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Inactivation of Listeria monocytogenes by cinnamon extract and essential oil in apple juice, production of cinnamon apple juice

Nadia Fiji Vari[1]*, Hamdollah Mushtaghi[2], Maryam Abbas Vali[3]

[1]Ph.D. student in food hygiene, Faculty of Veterinary Medicine, University of Mashhad
[2]Full Professor, Department of Health and Food Quality Control, Faculty of Veterinary Medicine, Shahrekord University
[3]Assistant Professor, Department of Health and Food Quality Control, Faculty of Veterinary Medicine, Shahrekord University

Abstract

Fruit juice is the liquid obtained from healthy, suitable and ripe edible parts of fresh or preserved fruits in ideal conditions and contains most of the salts and vitamins of the original fruit.

The low pH of these products and the high concentration of fermentable sugars have made their environment selective for the growth of certain groups of acid-resistant microorganisms.

The origin of microorganisms is in fruit juice, raw fruit and contaminated equipment. Listeria bacteria contaminates many foods and drinks, and swallowing contaminated food is the main source of infection. Listeria monocytogenes is the only important human pathogen among the other six Listeria species.

Cinnamon is used as a flavoring and food preservative. In this study, the antibacterial effect of cinnamon extract and essential oil in apple juice and the production of favorable taste were evaluated.

The diameter of Listeria monocytogenes non-growth halo was 10 mm by welling method in a concentration of 20% ethanol extract of cinnamon.The minimum Inhibitory concentration (MIC) of cinnamon ethanolic extract was 6 mg/ml (0.6%) and the minimum inhibitory concentration of cinnamon essential oil was 0.1 mg/ml (0.01%).The results of evaluating the number of bacteria in apple juice containing cinnamon extract showed that the number of bacteria decreased by 4 logarithms from day zero to the third day and then increased by 1 logarithm until the tenth day.In the control group, from day 0 to day 1, the number of bacteria decreased by 1 logarithm, and then it decreased by 1 logarithm until day 10. Statistically, there was a significant difference between the tested group and the control group ($P<0.05$).

Keywords: Listeria monocytogenes, cinnamon extract and essence, apple juice

Introduction

Fruit juice is the liquid obtained from healthy, suitable and ripe edible parts of fresh or preserved fruits in ideal conditions and contains most of the salts and vitamins of the original fruit.

Currently, the use of fruit juice is seen in the diet of many societies. On the other hand, in many countries with hot climates or in the hot seasons of the year when the body needs to receive plenty of fluids to compensate for lost water, consuming fruit juice is unavoidable (Al-jedah, 2002).

Fruit juices have a low pH due to the presence of large amounts of organic acids such as malic and citric acid. The low pH of these products and the high concentration of fermentable sugars have made their environment selective for the growth of certain groups of acid-resistant microorganisms (Iran Standard Institute).The origin of microorganisms is in fruit juice, raw fruit and contaminated equipment. Listeria bacteria contaminates many foods and drinks, and swallowing contaminated food is the main source of infection.This bacterium is found everywhere, including soil, plants, water, surfaces, milk, sewage from slaughterhouses, human and animal feces. Listeria genus includes 6 Listeria species (Punder, 2010). Listeria monocytogenes is the only important human pathogen among the other six species of Listeria (Mortazavi and Sadeghi Mahong, 2013).

Listeria monocytogenes grows in a wide range of temperatures between 0-42 with the optimum temperature between 30-35 and it grows extremely slowly at temperatures below 50 C and its lag phase is between 1-33 days and the division time is from 13 to more than 130 hours.

HTST pasteurization reduces the number of living cells of this organism by 2.5 logarithmic cycles. All strains of this organism are unable to grow in the pH range below 5.5, but the minimum pH for growth depends on the strain and the acid

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used to adjust the pH varies between 4.4 and 5.6 (Mortazavi and Sadeghi Mahong, 2014).

Cinnamon with its scientific name (Zeylanicum Blum Cinnamomum) is a shrub belonging to the family of oleanders and is native to Sri Lanka and South India and is used as a food flavoring and preservative.

It has also been used since ancient times to treat some diseases such as indigestion, inflammation of the stomach and intestines, and blood flow disorders in many countries. In addition, it has been used as an antipyretic, anti-allergic and anti-cough medicine.

The important compounds of cinnamon are cinnamic aldehyde and eugenol, which has the most antibacterial effect related to cinnamic aldehyde (Hamidpour et al., 2013).

Research Methodology

Raw materials include Listeria monocytogenes standard strain ATCC 1297, Tryptose Soy Agar culture medium, Tryptose Soy Broth culture medium from Merck, Germany, Plate Count Agar culture medium from Ibersco company, Palcom culture medium from Ibersco company. Arman Sina's pure ethanol was ground cinnamon wood.

Investigating the inhibitory effect of cinnamon extract by welling method

First, Mueller Hinton Agar culture medium was poured into several plates with a large thickness, then a swab was dipped into the tube containing Listeria monocytogenes bacteria and drawn on the surface of Mueller Hinton Agar in four lines so that the entire medium was covered with bacteria.

Then a yellow sampler head was cut with a sterile scalpel and pressed into the Mueller Hinton agar medium and several wells with a distance of 5 mm were created with a sampler of 100 microliters of different concentrations of cinnamon extract (5, 10, 15 and 20%) in the well. The numbered cards were thrown. Then it was placed in a 37 degree greenhouse for 24 hours. After 24 hours, the plate was checked and the diameter of the growth halo around the well was measured (Soleimani and Abrazah, 2014).

Determination of minimum inhibitory concentration (MIC) of cinnamon extract

For this purpose, the microdilution method was used. First, next to the flame, with a sampler, one milliliter of bacteria with a dilution of 105 was taken from the TSB culture medium and poured into the wells of the 96 microplate. The order of concentrations included 0.2, 0.4, 0.6, 0.8, 1 and 1.2% of cinnamon extract was 20% (equivalent to 2, 4, 6, 8, 10 and 12 microliters).

Then 188, 186, 184, 182, 180, 178 and 176 microliters of TSB culture medium were added to the wells and finally 10 microliters of bacteria were added to all the wells (the capacity of the wells is 200 microliters).

Three replicates were done for each concentration. In addition, 3 wells were considered as controls, and 100 microliters of cinnamon extract and 100 microliters of TSB culture medium (extract control and culture medium) were poured into the first well.

190microliters of culture medium and 10 microliters of bacteria (control culture medium and bacteria) were poured into the second well, and 200 microliters of TSB culture medium was poured into the third well for control.

Controls were also repeated three times. Then the microplate was placed in a 37°C greenhouse for 24 hours. The microplate was examined after 24 hours. The first clear well was identified as the minimum inhibitory concentration of cinnamon extract (Mousavi and Shavisi, 2012).

Determining the minimum bactericidal concentration (MBC) of cinnamon extract

From the wells containing 0.6% concentration of cinnamon extract (MIC) and the wells after that, 20 microliters were removed and poured into plates containing TSA culture medium and cultured superficially (spread with a Pasteur pipette).

And placed in a greenhouse at 37 degrees Celsius for 24 hours. Plates were counted after 24 hours. The plate in which the bacteria did not grow was determined as the minimum lethal concentration of cinnamon extract (Mousavi and Shavisi, 2012).

Impregnation

After determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of cinnamon extract and essential oil, 19 ml of apple juice was poured into a sterile container, then 1 ml of Listeria monocytogenes bacteria with a dilution of 105

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was added to it, which was considered as day zero witness.

18.6 ml of apple juice plus 0.4 ml of cinnamon extract (a concentration before MIC) and finally 1 ml of Listeria monocytogenes bacteria with a dilution of 105 were added in 3 other sterile containers, which were considered as treatments. Immediately from 4 dishes, it was directly poured into the plates and it was cultured on the surface. In addition, 1 ml from the dishes was transferred into a tube containing 9 ml of physiological serum and it was diluted to 0.1 ¬¬ It was obtained that from this dilution, it was cultured on the surface of the plate. From one of the dilutions, it was cultured superficially in Palcom culture medium.

Then the plates were placed in a greenhouse at 37 degrees Celsius for 24 hours. Also, containers containing apple juice, cinnamon extract and bacteria were placed in a 15°C refrigerated greenhouse.

The result of counting the bacteria in the plate after 24 hours was considered day zero. After 24, 48, 72, 120, 168 and 240 hours, it was cultured from the containers inside the greenhouse at 15°C into the PCA plate and placed in the greenhouse at 37°C for 24 hours.

The result of counting the bacteria inside the plates was considered as culture on the 1st, 2nd, 3rd, 5th, 7th and 10th day. The same method was used for cinnamon essential oil. With the difference that 18 ml of apple juice plus 1 ml of dimethyl sulfoxide (DMSO) along with 0.05 mg/ml of cinnamon essential oil and finally 1 ml of Listeria monocytogenes bacteria were poured into the containers.

Finally, the results of this study were repeated 3 times at two temperatures of 4 and 15 degrees Celsius using SPSS version 20 software, the repeated measurements ANOVA test was performed.

Findings

Minimum inhibitory and bactericidal concentration of cinnamon extract and essential oil

MIC of cinnamon ethanolic extract mg/ml 6 (0.6%), MIC of cinnamon essential oil 0.1 mg/ml (0.01%), MBC of cinnamon ethanolic extract mg/ml 8 (0.8%) and MBC of cinnamon essential oil mg/ml ml became 0.1 (0.01 percent).

Antibacterial effect of 0.4% concentration of cinnamon extract at 4°C

According to Figure 1, the number of bacteria in the apple juice sample in the presence of 0.4% cinnamon extract decreased by 4 logarithms from day zero to the third day and then increased by 1 logarithm until the tenth day.

In the control group, from day 0 to day 1, the number of bacteria decreased by 1 logarithm, and then it decreased by 1 logarithm until day 10.

Statistically, there was a significant difference between the tested group and the control group ($P<0.05$). As a result, cinnamon extract had antibacterial effect on Listeria monocytogenes bacteria in apple juice at 4 degrees Celsius.

Antibacterial effect of 0.4% concentration of cinnamon extract at 15°C

According to Figure 2, in the tested group (apple juice, cinnamon extract and Listeria monocytogenes bacteria) on the first day, the number of bacteria decreased by 2 logarithms, and this trend of decreasing bacteria continued on the second and third day, and the number of bacteria decreased to cfu reached 102/ml, then on the fifth and seventh days we saw an increase of bacteria by 1 logarithm and finally on the tenth day the number of bacteria decreased from 103 cfu/ml to 101 cfu/ml. In fact, the number of bacteria on the tenth day compared to on day zero, it decreased by 4 logarithms.

In the control group (apple juice and Listeria monocytogenes bacteria), a 1 log reduction of bacteria was observed from day 1 to day 10. According to the analysis of variance, the difference between the tested group and the control group was statistically significant ($P<0.05$).

Antibacterial effect, 0.005% concentration of cinnamon essential oil at 4 degrees Celsius

According to Figure 3, the concentration of 0.005% cinnamon essential oil caused a decrease of 1 logarithm in the number of bacteria from the first day to the third day, and another 1 logarithm decrease from the fifth day to the tenth day.

In the control group (apple juice and Listeria monocytogenes bacteria), the process of bacteria reduction was as follows: on the first, second and third day, there was a 1 logarithm reduction, and then on the fifth, seventh and tenth day, we had another 1 logarithm reduction.

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According to the analysis of variance test, there was no statistically significant difference between the tested group and the control group (P≤0.05).

Antibacterial effect of 0.005% concentration of cinnamon essential oil at 15°C

Figure 4 shows that in the tested group, the number of bacteria decreased by 1 logarithm on the first day and this decrease trend remained constant until the fifth day. Finally, on the tenth day, the number of bacteria reached zero.

In the control group, there was no reduction of bacteria on the first day, but on the second, third, fifth and seventh days, 1 logarithm of bacteria was reduced, and on the 10th day, another 2 logarithms of bacteria were also reduced. According to the analysis of variance, the difference between the case and control groups was statistically significant (P<0.05).
<0.05).

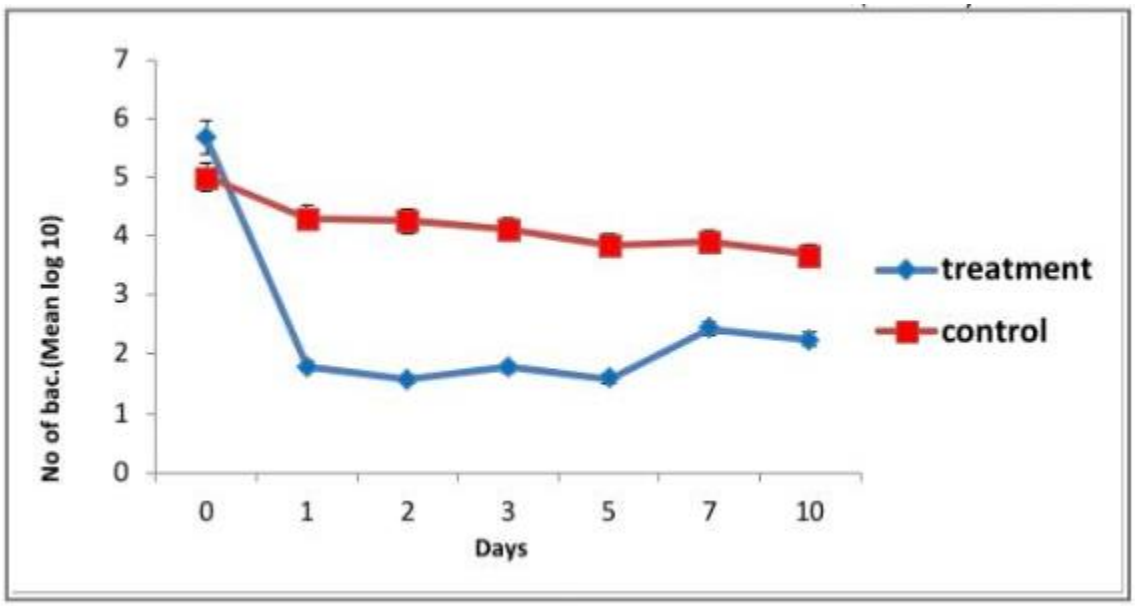

Fig 1 The Effect of concentration 0/4 % Cinnamon Extract on Listeria monocytogenes at 4 ° C

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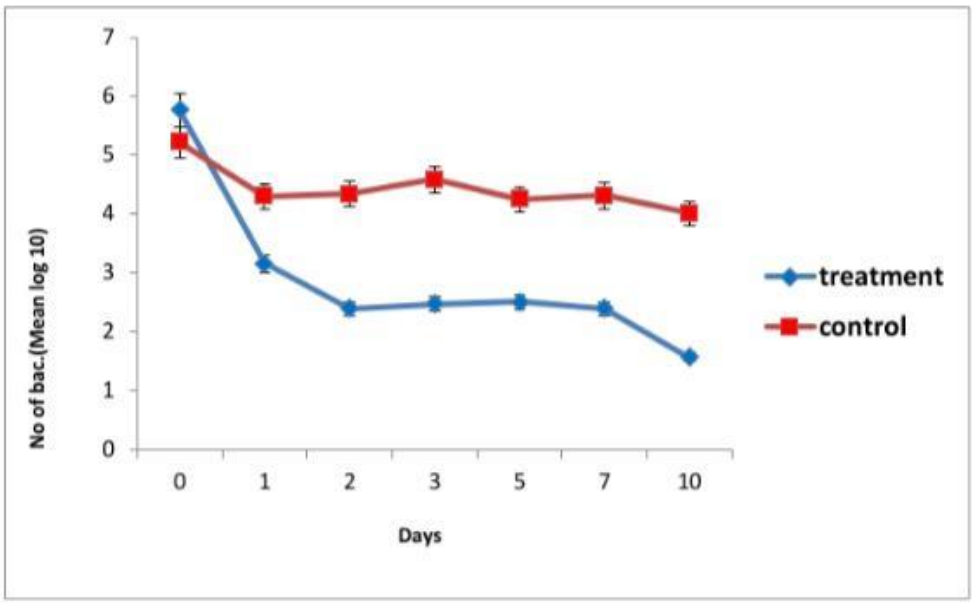

Fig 2 The Effect of concentration 0/4 % Cinnamon Extract on Listeria monocytogenes at 15 ° C

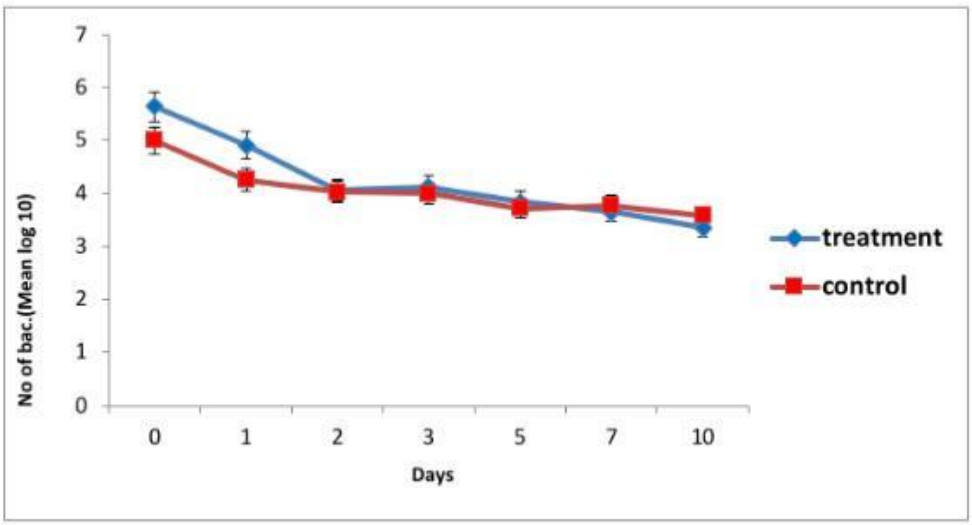

Fig 3 The Effect of concentration 0/005 % Cinnamon essential oil on Listeria monocytogenes at 4 ° C

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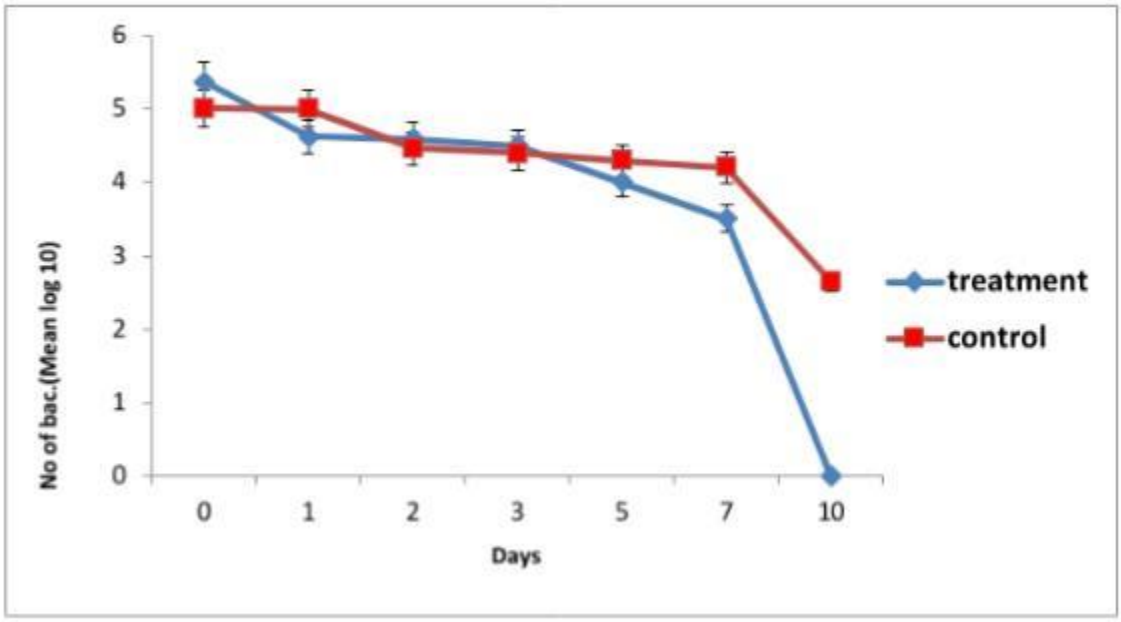
Fig 4 The Effect of concentration 0/005 % Cinnamon essential oil on Listeria monocytogenes at 15 ° C



Discussion

Yusti et al investigated the inactivation of Listeria monocytogenes in apple juice supplemented with cinnamon and found that Listeria monocytogenes is completely inactivated in apple juice at 20°C with 1% cinnamon (Yusti et al, 2002). In the present study, cinnamon extract and essential oil caused the inactivation of Listeria monocytogenes in apple juice at two temperatures of 4 and 15 degrees Celsius.

Surre et al. investigated the antimicrobial effect of cinnamon essential oil on Escherichia bacteria strains and concluded that cinnamon compounds have an inhibitory effect on this bacterium (surre et al, 2005).

Simanga and colleagues investigated the antimicrobial effect of cinnamon essential oil on Escherichia coli and Salmonella typhimurium bacteria and found that cinnamon essential oil has an inhibitory effect on these bacteria. (Cimanga et al, 2006) Bin et al.

They investigated bacteria (Bacillus cereus, Listeria monocytogenes, Escherichia coli, Staphylococcus aureus and Salmonella anatomum) and found that cinnamic aldehyde and several polyphenolic compounds of cinnamon have an inhibitory effect on these bacteria and can be used as natural preservatives. Bin et al., 2007).

Becerril et al investigated the antimicrobial properties of cinnamon and oregano on Escherichia coli and Staphylococcus aureus in food packaging and proved the inhibitory effect of these compounds on bacteria. (Becerril et al, 2007).

The use of medicinal plants and spices can control bacteria in food, in addition to creating a pleasant taste and aroma, based on the results of this research, the concentration of 0.4% cinnamon extract in apple juice reduces Listeria monocytogenes was observed at two temperatures of 4 and 15 degrees Celsius.

The concentration of 0.005% of cinnamon essential oil at 4°C did not have much effect in preventing the growth of Listeria monocytogenes bacteria in apple juice. But the concentration of 0.005% of cinnamon essential oil at 15 degrees Celsius decreased the number of Listeria monocytogenes bacteria in apple juice.

Based on the obtained conclusions, cinnamon extract and essential oil can be used as a natural preservative and flavor to replace chemical preservatives in fruit juices.

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