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# Comparison of the Antibacterial Effects of Metabisulfite and *Mentha longifolia* L. Essential Oil in Giant Freshwater Prawns

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**ABSTRACT**

**Introduction:** Using herbal essential oils and extracts as antibacterial agents has attracted great attention for preventing the growth of pathogenic bacteria. The present study aimed to compare the effects of sodium metabisulfite and *Mentha longifolia* L. essential oil (MEO; 0%, 0.5%, 1%, and 2%) in the growth prevention of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in peeled giant freshwater prawns in cold storage for two weeks.

**Methods:** The antimicrobial effects of MEO against *L. monocytogenes* and *E. coli* O157:H7 were investigated *in-vitro* using the disk diffusion method. In addition, the effects of the direct addition of MEO (0%, 0.5%, 1%, and 2%) and sodium metabisulfite (1.25%) to prawn samples were evaluated.

**Results:** The major chemical constituents of MEO were pulegone (47.20%), eucalyptol (22.72%), and menthone (13.44%). The mean diameter of the inhibition zone of MEO against *L. monocytogenes* and *E. coli* O157:H7 was determined to be 9.45±0.23 and 6.37±0.02 millimeters, respectively. MEO concentrations of 0.5%, 1%, and 2% significantly reduced the growth of *L. monocytogenes* and *E. coli* O157:H7 compared to the control group ($P<0.05$). However, sodium metabisulfite was more effective than MEO in inhibiting the growth of *L. monocytogenes* and *E. coli* O157:H7 in raw freshwater prawns.

**Conclusion:** According to the results, MEO could effectively prevent the growth of *L. monocytogenes* and *E. coli* O157:H7 and improve the safety of raw freshwater prawns during prolonged refrigerated storage.

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## Introduction

Fishery products such as prawn, shrimp, and rainbow trout , are valuable foods and major sources of proteins, essential amino acids, minerals, and vitamins (1). The farming of giant freshwater prawns has recently received increasing attention owing to its low environmental concerns, suitability for small-scale systems, compliance with the precepts of sustainable aquaculture, economic viability, and potential export (2, 3). However, these products could rapidly deteriorate due to chemical oxidation and unavoidable microbial contamination (4). Seafood products may also carry various pathogenic microorganisms, including *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* (1). Epidemiological evidence suggests that these pathogenic microorganisms could be transferred to the human body through the cross-contamination of raw and minimally processed fishery products and seawater. Therefore, they are considered high-risk microbial hazards to immunocompromised patients, pregnant women, and the elderly (5). Listeriosis is a perilous infectious disease with a 20% mortality rate in immunocompromised patients and pregnant women (1, 5). *E. coli* infection is also considered as a major contributing factor to severe infectious gastrointestinal diseases in developed countries, and the rate of these infection outbreaks has been estimated at 2-28% (7). Various decontamination approaches are currently

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applied in food industries, such as modified atmosphere packaging, spray washing, active packaging, and chemical and natural antibacterial preservatives (6). Moreover, there are increasing consumer concerns regarding the adverse effects of synthetic additives on raw, cooked, and processed foodstuffs (7, 8). For instance, sodium metabisulfite is commonly used to inhibit the melanosis of shrimps and prawns in refrigerated storage, while it could lead to anaphylactic reactions and bronchoconstriction in asthmatic patients (7).

Using herbal essential oils (EOs) and extracts as antibacterial agents has attracted great attention to for inhibiting the growth of pathogenic microorganisms (9). Herbal EOs and extracts could be used as natural alternative additives to increase the storage time of foodstuffs without negative effects on their organoleptic properties (10). The key benefits of *Mentha longifolia* as a medicinal plant are lipid metabolism, ability to stimulate digestion, and antimicrobial and antioxidant properties (11). *M. longifolia* belongs to the Lamiaceae family and is widely distributed in Europe, Australia, Central Asia, and North Africa (not found in South America and Antarctica) (8). These species have antibacterial, antifungal, and antioxidant activities *in-vitro* (12-14).

A recent study has shown that carboxymethyl cellulose-gelatin nanofiber mats containing *M. longifolia* EO (MEO) could effectively delay microbial and chemical spoilages and extend the shelf life of peeled giant freshwater prawns during prolonged cold storage (15). In another study (16), the direct addition of MEO exerted no significant adverse effects on the physicochemical and sensory properties of Ayran (Turkish yoghurt drink) and improved the survival of *Lactobacillus casei* in this product. To the best of our knowledge, no published studies are available regarding the potential effects of MEO on the growth of *E. coli* O157:H7 and *L. monocytogenes* during the refrigerated storage of raw giant freshwater prawns.

The present study aimed to compare the effects of sodium metabisulfite (1.25%) and MEO (0%, 0.5%, 1%, and 2%) on preventing the growth of *L. monocytogenes* and *E. coli* O157:H7 in peeled giant freshwater prawns in cold storage for two weeks.

## Materials and Methods

### *Isolation and Chemical compound's Analysis of MEO*

In this study, *Mentha longifolia L.* Huds was procured from by Gilan-e Gharb, which is located in the west part of Kermanshah, Iran, in spring (April-May, 2020). To analyze the chemical compounds of MEO, we used an Agilent 6890 GC-MS system equipped with a BPX5 phenyl methylsiloxane capillary column (length: 30 m, ID: 0.25 mm, film thickness: 0.25 µm). The temperature of the column was set, as follows: initial temperature: 50°C, increasing rate: 3°C/min, final temperature: 300 ºC. Helium was utilized as a carrier gas with the sustained flow rate of 0.5 ml/min. In our previous study (15), we have explained the isolation and identification of the chemical compounds of MEO.

### *Collection of Giant Freshwater Prawns*

Freshwater prawns (*Macrobrachium rosenbergii*) were procured by a regional farm in Ghasr-e Shirin, which is located in the west part of Kermanshah, Iran. The samples were preserved at a fairly low temperature, put inside a closed, clean polyethylene bag, and transferred to the laboratory of the university. After cleaning with chilly sterile distilled water, the tails and heads of the prawns were peeled off under sterile conditions and dried on a layer steel wire grid rack for less than one hour (15).

### *Preparation of Pathogenic Microorganisms*

*L. monocytogenes* (ATCC 19118) and *E. coli* O157:H7 (ATCC 10536) were supplied from the culture collection of the Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran. To prepare both bacteria, 100 microliters of each bacterium was added to the brain heart infusion (BHI) agar, and the plates were incubated at the temperature of 37±1°C for 24 hours. Following that, bacterial colonies were transferred to the BHI broth (Merck, Germany), and the culture was incubated at the temperature of 37±1°C overnight. At the next stage, the BHI broth containing the cultured microorganisms was centrifuged at 4000×g for 20 minutes in cooled conditions. Afterwards, the supernatants were removed, and the pellets were cleaned three times with buffered peptone water. The final pellets were re-suspended in 10 milliliters of 0.1% buffered peptone water, and the absorbance at 660 nanometers was determined

to be 0.2 for *E. coli* O157:H7 (~$10^9$ CFU/ml) and 0.1 for *L. monocytogenes* (~$10^9$ CFU/ml) (17).

***In-vitro* Antimicrobial Effects of MEO**

The antimicrobial effects of MEO against *L. monocytogenes* and *E. coli* O157:H7 were evaluated *in-vitro* using the disk diffusion method. Initially, 100 microliters of the BHI broth (9 log CFU/ml) was cultured on the BHI agar using a sterile cotton swab, and the blank disk (diameter: 6 mm) was placed on the cultured plates. Following that, 10 microliters of MEO and 10 microliters of sodium metabisulfite (1.25%) were added onto the blank disks. The cultured plates were grown at the temperature of 37±1°C overnight, and the diameters of the inhibition zones were measured (18).

**Treatment of Giant Freshwater Prawns with Sodium Metabisulfite and MEO**

In this study, we evaluated the direct incorporation of MEO (0%, 0.5%, 1%, and 2%) and sodium metabisulfite (1.25%) into the prawn samples. For this purpose, 100 grams of the prawns was maintained in the diluted bacterial suspension (5 log CFU/ml) at an ambient temperature for 10 minutes. Afterwards, the samples were dried for 45 minutes at a refrigeration temperature. At the next stage, the prawns were placed in sterile stomacher bags, and the determined concentrations of MEO and sodium metabisulfite were directly added to the samples and spread on their entire surface. PALCAM *listeria* selective agar and eosin methylene blue agar were used to enumerate *L. monocytogenes* and *E. coli* O157:H7, respectively. For this purpose, the bacteria were cultured on the surface of the plate and incubated at the temperature of 37±1°C for 24 hours (1).

**Statistical Analysis**

Data analysis was performed in SPSS version 25 (Chicago, IL, USA) in triplicate using two-way repeated measures analysis of variance (ANOVA) with a within-subject parameter (eight levels of storage time) and a between-subject parameter (five different treatments). In addition, Duncan's multiple range test was used to compare the means at 95% confidence level. In all the statistical analyses, differences were considered significant at $P<0.05$.

## Results & Discussion

The main chemical constituents of MEO were bepulegone (47.20%), eucalyptol (22.72%), and menthone (13.44%) (15). In South Africa, Oyedeji and Afolayan (2006) also reported the main chemical constituents of MEO to be menthone (50.9%) and pulegone (19.3%) (14). In the study by Hajlaoui et al. (2008), the major chemical compounds of MEO extracted from leaves and stems of the plant were reported to be menthol (19.4-32.5%) and menthone (20.7-28.8%) (19). Furthermore, Golparvar et al. (2017) reported 1,8-cineole (37.16%), piperitenone oxide (18.97%), and sabinene (13.94%) to be the main compounds of MEO obtained from the leaves of the plant (20). In the study by Mahmoudi (2014), pulegone (31.5%), 1,8-cineole (15.9%), menthofuran (11.8%) and *cis*-isopulegone (9.7%) were also identified as the main components of MEO (16).

**Table 1.** *In vitro* antibacterial effect of *Mentha longifolia* L. essential oil against *L. monocytogenes* and *E. coli* O157:H7

| | Diameter of inhibition zone (mm) | |
|---|---|---|
| | ***M. longifolia* L. essential oil** | **Tetracycline** |
| ***L. monocytogenes*** | 9.45 ± 0.23 | 13.22 ± 0.02 |
| ***E. coli* O157:H7** | 6.37 ± 0.02 | 10.05 ± 0.01 |

In the present study, the mean diameters of the inhibition zone of MEO against *L. monocytogenes* and *E. coli* O157:H7 were 9.45±0.23 and 6.37±0.02 millimeters, respectively (Table 1). Al-Bayati (2009) reported that MEO obtained from the leaves grown wild in Iraq showed antimicrobial activity against *S. aureus, S. mutans, S. faecalis, L. acidophilus,* and *P. aeruginosa* (21). Moreover, the findings of Viljoen et al. (2006) indicated that MEO exerted moderate inhibitory effects against *S. aureus, S. epidermidis, B. cereus,* and *Y. enterocolitica* (22). According to Rasooli and Rezaei (2002), MEO had antimicrobial activity against the growth of *S. aureus* and *E. coli* O157:H7 (7 log CFU/ml) *in-vitro* (23).

Given the numerous chemical compounds found in herbal EOs, it is impossible to consider a single mechanism for their antibacterial effects as these compounds have multiple sites in bacterial cells. Hydrophobicity is one of the important properties of EOs and their constituents, which penetrate into the lipids of bacterial cell membranes and mitochondria, thereby disrupting their structures and create more

permeability. This phenomenon leads to the release of ions and other cell contents. Although the release of these substances in limited amounts is tolerable for the bacterium, it affects bacterial bioavailability and the release of large amounts of cellular contents, and the leak of critical ions and molecules causes bacterial cell death (10).

According to the current research, the diameter of the inhibition zone of sodium metabisulfite against *L. monocytogenes* and *E. coli* O157:H7 was 14.29±0.06 and 12.17±0.34 millimeters, respectively. The antimicrobial activity of sodium metabisulfite could be attributed to the inhibit ATP synthesis and bacterial metabolism (24). The results of the present study are generally consistent with the previous findings regarding the antimicrobial effects of sodium metabisulfite against *S. aureus* (25), *E. coli*, *Aspergillus flavus*, and *Candida albicans* (26).

**Table 2**. Effect of sodium metabisulfite and *Mentha longifolia* L. essential oil on growth of *L. monocytogenes* in giant freshwater prawns

| | | Essential oil | | | Sodium metabisulfite |
|---|---|---|---|---|---|
| **Day** | **Control** | **0.5%** | **1%** | **2%** | **1.25%** |
| **0** | 5.00 ± 0.03$^{a}$ | 5.00 ± 0.03$^{a}$ | 5.00 ± 0.03$^{a}$ | 5.00 ± 0.03$^{a}$ | 5.00 ± 0.03$^{a}$ |
| **2** | 5.21 ± 0.02$^{a}$ | 4.76 ± 0.02$^{ab}$ | 4.21 ± 0.04$^{bc}$ | 3.75 ± 0.02$^{c}$ | 3.02 ± 0.02$^{d}$ |
| **4** | 5.34 ± 0.01$^{a}$ | 4.12 ± 0.04$^{b}$ | 3.87 ± 0.02$^{b}$ | 3.21 ± 0.03$^{c}$ | 2.24 ± 0.01$^{d}$ |
| **6** | 5.45 ± 0.04$^{a}$ | 3.45 ± 0.95$^{b}$ | 2.11 ± 0.05$^{c}$ | 2.00 ± 0.03$^{c}$ | 2.11 ± 0.03$^{c}$ |
| **8** | 5.47 ± 0.01$^{a}$ | 3.14 ± 0.04$^{b}$ | ND | ND | ND |
| **10** | 5.52 ± 0.01$^{a}$ | 2.31 ± 0.01$^{b}$ | ND | ND | ND |
| **12** | 5.56 ± 0.02 | ND | ND | ND | ND |
| **14** | 5.61 ± 0.07 | ND | ND | ND | ND |

Means with different letters in the same row are significantly different (P < 0.05).
ND: Not detected.

**Table 3**. Effect of sodium metabisulfite and *Mentha longifolia* L. essential oil on growth of *E. coli* O157:H7 in giant freshwater prawns

| | | Essential oil | | | Sodium metabisulfite |
|---|---|---|---|---|---|
| **Day** | **Control** | **0.5%** | **1%** | **2%** | **1.25%** |
| **0** | 5.00 ± 0.02$^{a}$ | 5.00 ± 0.02$^{a}$ | 5.00 ± 0.02$^{a}$ | 5.00 ± 0.02$^{a}$ | 5.00 ± 0.02$^{a}$ |
| **2** | 4.87 ± 0.11$^{a}$ | 4.55 ± 0.07$^{ab}$ | 3.99 ± 0.08$^{b}$ | 3.11 ± 0.05$^{c}$ | 2.87 ± 0.09$^{c}$ |
| **4** | 4.75 ± 0.09$^{a}$ | 3.88 ± 0.02$^{b}$ | 3.55 ± 0.34$^{b}$ | 2.89 ± 0.09$^{c}$ | 2.23 ± 0.06$^{c}$ |
| **6** | 4.51 ± 0.05$^{a}$ | 3.11 ± 0.03$^{b}$ | 2.43 ± 0.03$^{b}$ | 2.11 ± 0.12$^{b}$ | ND |
| **8** | 4.27 ± 0.03$^{a}$ | 2.55 ± 0.02$^{b}$ | 2.12 ± 0.01$^{b}$ | ND | ND |
| **10** | 3.99 ± 0.14 | ND | ND | ND | ND |
| **12** | 3.76 ± 0.26 | ND | ND | ND | ND |
| **14** | 3.69 ± 0.05 | ND | ND | ND | ND |

Means with different letters in the same row are significantly different (P < 0.05).
ND: Not detected.

Tables 2 and 3 respectively show our findings regarding the effects of sodium metabisulfite and MEO on the growth prevention of *L. monocytogenes* and *E. coli* O157:H7 in the peeled giant freshwater prawn samples during refrigerated storage. Accordingly, the initial count of 5 log CFU/g was determined for both *L. monocytogenes* and *E. coli* O157:H7, which reached 5.61 and 3.69 log CFU/g at the end of the study period (day 14). Previous studies have indicated that *L. monocytogenes* is a psychrotrophic bacterium, which extensively grows in cheese (6), meatballs (4), and minced rainbow trout (27) during refrigerated storage. *E. coli* O157:H7 is a mesophilic bacterium, which cannot grow in refrigerated food products, and the reduction of this bacterium was rather expected in our study (1). Our findings also indicated that different concentrations of MEO (0.5%, 1%, and 2%) significantly decreased the growth of *L. monocytogenes* and *E. coli* O157:H7 compared to the control group (P<0.05). The population of *E. coli* O157:H7 reached the below-detection limit of 1 log CFU/g after 10 days in all the treated samples enriched with MEO (Table 3). Moreover, addition of 1% and 2% MEO to the prawns led to the reduction of *L. monocytogenes* to below 1 log CFU/g after eight days (Table 2).

According to the literature, herbal EOs/extracts such as *Zataria multiflora* (27), *Citrus latifolia* (7), *Ocimum basilicum* (10), and *Artemisia dracunculus* (28) could effectively inhibit the growth of pathogenic and spoilage microorganisms in raw foodstuffs, which is consistent with our findings.

As was expected, 1.25% sodium metabisulfite was more effective than MEO in inhibiting the growth of *L. monocytogenes* and *E. coli* O157:H7

inoculated into the raw freshwater prawns. At the end of the study period (day 14), the population of both microorganisms was lower than the detection limit of 1 log CFU/g in the samples treated with 1.25% sodium metabisulfite (tables 2 & 3). In similar studies, Khaledian et al. (2021) (7) and Basiri et al. (2015) (29) also reported that 1.25% sodium metabisulfite was more effective than Persian lime peel and pomegranate peel extracts in preventing the growth of spoilage microorganisms in pacific white shrimp during refrigerated storage.

## Conclusion

According to the results, the main chemical constituents of MEO were pulegone (47.20%), eucalyptol (22.72%), and menthone (13.44%). Furthermore, 0.5%, 1%, and 2% MEO could significantly inhibit the growth of *L. monocytogenes* and *E. coli* O157:H7 in the peeled giant freshwater prawns during refrigerated storage (14 days) without affecting the sensory properties of the samples (data of sensory analysis not presented). Further investigation is required on the effects of MEO on the growth of spoilage microorganisms and the chemical contamination of peeled giant freshwater prawns at refrigerated temperatures.

## Conflicts of Interest

None declared.

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