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Effect of Mint Essential Oil on Growth of Listeria monocytogenes during the Ripening and Storage of Iranian White Brined Cheese

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ABSTRACT

Introduction: Listeria monocytogenes is a Bactria that can result in Listeriosis disease by food contamination. This bacterium is potent to cause diseases in human and animals. This organism for causing sever diseases and high mortality with long incubation period and intending in causing diseases in individuals who have disorder backgrounds in cellular immunity are important with respect to human health.
Method: in this study to investigate anti-bacterial effect of peppermint essential oil in white cheese, the essential oil was extracted from the mint plant and then its components were analyzed by chromatographic gas. The effect of the anti-listeria in cheese with different densities of peppermint essential oil was analyzed after the sampling and proliferating in specific culture medium.
Findings: the results of the analysis of chromatographic gas showed that there are neo- menthol components, iso-menthone and cineole 1.8 in peppermint essential oil. The results of the culture for different densities of 0.03, 0.015 and 0.0075 per cent of mint essential oil indicates of the significant increasing of the anti-listeria nature along with increasing the essential oil as well as growth retardation of one logarithm on seventh and fifteenth days, 65% of growth retardation on thirties day and growth retardation of one logarithm on sixties day for different densities ($P < 0.05$).
Conclusions: based on the findings, mint essential oil in different densities of 0.03, 0.015 and 0.0075 per cent contains anti-listeria monocytogenes nature that reduces the number of listeria monocytogenes bacteria significantly in samples with essential oil in comparison with samples without essential oil. The most nature of anti-listeria relates to 0.03 per cent- density mint essential oil. There is a significant difference between the different varieties of densities in terms of sensory features and all the densities were acceptable in panelists'.

1. INTRODUCTION

listeria monocytogenes, is one of the most important pathogenic factorsthat is food poisoning caused by eating foods, especially dairy products can be transferred to human, contaminated with the *Listeria monocytogenes*. This pathogenic bacterium is capable to spread through the mammary glands, feces and other secretions of infected animals or animals without clinical symptoms, human resources and also environmental sources and contaminated instruments cause to contaminate the milk [1, 2]. Since the bacterium is capable to proliferate at 1 to 45° C and pH range of 4.1 to 9.6, it is supposed that to survive for a long time in food products [3, 4].

Today, it is mostly used cheese made byunpasteurized and local milk. Also, Iranian traditional white cheese is produced in Iranian families extendedly; to make these kinds of cheese unpasteurized milk with *listeria monocytogenes* is used or it may be contaminated in time of making cheese which results in disease due to the microorganisms in food.

There are several serious infections in the world usually by eating unpasteurized and pasteurized cheese contaminated with the bacterium *listeria monocytogenese*; therefore, it is logical to use chemical preservative and natural essential oil to prevent the listeriosis through consumption of cheese contaminated with *Listeria monocytogenes* [6, 5].

Using chemicals to prevent or delay food spoilage is widely prevalent today. There are studies in relation to side effects of chemicals that are carcinogenic for human being [7, 8].

Nowadays, most of the consumers in worldwide are interested to use natural essential oils as preservatives. These essentials are effective over the wide range of positive and negative warm bacteria. However negative warm bacteria seem to be partly more resistant due to having lipopolysaccharide in the outer membrane [5, 6].

According to studies, the possibility of being *listeria monocytogenes* bacterium in different kinds of cheese and the effects of anti-listeria in mint essential oil over this bacterium in Iranian white cheese is investigated.

2. MATERIALS AND METHODS

The purpose of this study is to investigate the effect of essential oil over the growth of *listeria monocytogenes* in Iranian white cheese with different kinds of densities, including 0.03, 0.015 and 0.0075 that are tested three times. First

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of all Iranian white cheese was made traditionally and after pasteurizing the *listeria monocytogenes* bacterium was added to cheese. It was completely standardized to produce the cheese and finally it was dehydrated and preserved in common condition. Mint essential oil that is prepared through the steam distillation method, was added to cheese. At the same time the cheese without *listeria monocytogenes* bacterium, as a sample to make sure about the producing cheese properly, was provided. One of the samples was without essential oil. At specified intervals, it was sampled from the cheeses with different densities of essential oil and they were compared with the sample including no essential oil and the results were analyzed [9]; mint essential oil was prepared by soxhlet extractor [9, 10].

The standard peppermint essential oil was analyzed in terms of its components (menthaspicata) after preparing through the gas chromatography-mass machine Agilent 6890 of a kind Hewlet-Packed with column of 30 m. length,inside diameter of 0.25 mm. and the layer thickness of 0.25 micrometer of a kind BPX5 [11,12]. The bacterium was studied in this research was of a kind *listeria monocytogenes* (ATCC 19115).

The intended amount of mint essential oil was added to cheese, containing bacterium to be achieved in 0.03, 0.015 and 0.0075 per cent of density [13]; then the cheeses were preserved at 15° C up to 15 days and then in a usual condition of fridge (temperature of the refrigerator, closed door and protect from light) were kept for 60 days. The sampling was conducted in specific times, while the microbiological safety conditions were considered to prevent any contamination during the experiment [13, 14]. In order to analyze the growth of *listeria monoctogenes* bacterium PALCAM Listeria-Selective agar (Base) made by Merck company of Germany was used. The plates were kept at the temperature of fridge until to be used. After sampling for culturing, 5 gr. Of each sample produced cheese was taken, weighed and sliced by mortar and Chinese sterile crucible and mixed with 45cc peptone water and 0.1 cc of the prepared solution was transferred to palcam listeria agar by the sampler to be cultivated via surface culture method. After 24 hrs the cultivated culture was incubated for 24 h at 37 ° C, the colonies were counted and according to the dilution the number of the bacteria per gram was calculated. The samplings were carried out about one-tenth, using the dilutions of 1-10 and 2-10. In PALCAM culture medium the incubated *listeria monocytogenes* bacteria formed the colonies that were white with black halo (figure1).

Figure1: white colonies of *listeria monocytogenes* with black halo in PALCAM culture medium.

In order to count the colonies of the *listeria monocytogenes*, plates including bacteria were analyzed and spherical colonies, tiny, green- gray with black margin were counted and the number of *listeria monocytogenes* bacteria per gram were reported. To count the *listeria monocytogenes* bacteria and measuring pH, biological and chemical tests were performed at the following time:

- zero day (after inoculation with *Listeria monocytogenes* and formation of cheese);
- Seventh day (168hrs);
- Fifteenth day (after the first stage of forming cheese at 14-15° C or 360 hrs.);
- Thirties day (720 hrs.);
- Forties day (1080 hrs.);
- Sixties day (1440).

To evaluate the effect of the essential oil over the sensory features including flavor, taste and general acceptance of cheese with different densities of essential oil along with a sample in control group under the equal condition by the seven panels of test were trained in terms of evaluated concepts of features; after preparing the samples offered to reviewers with a assumed code and the results were recorded in evaluation forms. In order to determine the features of favor and taste of samples Hedonic test was used (1- Intolerable, 2- bad, 3-not interesting, 4-average, 5-good, 6- very

good, and 7- excellent). Excel 2013 was applied in statistical analysis to draw diagram and tables. Analyzing the statistical data, the version 19 of SPSS was used. The amount of the P is less than 0.05 in terms of statistical analysis that is significant. Variance test analysis (ANOVA) was used to study the effect of the independent variables (essential oil amount, time maintenance and sampling) on dependent variable (logarithm of the number of bacterium).

3. RESULTS

The results of the analysis of gas chromatographic of mint essential oil indicate that that there are neo- menthol components, iso-menthone and cineole 1.8 respectively 38.7%, 31.32% and 7.87% in peppermint essential oil. The results of the culture for different densities of 0.03, 0.015 and 0.0075 per cent of mint essential oil indicates of the significant increasing of the anti-listeria nature along with increasing the essential oil as well as growth retardation of one logarithm on seventh and fifteenth days, 65% of growth retardation on thirties day and growth retardation of one logarithm on sixties day for different densities. The results of the sampling bacteria in test days have been shown in diagram of figure 2 to show the mean logarithmic growth of *Listeria monocytogenes* bacterium at the days of the test.

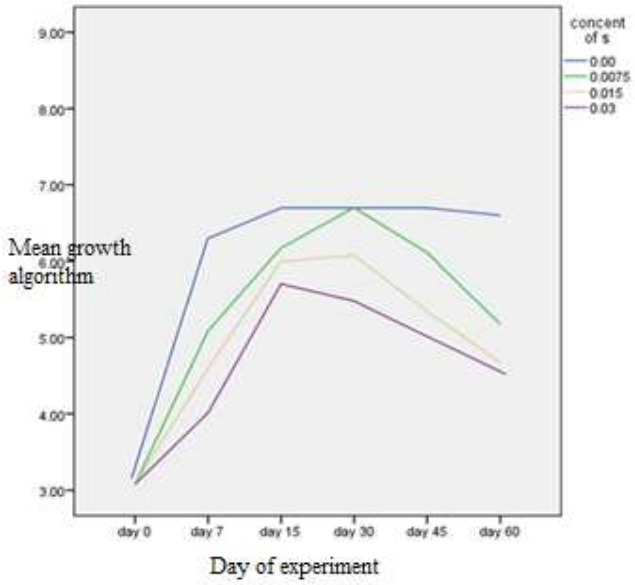

Figure 2: the mean logarithmic growth of *Listeria monocytogenes* bacterium at the days of the test.

The highest amount of anti-listeria characteristic relates to the density of 0.03% of mint essential oil during the sixty days. The results of the sensory features of cheese samples including essential oil was shown via panelists'. There are significant differences between the different densities of essential oil in terms of taste characteristics so that, there are no significant relationship between the densities of 0%, 0.0075%, 0.015%, and 0.03% in terms of general acceptance. Also, there is no significant relationship between the flavor characteristics in different densities of 0.0075%, 0.015% and 0.03% and it is acceptable. There is no significant difference between the densities in respect to general acceptance. The results of the sensory evaluation of cheese, including mint essential oil are represented in table1 and figure3.

Table 1: the mean results of sensory evaluation

Density of mint essential oil	taste	flavor	General acceptance
(control)	5.42A	5.7 A	0.99 A
%0.0075	5.85Aa	6.42Aa	1Aa
%0.015	4.14Ab	5.75Aa	0.97 Aa
%0.03	4.04Ab	5.55 Aa	0.96 Aa

The similar letters of each column introduces that there is no significant difference between two groups at the level of 5%.
Capital letters present the difference between treatment in control group and other treatments.
Small letters present the difference between the treatment groups.

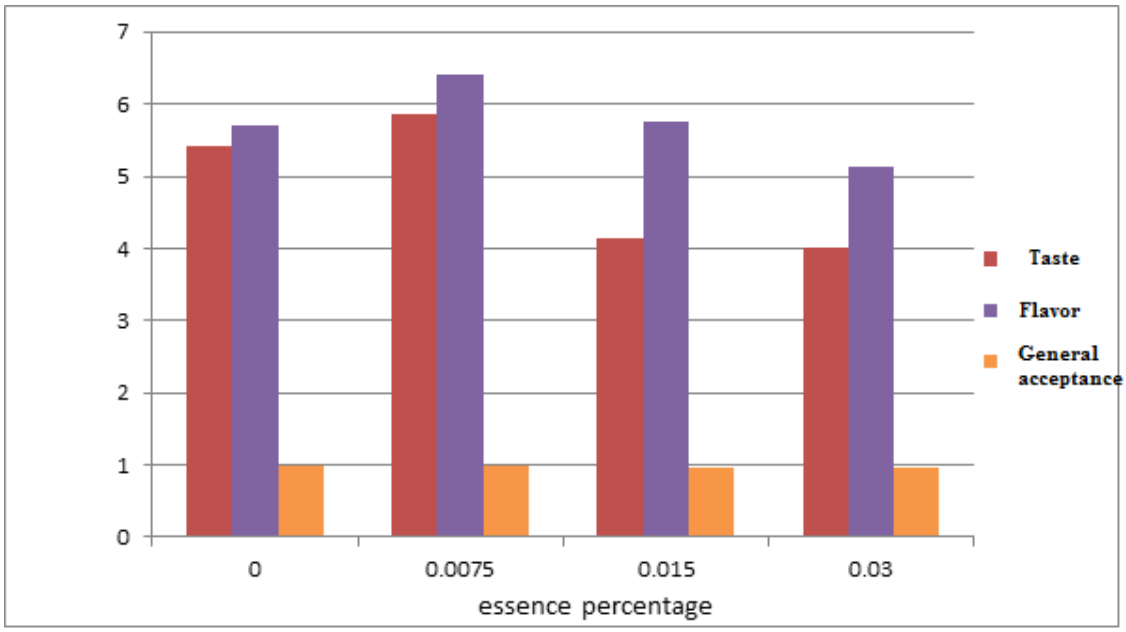

Figure 3: the comparison of sensory evaluation results of mint essential oil.

4. DISCUSSION AND CONCLUSION

It is estimated that more than 30% of people in the industrial countries are suffering from transmitted diseases by foods. It is declared that in 2000 at least 2million individuals all over the world have died because of food related diseases. Therefore, methods for decreasing or controlling transmitted pathogens by foods are necessary. One of these methods is adding anti-microbial compounds to food compounds, and one of these secure methods is using herbal essences [15-17]. Generally, biological activities of herbal essences are related to their chemical compounds. The existent compounds in herbal essences have anti-microbial activities and in categorizing materials are considered as immune materials (GRAS). Therefore, it is possible to use them for preventing the growth of pathogen bacteria and microbial flora in the foods [17]. Peppermint is to some extent different from other types in Mentha with respect to components of essence that the most important of them are components such as Carvone, limonene, piperitone and so on that these compounds according to seasonal conditions, life of plant, weather and climate, soil composition and other conditions includes different percentages [18], therefore, different researchers reported the main compositions of peppermint differently [19]. Composition of components of the essence used in this study during chromatography gas that has been performed by the researcher center of medical herbs of Jihad University includes neo Menthol, IsoMenthone and 1.8 Cineole are respectively 38.7%, 31.32%, and 7. 87%. Because of different compositions in different essences in different essences that are prepared from various row materials essences have different anti-microbial activities. In the present study 0.03, 0.015, and 0.0075% density peppermint essence were used to analyze anti-bacterial effects of this essence *on Listeria Monocytogenes*. ($P < 0.05$) As mentioned previously the peppermint essence for having aforementioned compositions has strong anti-bacterial on warm positive bacteria. *As Listeria Monocytogenes* is a positive warm bacteria and according to such decreasing a considerable growth of bacteria in densities of 0.03, 0.015, and 0.0075% peppermint essence that the sample without essence seems that peppermint essence has rapid antibacterial effects on *Listeria Monocytogenes.*

According to the result of this study we conclude that peppermint essence in the entire densities has appropriate anti- *Listeria Monocytogenes*, so that the entire applied densities in this study causes a significant decreasing in the number of *Listeria Monocytogenes* bacteria in samples with essence than samples without essence. Because against densities of 0.0075 and 0.015 until the sixth day the number of Listeria bacteria is in the logarithm form that somehow it should be emphasized that in a higher density the essence has a higher anti-listeria effect but it should be considered that higher densities will damage the flavor of cheese that is not appropriate in the view of consumers, therefore the results of sensual evaluation showed that there is no significant difference between three densities of essence, but analyzing three densities it is observed that beside lack of significance the difference sensual evaluations in applied densities, general acceptance of such densities from 0.0075% to 0.03% is decreasing that shows by increasing density of general acceptance this essence decreases and it is not possible to use high level of this essence in the cheese. Also it is inferred that anti *Listeria Monocytogenes* effects of peppermint essence in the common conditions of Iranian white cheese in the first 15 days has a more power than 30 last days of preservation.

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