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Does Thyme Essential Oil (Zataria Multiflora) Improve Durability, the Taste and Nutritional Value of Doogh?

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ABSTRACT

Introduction: Diseases caused by the consumption of bacteria-contaminated foods have been of great importance in public health. Therefore, control of dairy product contamination with coliforms is a concern for the durability of dairy producers. Control of the contamination by natural products may have dual action; nutritive value and natural antibiotics. In this study, the antimicrobial activity of thyme essential oil against *Escherichia coli* O157: H7 in doogh (Iranian fermented dairy drink) was examined and the MIC and MBC were determined.

Methods: Antimicrobial activity of thyme essential oil was examined using different concentrations (0, 30, 60, 90, and 120 µl/ml) against bacteria with a density of 10^5CFU/ml during the 60-day at 4 °C. Moreover, the effect of different concentrations of essential oils on the taste of doogh was also studied. Taste evaluation was performed based on 5 points hedonic test to evaluate the effect of Thyme oil on the sense of taste. Duncan software was used to determine the difference between test and control groups at a confidence level of 5%. The SPSS software was also used for statistical analysis.

Results: All concentrations of thyme essential oil could stop the growth of bacteria (P<0.05). The inhibitory effect of oil increased with increasing concentration and time. Based on the results of sensory (taste) evaluation, treatment 2 (containing 60 µl/ml of essential oil of thyme) was selected as the best treatment.

Conclusion: Thyme essential oil can be used as a natural preservative with a positive impact on taste and nutritive value.

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Introduction

Diseases born from the consumption of foods contaminated with pathogenic bacteria impose significant morbidity, mortality, medical costs, and financial damage to health systems annually (1). In the meantime, many cases of human disease and death have been attributed to bacterial infections that can enter the human body through various roots such as drinking water, breathing, and food (2-4). Centers for Disease Control and Prevention reported that 9.4 million people in the United States get sick from foodborne pathogens annually. Such diseases result in near to 60000 hospitalizations and 1300 deaths each year (5).

Escherichia coli is a highly pathogenic bacterium that originates in the gut of many animals. Children, the elderly and immunocom promised people are more susceptible to infection caused by this bacterium (6 and 7). It is found in a wide variety of food products such as yogurt, milk, fruit juices, and meat (8). Pasteurization is a method of choice for eliminating the bacterium. However, heat treatment is not suitable for all kinds of foods and cross-contamination cannot always be prevented. Therefore, controlling the growth of pathogenic bacteria in food is of great importance both in terms of food quality standards and in terms of public health. One usual way to control the growth of infections in food is to use antimicrobial preservatives and compounds (9 and 10). Since chemical additives lead to allergies, undesirable side effects, and emerging antibiotic-resistant strains, there is a tendency to use natural products that not only lack side effects but also have nutritional properties. Natural preservatives like herbal essences and extracts have been shown to possess anti-bacterial effects (10 and 11). Among medicinal plants, Thyme essential oil and extract

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have been extensively studied in vitro as a source of antibacterials (12). Using plant preservatives to control the growth of pathogenic bacteria of food origin has two benefits; avoiding undesirable side effects and receiving nutritional ingredients. The present study aimed to investigate the antibacterial effect of Thyme essential oil at different concentrations as a natural flavoring and preservative on *Escherichia coli* O157H7 in pasteurized doogh. In this way, minimum growth inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Moreover, the sensory test was performed to assess the sensory properties of thyme essential oil on the taste of doogh (Iranian fermented dairy drink).

Material and Methods

Antibacterial Susceptibility Assay

The antibacterial effect of thyme essential oil was evaluated using the disk diffusion method based on the National Committee for Clinical Laboratory Standards (NCCLS). Three milliliters of sterile distilled water were added to the lyophilized *Escherichia coli* O157H7 (Iranian Biological Resource Center, Iran) and was kept under ambient temperature for 4 hours under a sterile hood. The bacteria were cultured on MacConkey agar medium and incubated at 35 °C for 24 hrs. The cultured bacteria were used for making a suspension of bacteria equal to a 0.5 McFarland standard (in normal saline) (1.5×10^8 CFU/ml). From the suspension, 0.5 ml was transferred to 500 ml pasteurized doogh (Vijeh dairy, Iran). As a result, the final concentration of bacteria in doogh was 1.5×10^5. Inoculated doogh was incubated, and a sample was taken from the suspension at the first, twentieth, fortieth, and sixtieth days of incubation for an antimicrobial susceptibility test.

Preparation of discs containing Thyme essential oil: The blank filter paper discs (6 mm) (Padtanteb, Iran) were immersed in 20 µl of different concentrations of thyme essential oil (0, 30, 60, 90, and 120 µl from essential oil per 1 ml solvent) for 24 hrs. Samples from inoculated doogh were cultured on plates and antibacterial activity was performed using discs immersed in different concentrations of Thyme essential oil.

Disc diffusion method: According to the NCCLs (2000a) standard, 150µl of bacterial suspension containing 1×10^5 CFU/ml was poured onto Mueller-Hinton agar medium. The 6-mm discs containing thyme essential oils were placed on the plate at appropriate distances. A blank disc was included in each plate as a control. The plate was incubated at 37°C and the growth inhibition zone was measured after 24 hours (13). Antibacterial activity of essential oil in doogh samples was performed at first, twentieth, fortieth, and sixtieth days of storage.

Determination of MIC and MBC

Sixty microliters of Mueller-Hinton Broth medium, 20 µl of bacterial suspension containing 1.5×10^5 bacteria, and 20 µl of different thyme essential oil concentrations (30, 60, 90, and 120 µl/ml) were transferred to the tubes. One tube containing culture media and bacteria and one tube containing culture media and essential oil were included as controls. All the tubes were incubated at 35°C for 24 hours and then examined for turbidity caused by bacterial growth and the lowest concentration at which the bacterium did not grow visibly was considered MIC (14). To measure MBC, 5 µl from each tube in which bacterial growth was not observed was transferred to a nutrient agar medium and incubated at 35°C for 24 hours. The lowest concentration of thyme essential oil at which most bacteria were killed was considered MBC.

Sensory Evaluation

A sensory evaluation was performed to evaluate the effect of different concentrations of essential oils on the taste of doogh. The test was done on the mixture of thyme essential oil and doogh on the first, twentieth, forty-sixth, and sixtieth day of mixture preparation, by seven trained panelists of the Iranian Food and Drug Administration (based on national standards 3442, 2442, 3581, 4940) based on 5 points hedonic test (from 1 to 5). For this purpose, sterile doogh was mixed with Thyme essential oil at concentrations of 30, 60, 90, and 120 µl/ml and an equal sample from each concentration was served to the assessors. Water was used to rinse the assessor's mouths before and between sample evaluations (15).

Statistical Analysis

All the experiments were done in triplicate. Data were analyzed by SPSS software and mean data were compared using a t-test. A p-value less than 0.05 was considered statistically significant.

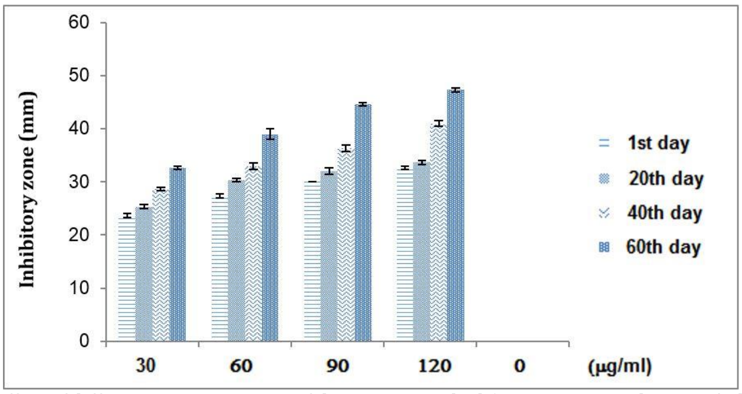

Figure 1. Antibacterial effect of different concentrations of thyme essential oil (0, 30, 60, 90, and 120 µg/ml) in different incubation times (1st, 20th, 40th and 60th day) on the growth of *Escherichia coli*. Antibacterial effects were assessed by the disk diffusion method are expressed as inhibitory zone (mm).

Results

Antibacterial susceptibility test by the disk diffusion method showed that thyme essential oil has a significant effect on bacteria. This antibacterial effect increased by increasing the concentration of the extract (data are missed apparently). MIC and MBC were measured to be 60 and 90 µl/ml respectively. Moreover, our data also show that thyme essential oil dissolved in doogh had a significant ($p <0.05$) effect on the growth of *Escherichia coli* O157H7. As shown in figure 1, increasing the essential oil concentration and increasing incubation time (from the first day to the 60th day) had a progressive antibacterial effect on bacterial growth, therefore the highest antibacterial effect was at 120 µl/ml on the 60th day.

The effect of thyme oil extract on the taste of doogh was investigated in the first, twentieth, fortieth, and sixtieth days. As shown in table 1, the concentration of 60 µl/ml obtained the highest taste score in all four experiments (first, twentieth, fortieth, and sixtieth day). Higher concentrations were not desirable for assessors. Therefore, the concentration of 60 µl/ml of essential oil in all the studied days has the best effect on the sensory properties of doogh.

Considering both results obtained from antibacterial susceptibility and sensory test, 60 µl/ml of thyme essential oil during 60 days incubation is suggested as optimum treatment.

Table 1. Scores assigned based on the sensory test to each concentration of thyme in doogh (0, 30, 60, 90, and 120 µl/ml) over 60 days of incubation (1st, 20th, 40th and 60th day).

	1st	**20th**	**40th**	**60th**
30 µl/ml	2.28±0.48^{b}	2.57±0.53^{b}	2.71±0.48^{b}	2.57±0.53^{b}
60	3.57±53^{a}	3.71±0.48^{a}	3.85±37^{a}	3.42±0.53^{a}
90	1±0.00^{c}	1±0.00^{c}	1±0.00^{c}	1±0.00^{c}
120	1±0.00^{c}	1±0.00^{c}	1±0.00^{c}	1±0.00^{c}
0	2.71±1.1^{b}	2.85±1.00^{b}	2.42±0.9^{b}	2.57±1.1^{b}

Discussion

In the present study, the effect of different concentrations of thyme essential oil on *Escherichia coli* O157H7 inoculated in doogh for different incubation times was assessed. The essential oil could reduce the growth of bacteria in a concentration-dependent manner. Plant essential oils have been used to control the growth of pathogenic bacteria of food origin or spoilage bacteria and also as a food preservative (16 and 17). With excessive use of chemical preservatives, some of which are suspected to have toxic and carcinogenic effects, therefore there is a tendency to reduce or eliminate these synthesized compounds and to replace them with natural compounds (18-20). Sagdic et al. found that thyme essential oil had a dose-dependent bactericidal effect against *Escherichia coli* O157H7 and suggested it as a food preservative (21). Mahboubi et al. reported linalool, thymol, and carvacrol as three major chemotypes with the highest antibacterial activity (22). Moreover, the antimicrobial effect of thyme essential oil has been assessed on *Escherichia coli* in white-brined cheese after 60 days of cheese storage and showed that storage time and thyme concentration had a significant effect on the logarithmic number of bacteria (23). In the present study, it was found that the storage time of inoculated doogh from first to 60th day and increasing essential oil concentration had an increasing and cumulative effect on the growth of bacteria. In another study, essential oils from four plants; bay, clove, cinnamon, and thyme showed antimicrobial effects against *Listeria monocytogenes* and *Salmonella enteritidis* in soft cheeses and reduced bacterial count to ≤1·0 log10 CFU ml−1 over 14 days (24). In another study, Shahbazi showed that Ziziphora clinopodioides essential oil could reduce 1 log CFU mL-1 of *Salmonella typhimurium* and *Staphylococcus aureus* in doogh (25). Antimicrobial effects of Thyme essential oil was also confirmed in another dairy product (Mayonnaise) against fungi and bacteria (26).

Studies show that essential oil is a rich source of thymol, which interacts with lipid bilayer resulting in changes in membrane stability, and elasticity (27) that causes disturbances in plasma membranes, loss of cellular constituents, and ultimately the death of pathogens (18, 28, and 29). Therefore, the antimicrobial activity of thyme essential oil tested in this study can be attributed to the presence of these compounds.

In the present study, MIC and MBC have measured at 60 and 90 µl/ml respectively. One of the drawbacks of aromatic compounds as food preservatives is high concentrations of them are needed to protect food against bacteria. However, it should be noted that when the amount of essential oil is high, it negatively affects the taste of the product which is not desirable to the customers. The main limiting factor to using thyme essential oil is negative organoleptic properties that lead to an unpleasant taste (30). The desirable concentration of Thyme oil seems to depend on the kind of food. Some studies have shown that a concentration of 3 µl/ml of Thyme oil is most desirable in meat products (31). However, the results of the sensory test indicated that the most desirable concentration of essential is 60 µl/ml that equal to MIC. There is no significant difference between the concentrations of 0 and 30 and also between the concentrations of 90 and 120 µl/ml. In total, considering both the antimicrobial potency and taste property, this study suggests a concentration of 60 µl/ml for doogh.

Conclusion

Our data showed that thyme essential oil can prevent the growth of bacteria and this effect is concentration-dependent, the most potent antimicrobial activity was observed to be 120 µl/ml. However, in the sensory evaluation, the most desirable concentration was 60 µl/ml. Since we cannot exceed the desirable concentration of essential oil that affects the quality of the product, we suggest the concentration of 60 µl/ml of essential oil to achieve both MIC and the best taste desirability.

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Conflict of interest

There is no conflict of interest to declare.

References

1- Scharff RL. Economic burden from health losses due to foodborne illness in the United States. J Food Prot. 2012;75(1):123-31.
2- Cabral JP. Water microbiology. Bacterial pathogens and water. Int J Environ Res Public Health. 2010;7(10):3657-703.
3- Bintsis T. Foodborne pathogens. AIMS Microbiol. 2017;3(3):529-63.
4- Dasaraju PV, Liu C. Infections of the respiratory system. In: Baron S, ed. Medical Microbiology. 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston. 1996.
5-Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. Foodborne illness acquired in the United States--major pathogens. Emerg Infect Dis. 2011;17(1):7-15.
6- Coia JE. Clinical, microbiological and epidemiological aspects of Escherichia coli O157 infection. FEMS Immunol Med Microbiol. 1998;20(1):1-9.
7- Bach SJ, McAllister TA, Veira DM, Gannon VPJ, Holley RA. Transmission and control of Escherichia coli O157:H7-a review. Can. J. Anim. Sci. 2002; 82:475–90.
8- Mead PS, Griffin PM. Escherichia coli O157:H7. Lancet. 1998; 352(9135):1207-12.
9- Jay JM. Antimicrobial food preservatives. In: Rossmoore H.W. (eds) Handbook of Biocide and Preservative Use. Springer, Dordrecht. 1995.
10- Davidson PM, Taylor TM, Schmidt SE. Chemical preservatives and natural antimicrobial compounds. Food microbiology: fundamentals and frontiers. 2012:765-801.
11- Lucera A, Costa C, Conte A, Del Nobile MA. Food applications of natural antimicrobial compounds. Front Microbiol. 2012; 3:287.

12- Burt SA, Reinders RD. Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7. Lett Appl Microbiol. 2003; 36(3):162-7.
13- Winn Jr W, Washington C, Elmer W. Koneman. Koneman's color atlas and textbook of diagnostic microbiology. Philadelphia: Lippincott Williams & Wilkins. 1983.
14- Pfaller MA, Messer SA, Boyken L. Evaluation of the NCCLS M44-P disk diffusion method for determining susceptibilities of 276 clinical isolates of Cryptococcus neoformans to fluconazole. J Clin Microbiol. 2004;42(1):380-3.
15- Watts BM, Ylimaki GL, Jeffery LE, Elias LG. Basic sensory methods for food evaluation. IDRC, Ottawa, ON, CA; 1989.
16- Moreira M R, Ponce A G, del Valle C E, Roura S I. Inhibitory parameters of essential oils to reduce a foodborne pathogen. LWT - Food Sci Tech. 2005;38 565-570.
17- Kostaki M, Giatrakou V, Savvaidis IN, Kontominas MG. Combined effect of MAP and thyme essential oil on the microbiological, chemical and sensory attributes of organically aquacultured sea bass (Dicentrarchus labrax) fillets. Food Microbiol. 2009;26(5):475-82.
18- Borugă O, Jianu C, Mişcă C, Goleţ I, Gruia AT, Horhat FG. Thymus vulgaris essential oil: chemical composition and antimicrobial activity. J Med Life. 2014; 7. 3(3):56-60.
19- Anzlovar, Sabina & Baricevic, Dea & Avguštin, Jerneja & Koce, Jasna. Essential oil of common thyme as a natural antimicrobial food additive. Food Technol Biotechnol. 2014;52. 263-8.
20- Sajed H, Sahebkar A, Iranshahi M. Zataria multiflora Boiss. (Shirazi thyme)-an ancient condiment with modern pharmaceutical uses. J Ethnopharmacol. 2013;145(3):686-8.
21- Sağdıç, A. Kuşçu, M. Özcan, S. Özçelik. Effects of Turkish spice extracts at various concentrations on the growth of Escherichia coli O157:H7. Food Microbiol. 2002;19, 473-80.
22- Mahboubi M, Heidarytabar R, Mahdizadeh E, Hosseini H. Antimicrobial activity and chemical composition of Thymus species and Zataria multiflora essential oils. Agric Nat Resour. 2017; 51(5):395-401.
23- Mehdizadeh T, Narimani R, Mojaddar Langroodi A, Moghaddas Kia E, Neyriz-Naghadehi M. Antimicrobial effects of Zataria multiflora essential oil and Lactobacillus acidophilus on Escherichia coli O157 stability in the Iranian probiotic white-brined cheese. J Food Saf. 2018.
24- Smith-Palmer A, Stewart J, & Fyfe L. The potential application of plant essential oils as natural food preservatives in soft cheese. Food Microbiolo. 2001; 18, 463-70.
25- Shahbazi Y. The antibacterial effect of Ziziphora clinopodioides essential oil and nisinagainst Salmonella typhimurium and Staphylococcus aureus in doogh, a yoghurt-based Iranian drink. Vet Res Forum. 2016; 7(3):213-9.
26- Hajimohammadi- Telkabadi, B., Ahmadi-Dastgerdi, A. The Effect of Thyme (Zataria multiflora boiss) Essential Oil against Bacterial and Fungal strains in Mayonnaise. J Food Microbiol. 2020; 7(1): 1-16.
27- Kowalczyk A, Przychodna M, Sopata S, Bodalska A, Fecka I. Thymol and Thyme Essential Oil-New Insights into Selected Therapeutic Applications. Molecules. 2020; 25(18):4125.
28- Nazer AI, Kobilinsky A, Tholozana JL, Dubois-Brissonneta F. Combinations of food antimicrobials at low levels to inhibit the growth of Salmonella sv. Typhimurium: a synergistic effect?. Food Microbiol, 2005; 22, 391-8.
29- Chouhan S, Sharma K, Guleria S. Antimicrobial activity of some essential oils-present status and future perspectives. Medicines (Basel). 2017; 4(3):58.
30- Nieto G. A Review on Applications and Uses of Thymus in the Food Industry. Plants (Basel). 2020; 9(8):961.
31- Boskovic M, Djordjevic J, Ivanovic J, Janjic J, Zdravkovic N, et al. Inhibition of Salmonella by thyme essential oil and its effect on microbiological and sensory properties of minced pork meat packaged under vacuum and modified atmosphere. Int. J. Food Microbiol. 2017; 258: 58-67.