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The effect of rosemary essential oil on Listeria monocytogenes bacteria in turkey meat extract

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Abstract

Meat is an important source of nutrition for humans, which plays an essential role in human daily nutrition due to the existence of rich sources of protein. Poultry meat is one of the most favorable foods for the growth of pathogenic bacteria such as Salmonella, Clostridium, Campylobacter, Escherichia coli and Listeria.

Today, the use of antibiotics has decreased due to their side effects and increased resistance of microorganisms to them, and medicinal plants are very popular in curing bacterial infections. In this study, the antibacterial effects of rosemary essential oil (Rosmarinus officinalis) on Listeria monocytogenes bacteria were investigated at 4 and 15 degrees Celsius in turkey meat extract.

The minimum inhibitory concentration (MIC) of rosemary essential oil was 0.5% by microdilution broth method and the minimum bactericidal concentration (MBC) of rosemary essential oil was 0.5% by plating method.

The results obtained from the inoculation of bacteria in the turkey meat extract at 4 and 15°C after ten days showed that at 4°C the number of bacteria in the experimental groups decreased by 2 logarithms on the first day. And after that, it decreased by another 1 logarithm until the seventh day, and finally, on the tenth day, bacteria decreased by another 1 logarithm.

Also, at a temperature of 15 degrees Celsius, the number of bacteria decreased by 1 logarithm on the first day, and another 1 logarithm decrease was observed on the second and third day. Then, on the fifth and seventh days, we saw an increase in bacteria by 1 logarithm, and finally, on the tenth day, the number of bacteria increased by another 1 logarithm ($P \leq 0.05$). The results showed that rosemary essential oil has antibacterial properties against Listeria monocytogenes bacteria.

Key words: Listeria monocytogenes, rosemary essential oil, turkey meat extract

Introduction

Meat is an important nutritional source for humans, which plays an essential role in human daily nutrition due to the existence of rich sources of protein.

All types of red and white meat are important sources of protein, and in today's societies, due to the prevalence of cardiovascular diseases and diabetes that occur after the excessive consumption of red meat, consumers' preference for white meat is increasing. Meanwhile, poultry meat has received more attention due to its acceptability and easier access.

Turkey meat is one of the common types of poultry meat, which is less consumed by people due to the fact that the unique characteristics of turkey meat are unknown.

Poultry meat is one of the most favorable foods for the growth of pathogenic bacteria such as Salmonella, Clostridium, Campylobacter, Escherichia coli and Listeria, which due to the special nature of poultry farming, the lack of correct farm management methods, the special way of killing and processing meat.

Extraction is exposed to pollution. Also, the indiscriminate use of antibiotics to control infectious agents or to increase the absorption and rapid growth in poultry threatens the health of consumers (Hosseini and Ebadi, 2015, Kashdozan et al., 2016, Melki et al., 2015).

Today, food safety is a major concern for both the consumer and the food industry, and despite the progress made in the food industry, diseases

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caused by microbial contamination of food have emerged as a serious problem.

Listeria are Gram-positive, sporeless, rod-shaped, cold-oriented, facultative anaerobic, catalase-positive, oxidase-negative bacteria that belong to the Lactobacillus family (Punder, 2010). This bacterium is widely distributed in nature and can be found on soil, water, manure, vegetables, sewage, fodder and plants (Mortazavi et al., 2013).

Since long ago, medicinal plants are widely used in traditional medicine and modern medicine due to their medicinal properties. Also, due to the side effects of antibiotics and the increase in the resistance of microorganisms against them, medicinal plants are very popular in curing bacterial infections (Melki et al., 2015).

Rosemary plant with the scientific name (Rosmarinus officinalis) is a member of the mint family. Rosemary plant contains oleoresin, tannin, pinene, camphor, cineol, ecanone, verbanone, borneol, linalool and camphene.

Rosemary actually contains variable amounts of aromatic and volatile substances, which can be referred to flavonoids such as diosmetin, hispidolin and apigen and phenolic groups such as caffeic, chlorogenic and rosmarinic acid. Rosemary hempenine contains large amounts of salicylates (Ahmadi Asbchin and Mostafavipour, 2014, Sai Dehkordi et al., 2018).

Research method

The required materials included Listeria monocytogenes standard strain ATCC 1297, Tryptose Soy Agar (TSB) culture medium from Merck, Germany, Plate Count Agar (PCA) culture medium from Ibresco, Palcam culture medium from Ibresco, rosemary plant leaves and turkey meat.

Rosemary essential oil method

Rosemary essential oil was prepared by steam distillation with a Clevenger machine. First, 40 grams of rosemary were dissolved in 700 milliliters of distilled water (in a 1000 milliliter round-bottomed George balloon), then the balloon was installed on the Clevenger heater. After two hours of heating, the volatile compounds evaporated and after passing through the machine's refrigerant, they were condensed. The obtained essential oil was poured into a sterile container (Ajaq et al).

Preparation of bacteria

First, a loop was removed from the vial containing the standard strain of Listeria monocytogenes (1297) and cultured on the solid culture medium of Tryptose Soy Agar in a streak form and placed in a 37°C incubator. After 24 hours, one colony of bacteria colony was transferred to the Tryptose Soy Broth culture medium by a sterile instrument and next to the flame, and it was placed in a greenhouse at 37 degrees Celsius for 24 hours (Mousavi and Shavisi, 2012).

Determining the minimum inhibitory concentration (MIC) of rosemary essential oil by microdilution method

First, 2.5 ml of dimethyl sulfoxide (DMSO) was dissolved in 50 ml of TSB medium (5% solution), then 5 ml of this solution was poured into 8 test tubes. The tubes were placed in an autoclave for sterilization. After cooling the tubes, with a sampler, concentrations of 0.25-2% of rosemary essential oil were poured into them and it was completely smooth (a tube without essential oil as a control).

Then, 180 microliters of each tube was poured into the microplate wells, and then 20 microliters of bacteria with a dilution of 105 was added to each well. For control, only 200 microliters of TSB solution with DMSO without rosemary essential oil was poured into one well, and 180 microliters of TSB solution with DMSO without rosemary essence was poured into the other well along with 20 microliters of Listeria monocytogenes bacteria.

The tests were done with 3 repetitions. The microplate was slightly shaken and then placed in a 37°C incubator for 24 hours. Then the microplate was taken out of the greenhouse and the first well that did not have turbidity was considered as MIC (Mousavi and Shavisi, 2013).

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Determining the minimum bactericidal concentration (MBC) of rosemary essential oil

To determine MBC, 20 microliters were taken from the wells containing the MIC concentration and subsequent concentrations (0.5, 0.75, 1, 1.25, 1.5 and 2%) with a sampler and placed In the plates. Containing TSA culture medium was poured and spread with a Pasteur pipette. The plates were placed in a greenhouse at 37°C for 24 hours. After leaving the plates from the greenhouse, any concentration in which the bacteria did not grow was considered as the minimum bactericidal concentration (MBC) of the essential oil (Mousavi and Shaveisi, 2012).

Preparation of turkey meat extract

First, half a kilo of turkey meat was bought from the meat shop and transported to the food laboratory following the cold chain. Some of the meat was minced and 50 grams of this meat was weighed with a scale and poured into the Erlenmeyer flask along with 450 ml of distilled water. Arlen stood on the flame.

After boiling for half an hour, the flame was turned off and Erlenmeyer flask was placed in the refrigerator after cooling. After 24 hours, Arlen was removed from the refrigerator and the fat on it was smoothed with a tampon strainer and placed on the flame again for half an hour. The obtained meat extract was smoothed with filter paper (Whatman) and poured into an Erlenmeyer flask. And kept in the refrigerator until inoculation.

Evaluation of bacterial growth in meat extract containing rosemary essential oil

The turkey meat extract was taken out of the refrigerator and boiled for half an hour. Then its pH was read.

In 10 sterile containers with a lid, 18 ml of turkey meat extract plus 1 ml of rosemary essential oil along with dimethyl sulfoxide (DMSO) solution of a concentration before (MIC) and 1 ml of Listeria monocytogenes bacteria with 105 dilution was added.

Then the containers were placed in the greenhouse at 4 and 15 degrees Celsius for 10 days to count the bacteria at time zero, the first, second, third, fifth, seventh and tenth days, and serial dilutions were prepared from the sterile containers. 1 ml of the contents of the container was taken and transferred into a tube containing 9 ml of physiological serum and mixed with a vortex.

Then, 0.1 ml of the intended dilution was taken with a sampler and transferred to three plates containing Palcom culture medium by surface method. After 24 hours, the plates containing 30-300 colonies were counted and by multiplying the number of colonies in the dilution photo and multiplying by 10, the number of bacteria per milliliter of turkey meat extract was obtained. The control and treatment groups were prepared at two temperatures of 4 and 15 degrees Celsius as follows:

-ml/CFU 105 Listeria monocytogenes bacteria + 19 ml turkey meat extract (control)

CFU/ml- 105 Listeria monocytogenes bacteria + 18 ml turkey meat extract + 1 ml DMSO solvent (control)

105 CFU/ml of Listeria momenocytogenes bacteria + 18 ml of turkey meat extract + 1 ml of DMSO solvent with 2.5 mg/ml of rosemary essential oil

Finally, the results of this study were repeated twice at 4 and 15 degrees Celsius using SPSS version 20. The repeated measurements ANOVA test was performed and (0.05) P<) was considered for the significant level of difference between groups.

Findings

The minimum inhibitory and bactericidal concentration of rosemary essential oil

The minimum inhibitory concentration of rosemary plant essential oil was 0.5% and the minimum bactericidal concentration of rosemary plant essential oil was 0.5%.

Antibacterial effect of 0.25% concentration of rosemary essential oil at 4°C with control containing DMSO

In the control group, on the first day, the number of bacteria decreased by 1 logarithm, and on the

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second day, the bacteria decreased by 1 logarithm, which continued until the seventh day. And then on the 10th day, another 1 logarithm decrease in bacteria occurred, which statistically had a significant difference between the tested group and the control group (P<0.05). As a result, rosemary essential oil had antibacterial effect on Listeria monocytogenes bacteria in turkey meat extract at 4 degrees Celsius.

Antibacterial effect of 0.25% concentration of rosemary essential oil at 15°C with control containing DMSO

Figure contains the results of investigating the effect of rosemary essential oil on Listeria monocytogenes bacteria in turkey meat extract at 15 degrees Celsius.

The results show that in the tested group (rosemary essential oil, turkey meat extract, and Listeria monocytogenes bacteria), the number of bacteria decreased by 1 logarithm on the first day, and there was another 1 logarithmic decrease on the second and third day.

Then, on the fifth and seventh days, we saw an increase in bacteria by 1 logarithm, and finally, on the tenth day, the number of bacteria increased by another 1 logarithm.

In the control group (turkey meat extract, Listeria monocytogenes bacteria and DMSO), there was no decrease in bacteria on the first day and it decreased by 1 log from the second and third day. And on the fifth and seventh day, we saw an increase in bacteria by 1 logarithm, and on the 10th day, another 1 logarithm increase was also observed. According to analysis of variance, the difference between the experimental group and the control group was statistically significant (P<0.05).

Antibacterial effect of 0.25% concentration of rosemary essential oil at 4°C with control without DMSO

In graph 3, we can see the effect of 0.25% concentration of rosemary essential oil on Listeria monocytogenes bacteria in turkey meat extract at 4 degrees Celsius.

According to this graph, the concentration of 0.25% rosemary essential oil caused a decrease of 2 logarithms of bacteria on the first day, and another 1 logarithm was decreased from the second to the seventh day, and we also saw a decrease of 1 logarithm of bacteria on the tenth day.

In the control group (turkey meat extract and Listeria monocytogenes bacteria), the bacteria reduction process was as follows: on the first day, bacteria decreased by 1 logarithm, and on the second, third, fifth, and seventh days, the bacteria also decreased by 1 logarithm, and finally on the tenth day 1 more logarithm also reduced bacteria. According to the analysis of variance test, there was a statistically significant difference between the tested group and the control group (P<0.05).

Antibacterial effect of 0.25% concentration of rosemary essential oil at 15°C with control without DMSO

Figure 4 contains information related to the average number of bacteria in the samples of the tested group (rosemary plant essence, turkey meat extract and bacteria) and the control (turkey meat extract and bacteria) during the studied times at a temperature of 15 degrees Celsius.

Based on this graph, the number of bacteria in the tested group decreased by 1 logarithm on the first day. On the second and third day, the number of bacteria decreased by 1 logarithm, and on the fifth and seventh day, it increased by 1 logarithm.

Finally, on the 10th day, bacteria increased by 1 logarithm. In the control group, bacteria decreased by 1 logarithm on the first, second and third day, and another 1 logarithmic decrease was observed on the fifth and seventh day, and on the tenth day we saw an increase of 1 logarithm of bacteria. According to the analysis of variance, the difference between the case and control groups was statistically significant (P<0.05).

According to the previous results, the effect of temperature on the antimicrobial effectiveness of rosemary essential oil in reducing Listeria monocytogenes bacteria was also investigated. The results showed that there was a statistically significant difference between the effectiveness of rosemary essential oil at two temperatures of 4°C and 15°C (P<0.05).

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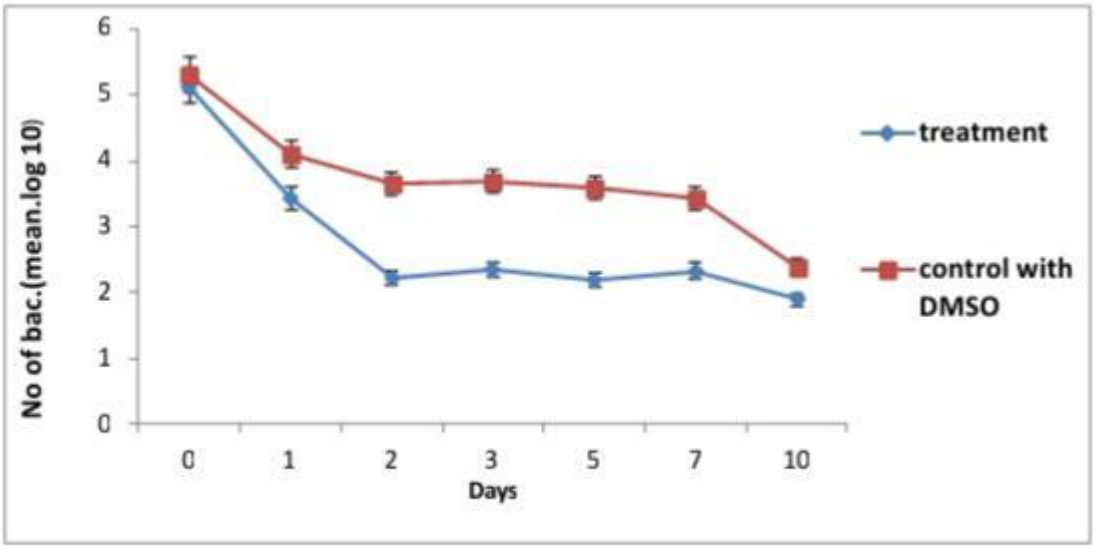

Fig 1 The Effect of concentration 0/25 % Rosemary essential oil on Listeria monocytogenes at 4 ° C

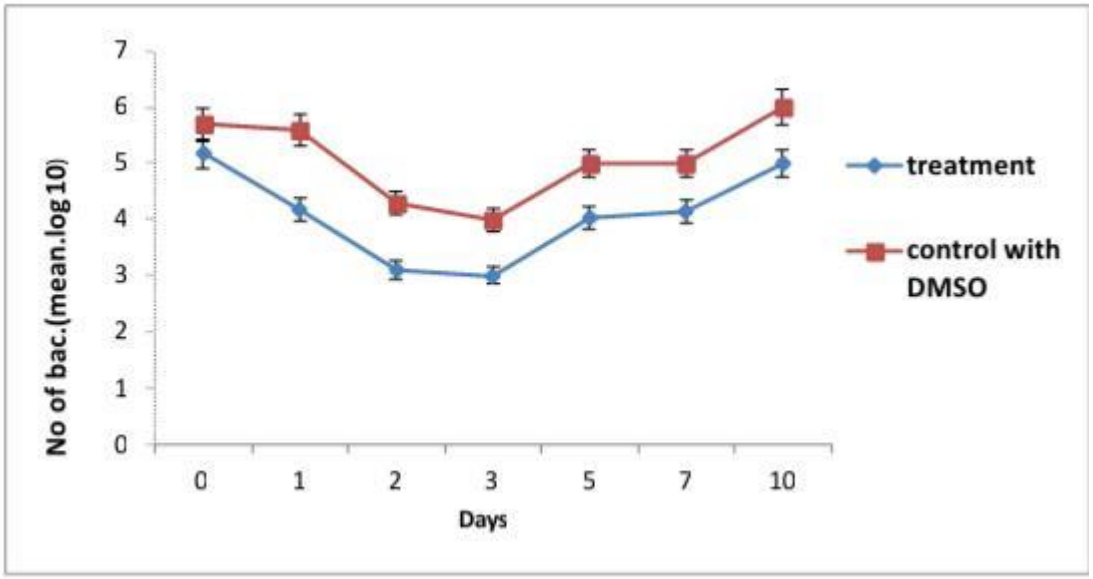

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Fig 2 The Effect of concentration 0/25 % Rosemary essential oil on Listeria monocytogenes at 15 ° C

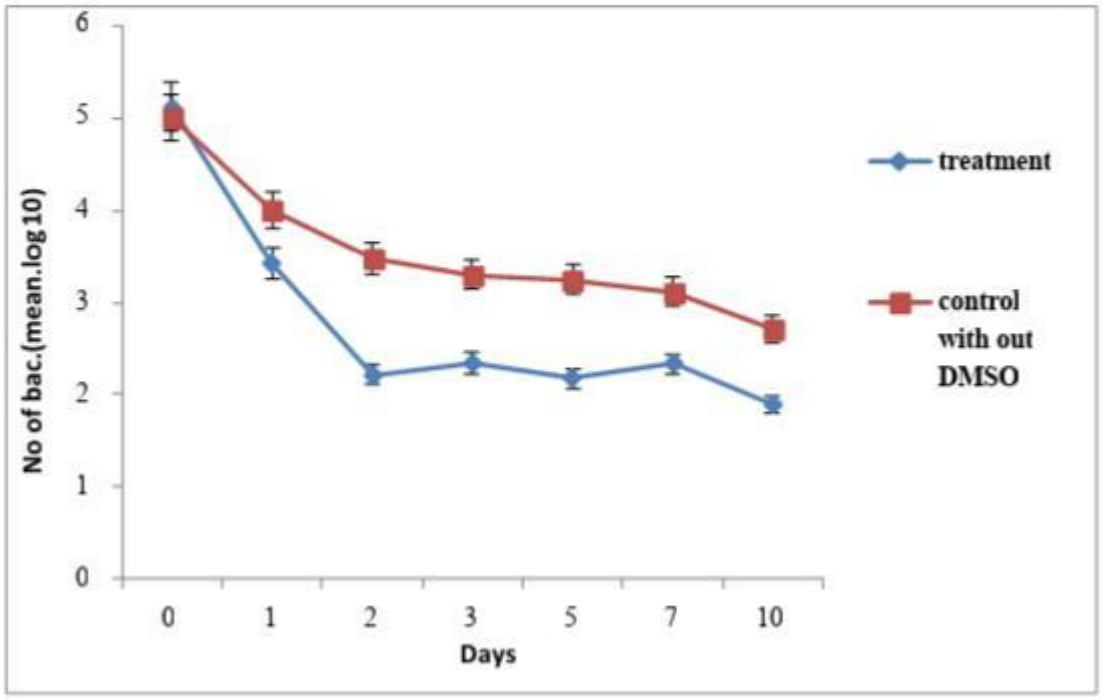

Fig 3 The Effect of concentration 0/25 % Rosemary essential oil on Listeria monocytogenes at 4 ° C without DMSO

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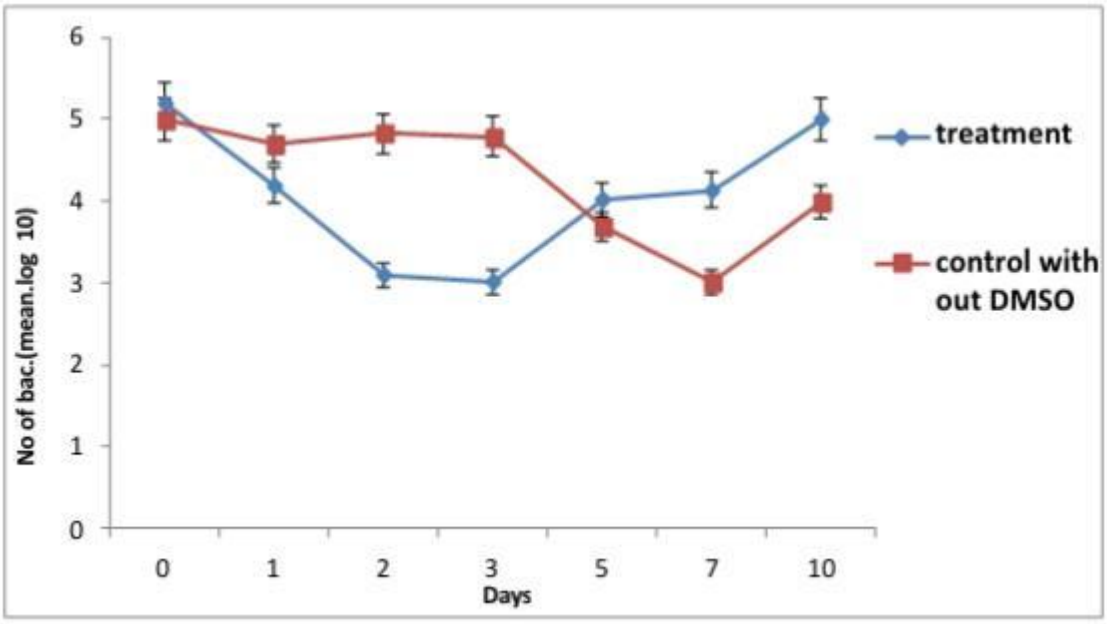

Fig 4- The Effect of concentration 0/25 % Rosemary essential oil on Listeria monocytogenes at 15 ° C without DMSO

Discussion and conclusion

In a study, the antibacterial effect of rosemary extract on gram-negative and gram-positive bacteria was evaluated. For gram-positive bacteria, the concentration of Staphylococcus aureus was 0.5%, the concentration of Streptococcus mutans was 0.13%, the concentration of Leuconostoc mesonteroides was 1%, the concentration of 5% .

It inactivates Listeria monocytogenes at 0.06% and Bacillus cereus at a concentration of 0.06%, and up to 1% concentration of rosemary ethanolic extract has no effect on Gram-negative bacteria such as Escherichia coli and Salmonella enteritis (Campose et al, 2000).

In the current study, the minimum inhibitory concentration and the minimum lethal concentration of rosemary on Listeria monocytogenes bacteria were reported to be 0.5%, which is consistent with this study.

Antioxidant and antibacterial properties of rosemary extract on Escherichia coli, Salmonella and Listeria monocytogenes were investigated in laboratory conditions and beef. The results showed that 1% concentration of rosemary extract leads to a log 1 reduction of the population of all pathogens (Ingolf et al. , 2003).

In the present study, rosemary essential oil at a temperature of 4 degrees Celsius and at a concentration of 0.25% led to a log 1 decrease in the bacterial population on the first day. This difference in concentration can be due to the difference in the composition of essential oil with rosemary extract, as well as the difference between beef and turkey meat.

In a study, the antibacterial activity of rosemary extract was evaluated against Listeria monocytogenes species. The results showed that the minimum inhibitory concentration of rosemary extract is between 625-5000 µg/ml. Also, the minimum lethal

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concentration of rosemary extract is between 15.63-98.5 µg/ml (Rozman and Jersek, 2009). In the current study, the minimum inhibitory and bactericidal concentration of rosemary essential oil was 5000 µg/ml, which is consistent with the minimum inhibitory concentration of this study.

In a review of the chemical composition and antimicrobial properties of rosemary plant essential oil alone and in combination with lysozyme against Listeria monocytogenes bacteria, the best MIC for lysozyme was 160 µg/ml at 80°C and pH=5, and the best MIC for Rosemary oil at pH = 5 was equivalent to 225 µg/ml (Saee et al., 2008).

In the present study, the MIC of rosemary essential oil at pH 2.6 was equal to 5000 µg/ml, which could be due to the difference in ph. The chemical composition and antibacterial properties of rosemary essential oil on Escherichia coli bacteria and its synthetic properties were investigated.

The results showed that rosemary essential oil collected in Kerman city had very good antibacterial properties against Escherichia coli, so that compared to some antibiotics Antibiotics such as gentamicin, penicillin, streptomycin and erythromycin have more antibacterial properties (Melkoutian et al., 2012).

In the present study, rosemary essential oil showed a very good antibacterial effect, which was consistent with this study. Due to the medicinal and antimicrobial properties in their compounds, as well as the aromatic substances, medicinal plants are a suitable additive for controlling food microbes, increasing the shelf life of foods, and improving the taste of food.

On the other hand, according to the results obtained from the evaluation of the antibacterial effect of rosemary essential oil on Listeria monocytogenes bacteria in turkey meat extract, it can be concluded that the concentration of 0.25% of rosemary essential oil has an antibacterial effect on the desired bacteria.

This effect was greater in the tested group at a temperature of 4 degrees Celsius. Therefore, it can be concluded that rosemary plant has antimicrobial properties and can replace chemical preservatives, especially food items stored in cold in the food industry.

References

احمدی اسب چین، محمدرضا و همکاران، 1395، بررسی اثرات مهاری عصاره رزماری بر روی باکتریهای گرم منفی و مثبت در شرایط آزمایشگاهی، مجله علمی دانشگاه علوم پزشکی ایلام، دوره بیست و چهار، شماره دوم، تیر 95 ، 89-80.

اجاق، سید مهدی و همکاران، 1388، بررسی فعالیت ضد باکتریایی اسانس پوست دارچین در شرایط آزمایشگاهی علیه پنج باکتری فاسد کننده مواد غذایی، مجله علوم و صنایع غذایی، شماره 35، دوره 9، تابستان 1391، 76-67.

حسینی، ساره، علی آبادی، مجید، 1395، بررسی اثر جایگزینی گوشت گاو با بوقلمون بر خصوصیات فیزیکوشیمیایی و حسی برگر، مجله تغذیه و صنایع غذایی ایران، 69، 227-215.

ساعی دهکردی، سید سیاوش، 1388، بررسی ترکیبات و خصوصیات ضدمیکروبی روغن فرار گیاهه رزماری به تنهایی و در ترکیب با لیزوزیم علیه لیستریا مونوسیتوژنز، مجله ارمغان دانش، دوره 14، شماره 3، پاییز 1388، 11-1.

کفشدوران، خاطره و همکاران، 1391، مروری بر نقش پروبیوتیکهای مورد استفاده در جیره غذایی طیور در ارتقای سلامتی گوشت مرغ، مجله تغذیه و صنایع غذایی ایران، دوره 7، شماره 5، بهار 1391، 828-821.

مرتضوی، سید علی و معتمدزادگان، علی، 1381، میکروبیولوژی مواد غذایی، 1: 416-381.

ملکوتیان، محمد، حاتمی، بهنام، 1392، بررسی ترکیب شیمیایی و خواص ضدباکتریایی اسانس رزماری بر باکتری

International Journal of Innovation in Agriculture Sciences and Rural Development
Vol.4, NO.2 , P:19 - 27
Received: 21 March 2022
Accepted: 10 June 2022

اشرشیاکلی و تعیین سنتتیک آن، فصلنامه علمی پژوهشی دانشکده بهداشت یزد، شماره اول، مسلسل 38، بهار 1392، 13-1.

ملکی کاهکی، علیرضا و همکاران، 1395، بررسی اثر جایگزینی نیتریت با نگهدارنده عصاره طبیعی کرفس بر خواص آنتی اکسیدانی ضد میکروبی، حسی و آنتی اکسیدانی سوسیس کوکتل گوشت مرغ، مجله تغذیه و صنایع غذایی ایران، 281-269.

موسوی م و شاویسی ن، خواص ضدلیستریایی اسانس نعناع در مقادیر مختلف دما، PHو نمک. دو ماهنامه علمی-پژوهشی تحقیقات گیاهان دارویی و معطر ایران، 1393؛5: 810-802.

Campo, Jose Del . Amlot, Mare Josephe. And Nguyen, Cherestophes. (2000). Antimicrobial Effect of Rosemary Extracts. Journal of Food protection.63: 1359-1368.

.

Ingolf, J.A. Grun, U. and Mustapha, A. (2003). Antimicrobial and Antioxidant Activities of Natural Extract In vitro and in Ground Beef. 67: 148-155.

Punder, R.K. and Jain, P. (2010). Comparative studied on the antimicrobial activity of black piper (piper nigrum) and turmeric (curcumalonga) extracts. International Journal of applied Biology and Pharmaceutical Technology.2: 491-501.

Rozman, T. and Jersek, B. (2009)s. Antimicrobial activity of Rosemary extract (Rosmarinus officinalis L) againts different species of Listeria. Journal Acta agriculturae Slovenica. 93: 51-58