oils of thyme and mint separately and doses of 10^3 and 10^5 $cfugr^{-1}$ from the *E. coli* O157:H7 bacterium. In zero, 30, 60 and 90 days of ripening period, the number of *E. coli* O157:H7 studied in traditional Lighvan cheese samples. The results showed that the use of essential oil of mint and thyme with concentrations of 100 and 200 ppm compared to non-oil samples, reduced the log mean of the number of *E. coli* O157:H7 significantly (P<0/05). Also, the cheese storage time had a significant effect in reducing the log mean of the number of *E. coli* O157:H7 (P<0/05).

Conclusion: The general results show that essential oil of Mint and Thyme can be used as a natural preservative and flavoring in the traditional Lighvan cheese but should be used in concentrations such that lacks no adverse effects on the taste and smell of food.

Keywords: Mint essential oil; Thyme essential oil; *E. coli* O157:H; Traditional Lighvan cheese

1. Introduction

Today, diseases of food origin in the whole world, even in developed countries like America, brings irreparable damages in various fields of economic, social and health. Rotten foods, in addition to damage to the health of the consumer, hurt the manufacturer also economically.

According to the Center report for Disease Control and Prevention (CDC), in 2012, food infections such as Salmonella, *Escherichia coli* (*E. coli*) O157, Campylobacter, Listeria, Shigella, Vibrio and Yersinia causes the disease about 15 percent of the population that has a population of over 48 million people [1]. *E. coli* O157:H7 as one of public concern is known at a global level and can be found in certain foods, including meat and meat products, milk, vegetables, salad and fruit juice [2]. The diseases resulting from the bacterium reported individually and epidemic in different parts of the world [3]. Hemolytic Uremic Syndrome (HUS) and Hemorrhagic Colitis (HC) through the production of toxins like Shigella (especially toxin STX) caused by this strain. Hemolytic uremic syndrome causes hemolytic anemia, thrombocytopenia and acute renal failure and hemorrhagic colitis can cause bloody diarrhea, severe abdominal cramps, nausea, vomiting and rarely fever [4]. Lighvan cheese is a ripe white cheese in brine and one of the most widely used traditional cheese that is produced in Lighvan village located in the south-east of Tabriz in the north west of Iran. In producing this cheese, raw sheep's milk used with accompany of 20-30% goat's milk without yeast. Most manufacturers are convinced that using raw milk cause good flavor and taste in the cheese that this may in fact be due to proteolytic and lipolytic enzymes in the milk and produced by raw milk micro flora [5]. When the quality of raw milk is not suitable, the risk of common disease transmission between humans and animals is followed. In addition, many of the cheese production processes is done manually and with the use of

traditional equipment, so all of these cases increase the risk of product contamination by spoilage bacteria, especially pathogens such as Salmonella, *Listeria monocytogenes*, *E. coli* producing Verocytotoxin and *Staphylococcus aureus* by animals, the environment and particularly worker's carriers and the collection of these factors reduce the health and long shelf life of the product [6,7]. Vegetable essential oils and its components have known anti-bacterial effects. Many applications in order to control the growth of pathogenic bacteria originating from food or spoilage bacteria, leading to its use as food preservatives [8]. The Mint plant has 4000 species which have been placed in 200 genera. Its distribution is that, in most regions of the globe can be found but most of it is in the Mediterranean region [9]. Among the different genera of the plant, menth is of important genus of the mint family. *Mentha spicata* has differences with other mint species that the main differences are the lack of menthol and composing of a compound called carvon that included a high percentage of essential oils [10,11]. Thyme (*Zataria multiflora*) is one of the Lamiaceae family plants that geographically planted only in Iran, Pakistan and Afghanistan [12]. Thymol and carvacrol are the main constituents of the thyme essential oil which is well dissolved in alcohol and organic solvents and the materials are stored mainly in young leaves during plant growth [13,14]. The aim of this study was to evaluate the antimicrobial effect of different concentrations of thyme essential oil and mint on the *E. coli* O157: H7 survival in traditional Lighvan cheese during ripening.

2. Materials and Methods

2.1 Preparation of herbs and essential oils

The thyme prepared in dried form and Mint in fresh form and then was confirmed by Institution of medicinal plants of University Jihad for the scientific name morphologically. The mint was dried in the shadow. Thyme and mint essential oils prepared by steam distillation using Clevenger apparatus. Essential oils dehydrated by anhydrous sodium sulfate and were kept in dark sealed glass containers away from light in the refrigerator. To evaluate the organoleptic properties of made essential oils, the Lighvan cheese with no *E. coli* O157:H7 prepared with different concentrations of essential oils of mint and thyme. Then sensory evaluation was conducted by test panel. Panel members determined their criterion of the sensory evaluation containing essence using a 9-point sensory scale. On this scale, 9 point was incredibly great score, 8 great score, 7 good score, 6 relatively good score, 5-neither good nor bad, 4 quite bad, 3 bad score, 2 very bad score and 1 incredibly bad [12]. The highest concentration of the essential oils that the taste is tolerable for assessors, with half of the highest concentration of essential oils considered as an admission rate (score 5).

2.2 Studied bacterium

In this study, *E. coli* O157:H7 (ATCC 35218) was obtained from the Department of Microbiology of veterinary faculty of Tehran University. To study, 10^3 and 10^5 cfu gr^{-1} was used, for this purpose bacteria cultured on two consecutive occasions by 20 and 24 h intervals in a nutrient broth culture and were incubated for 20 h at 37°C. In order to achieve the desired values for inoculation, standard 0.5 of McFarland was used. Using 20 h culture by spectrophotometer at a wavelength of 600 nm, the number of bacteria per ml was calculated.

2.3 Traditional cheese production of Lighvan

For the production of cheese, ewe's fresh and raw milk was used of Lighvan area. Milk for the presence of antibiotic residues was tested with the Beta Star testing. The search of *E. coli* O157:H7 in milk was performed using surface culture. For cheese production, first besides the observation of the manufacturing process in cheese production workshops in Lighvan area and talking to expert manufacturers and using the local manufacturer of Lighvan village help and guidance, the cheese was produced entirely by traditional methods. In this way that at first raw milk temperature set about 23°C and the bacterium in the desired dose inoculated in the milk, then rennet added at 0.001 percentage to the milk, at the same time, Mint and Thyme essential oils added to milk at concentrations of zero, 100 and 200 ppm. During about 2 h the clot was formed. The clots were maintained in 80 × 80 cm fabrics for 1-1.5 h. The thin edge of clot was cut and transferred to the middle of fabric and fabrics were closed again for 1-1.5 h. Then 12.6 kg weights were placed on fabrics for 1-1.5 h. The clots cut in at least 10 × 10 cm. Pieces of cheese placed in 18-24% brine for 6-4 h. Then grains of dry salt sprinkled 6 times during 3-6 days. Finally, cheese put in 500g packages and filled with 11 percent brine. And on days zero, 30, 60 and 90 tested and the test was repeated three times.

2.4 Microbial count and verifying *E. coli* O157:H7

To count of *E. coli* O157:H7, surfaced culture method was used and by transferring 1.0 ml of the prepared dilution to double plates containing medium of Cefixime Tlurit- Surbitol Mac agar (CT-SMAC) possessing 0.05 mg in liter of cefixime and 2.5 mg in per liter of tellurite potassium were cultured and after 24 hours of incubation at 35°C, colonies of negative sorbitol (colorless) were counted. 5-10 colonies were chosen to confirm by biochemical tests of agar citrate Simon, MR-VP broth, SIM agar, TSI agar [15].

2.5 Statistical analysis

To analyze data, ANOVA test (One-way ANOVA) and Tukey post hoc test (Tukey) were used in the level of α=0.05.

3. Results

3.1 Antimicrobial effects of extracts

The results of the initial sensorial evaluation by

panel test showed that mint and thyme essences in concentration of 200 ppm in terms of taste have the highest ability for tolerance to assess. Therefore, both mentioned essences of zero concentrations, 100 and 200 ppm in traditional Lighvan cheese samples were used.

The results of this study showed that the use of mint and thyme essences in concentration of 100 and 200 ppm compared to non-essence samples, the log mean of the number of *E. coli* O157:H7 has significantly reduced (P<0.05).

The log mean of the number of *E. coli* O157:H7 with inoculated dose of 10^3 cfuml^{-1} at traditional Lighvan cheeses by concentrations of 100 and 200 ppm of mint essence in comparison with non-essence samples on the ninetieth day of maintenance has significantly decreased (P<0.05). The effect of the concentration of 200 ppm of mint essence compared with the effect of 100 ppm concentration of mint on logarithmic decrement of the number of *E. coli* O157:H7 with inoculated dose of 10^3 cfu ml^{-1} in traditional Lighvan cheeses is significant (P<0.05) (Table 1). The log mean of the number of *E. coli* O157:H7 with inoculated dose of 10^5 cfu ml^{-1} at traditional Lighvan cheeses by concentrations of 100 and 200 ppm of mint essence in comparison with non-essence samples on the ninetieth day of maintenance has significantly decreased (P<0.05) (Table 2).

The log mean of the number of *E. coli* O157:H7 with inoculated dose of 10^3 cfu ml^{-1} at traditional Lighvan cheeses by concentrations of 100 and 200 ppm of thyme essence in comparison with non-essence samples on the ninetieth day of maintenance has significantly decreased (P<0.05) (Table 3).

The log mean of the number of *E. coli* O157:H7 with inoculated dose of 10^5 cfuml^{-1} at traditional Lighvan cheeses by concentrations of 100 and 200 ppm of thyme essence in comparison with non-essence samples on the ninetieth day of maintenance has significantly decreased (P<0.05) (Table 4). There is not any significant difference in impact of thyme and mint essences on the survival scale of *E. c*oli O157:H7 based on 10^5 and 10^3 cfu ml^{-1}.

Table 1. The comparison of (Mean ± SD) logarithm mean of the number of *E. coli* O157:H7 with inoculated dose of 10^3 cfu ml^{-1} at traditional Lighvan cheese containing different amounts of mint essence in different times of ripening period.

Essence concentration	Ripening Period (day)			
	zero	30	60	90
zero	5.67 ± 0.03 A	5.25 ± 0.05 A	4.44 ± 0.20 A	2.88 ± 0.01 A
100	5.54 ± 0.06 A	5.17 ± 0.05 B	4.33 ± 0.07 A	2.04 ± 0.04 B
200	5.52 ± 0.04 A	5.10 ± 0.07 B	4.22 ± 0.13 A	1.51 ± 0.007 C

A, B, C: There are significant differences among means in each column that hasn't any common letters are (P<0/05)

Table2. The comparison of (Mean ± SD) logarithm Mean of the number of *E. coli* O157:H7 with inoculated dose of 10^5 cfu ml^{-1} at traditional Lighvan cheese containing different amounts of mint essence in different times of ripening period.

Essence concentration	Ripening Period (day)			
	zero	30	60	90
zero	6.69 ± 0.01 A	6.37 ± 0.30 A	5.23 ± 0.30 A	4.46 ± 0.41 A
100	6.74 ± 0.04 A	6.20 ± 0.05 A	4.74 ± 0.12 B	0 B
200	6.70 ± 0.03 A	6.10 ± 0.02 A	4.37 ± 0.35 B	0 B

A, B: There are significant differences among means in each column that hasn't any common letters are (P<0/05)

Table 3. The comparison of (Mean ± SD) logarithm Mean of the number of *E. coli* O157:H7 with inoculated dose of 10^3 cfu ml^{-1} at traditional Lighvan cheese containing different amounts of thyme essence in different times of ripening period.

Essence concentration	Ripening Period (day)			
	zero	30	60	90
zero	5.67 ± 0.03 A	5.25 ± 0.05 A	4.43 ± 0.20 A	2.88 ± 0.01 A
100	5.63 ± 0.02 A	5.21 ± 0.10 A	4.85 ± 0.11 B	1.36 ± 0.32 B
200	5.59 ± 0.05 A	5.13 ± 0.06 A	4.48 ± 0.21 B	1.30 ± 0.42 B

A, B: There are significant differences among means in each column that hasn't any common letters are (P<0/05)

Table4. The comparison of (Mean ± SD) logarithm Mean of the number of *E. coli* O157:H7 with inoculated dose of 10^5 cfu ml^{-1} at traditional Lighvan cheese containing different amounts of thyme essence in different times of ripening period

Essence concentration	Ripening Period (day)			
	zero	30	60	90
zero	6.69 ± 0.01 A	3.37 ± 0.30 A	5.23 ± 0.30 A	4.46 ± 0.41 A
100	6.75 ± 0.03 A	5.24 ± 0.22 B	4.66 ± 0.35 A	1.56 ± 0.23 B
200	6.72 ± 0.04 A	5.27 ± 0.04 B	4.57 ± 0.30 A	1.15 ± 0.21 B

A, B: There are significant differences among means in each column that hasn't any common letters are (P<0/05)

4. Discussion

The results of this study showed that according to Tables 1 and 2, mint essence in doses of 100 and 200 ppm during reaching period causes to decreasing the log mean of the number of *E. coli* O157:H7 in the traditional Lighvan cheese. Also, according to Tables 3 and 4, mint essence in doses of 100 and 200 ppm during reaching period causes to decreasing the log mean of the number of *E. coli* O157:H7 in the traditional Lighvan cheese. Study on the antimicrobial properties of herbs and their essence have been conducted in various countries, in a study, Foda et al. [11] studied the effect of mint as aqueous extracts, alcoholic extracts and essence against twelve genera of bacteria including *E. coli*, molds and yeasts in white cheese in 2009, that aqueous extracts of mint showed no inhibitory effect on any of the studied materials. A mild effect against gram-positive bacteria such as *Bacillus cereus* and *S. aureus* was observed by alcoholic extracts of mint, while mint essence was the strongest inhibitor of antimicrobial and it causes to a significant reduction in counting bacterial population, proteolytic and lipolytic bacteria compared to the control group, that is consistent to the results of the present study in view of the antimicrobial effect of mint essence [11].

Karim et al. [16] have examined the antimicrobial effect of mint, tarragon, cumin, oregano and thyme on *E. coli* bacteria in Iranian white cheese that the results of this study showed the highest antimicrobial effect of inoculated cheese in volatile oils of thyme, oils of mint, cumin and oregano showed the same effect on inoculated bacteria and tarragon was showed the least effect of volatile oil more than others others. In another study, Selim [3] studied the impact of essence of eucalyptus, juniper, mint, rosemary, sage, clove and thyme with concentrations in 1.0, 5.0 and 1 percent against *E. coli* O157:H7 that the considered bacteria with concentrations (10^3 cfu/g) are incubated in soft white cheese (feta) and was maintained at the temperature of 7°C for 14 days. Among the mentioned essences, thyme and sage have the best effective against aimed bacteria. Thyme essence with concentrations of 0.5 and 1 percent showed the most significant effect on reducing *E. coli* O157:H7. The results of this study, the potential of thyme essence revealed as a natural preservative in contamination of soft white cheese (feta) with *E. coli* O157:H7. Mohammadi et al. [12] studied the effect of thyme essence with concentration of zero, 150, 100 and 200 ppm on *E. coli* O157:H7 in 10^4 cfu/ml dose in salt water white cheese during production and storage process that the effect of antibacterial thyme essence at a concentration of 200 ppm compared with the control group lower concentrations was significantly. In all these studies, antibacterial effects of mint and thyme have been observed that are consistent to the results of this study. Shan et al. [17] studied the bactericidal effect of cinnamon, cloves, oregano, pomegranate skin and grape seed against *L. monocytogene*, *S. aureus* and *S. enterica* in cheese, at temperature of room (23°C), the results showed that all five plant extracts used against these food pathogens are effective in cheese and in the meantime cloves indicated the most antibacterial and antioxidant effect.

Recently the serious debates were proposed about the safety aspects of the chemical preservatives, and because these materials have carcinogenic, teratogenic and toxigenic effects, consumers have a tendency to use natural preservatives. Herbal essences and their components have the known anti-bacterial effects. Many applications in order to control the growth of disease bacteria originating from food or spoilage bacteria, lead to its use as a food preservative. Because of new approach of public and also international and national organizations responsible for food health and food industry is welcomed to use of different natural preservatives instead of chemicals. Therefore, special interest focused on the potential applications of herbal essences and antimicrobial properties of herbal essences, against a wide range of micro-organisms (including bacteria, yeasts and molds) has been approved. Most studies have been conducted in the cultured media lab and acceptable antimicrobial effects of plant compounds have been observed but the effects of plant compounds have been evaluated in less food.

Goudarzi et al. [14] have studied the effect of aqueous and alcoholic extracts of thyme herb on enterohemorrhagic *E. coli in vitro*. The results of this study showed that alcoholic extract of thyme with concentration of 0.78 mg per ml have the inhibitory effect on the strain, while the aqueous extract was not effective in any concentration on the strains. In another study in 2012 Sheeladevi et al. [2] studied antibacterial activity of herbal essence of clove, oregano, rosemary, cinnamon and thyme in vitro against Campylobacter, Listeria, Yersinia, Salmonella and Pseudomonas that most essences showed relatively high antibacterial activity against the tested bacteria. In the meantime, cloves, cinnamon and thyme had the highest inhibitory activity. Boniadian et al. [18] have studied the impact of volatile essences of herbs (oregano, mint, tarragon, cumin and thyme) on the bacteria including *E. coli* and *S. aureus* in liquid cultured medium. The results indicated that volatile oil of thyme has the greatest impact on these two bacteria and Tarragon herb has the least effect and volatile essences of mint, oregano and cumin were demonstrated the moderate impact on bacteria. Similar results have been reported by Boniadian et al. [19]. In a study of Sagdıç [20], the effect of Turkish thyme and oregano against *E. coli* (ATCC 25922), *E. coli* O157:H7 (ATCC 33150), *S. aureus* (ATCC 2392) and *Yersinia enterocolitica* (ATCC 1501) has evaluated in vitro that all of these pathogenic bacteria were inhibited by these two extracts, among the studied bacteria, *S. aureus* was indicated as the most sensitive bacteria.

5. Conclusion

It can be concluded that the limited studies have been done about the effect of herbal essences on the survival scale of pathogenic microbes and spoilage in traditional Lighvan cheese. The essence of mint and thyme herbs has the killing an inhibitory effect on *E. coli* O157:H7 in traditional Lighvan cheese. The effect of the mentioned essences on the bacteria follows a time-dependent manner, so that whatever we reach to the final stages of reaching Lighvan cheese, it has significantly reduced the log mean of the number of bacteria. When using herbal essences as preservatives in food must be considered to organoleptic effects, because the use of the essences solely for the purpose of antimicrobial effect, can cause to taste and a bitter smell in food and makes it unusable, therefore, food sensory evaluation to determine tolerable concentration of essences is essential for consumers.

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