



Effects of Oregano Extract on the Inhibition of Selected Pathogens in Raw Beef Meat

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ARTICLE INFO	ABSTRACT
<p>Article type: Original article</p>	<p>Introduction: <i>Listeria monocytogenes</i>, <i>Salmonella</i> spp. and <i>Staphylococcus aureus</i> have been found in raw meat and meat products. The present study aimed to evaluate the effects of oregano extract (OE) at the concentrations of 0.1%, 0.2%, and 0.5% against <i>Listeria monocytogenes</i>, <i>Salmonella typhimurium</i>, and <i>Staphylococcus aureus</i> in raw beef meat during refrigerated storage ($4\pm 1^\circ\text{C}$) for 10 days.</p> <p>Methods: In this study, 10 grams of raw beef meat were added to 90 milliliters of sterile buffered peptone water and homogenized in a stomacher for two minutes. Proper decimal dilutions were prepared in buffered peptone water for microbial enumeration. <i>S. typhimurium</i> was cultured on Salmonella Shigella agar and incubated at the temperature of 37°C for 24 hours. In addition, <i>L. monocytogenes</i> was cultured on PALCAM listeria-selective agar and incubated at the temperature of 30°C for 48 hours, and <i>S. aureus</i> was cultured on Baird-Parker agar and incubated at the temperature of 37°C for 48 hours.</p> <p>Results: In all the experiments, no growth was observed in <i>L. monocytogenes</i>, <i>S. typhimurium</i>, and <i>S. aureus</i> in the non-inoculated control samples. Antimicrobial effects were more significant at the higher concentrations of OE ($P < 0.05$). Moreover, the addition of OEs to raw beef meat decreased the microbial counts of the tested pathogens during storage ($P < 0.05$).</p> <p>Conclusion: According to the results, utilization of OE in raw beef meat may be an effective strategy to assure safety against <i>L. monocytogenes</i>, <i>S. typhimurium</i>, and <i>S. aureus</i>. However, the associated sensory limitations do not allow the use of OE at higher concentrations, which are more effective in pathogen inhibition.</p>
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Introduction

Production and handling of raw meat and meat products depends on natural contamination by environmental microorganisms. Contamination may occur during slaughtering and increases during the manufacturing, handling, packaging, and storage of the products (1, 2).

Raw meat and meat products are recognized as microbiologically safe products, and the safety assurance depends on the appropriate anti-pathogen effectiveness of multiple antibacterial parameters based on hurdle technology (3, 4). However, considering the initial contamination of raw products with high levels of pathogenic bacteria and inappropriate

control of the antimicrobial parameters, the safety of these materials may be compromised (5, 6). Many researchers have reported the isolation of pathogenic microorganisms, such as *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*, in raw meat and meat products (3, 7-9). The global burden of the associated food-borne diseases is significant, and it is estimated that only in the United States of America (USA), approximately 48 million people acquire these diseases, 128,000 are hospitalized, and 3,000 die due to food-borne diseases every year (10).

Plant essential oils and extracts have

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exhibited remarkable antibacterial potential against spoilage and pathogenic bacteria in fresh meat and meat products (11, 12). Essential oils and extracts of aromatic plants are among the most important active constituents of herbs and spices (13). Furthermore, their application has increased in the food industry as flavoring compounds in order to avoid the use of traditional chemical preservatives (6). The differences in the antimicrobial activities of various herbal extracts are usually associated with their diverse chemical composition, which changes depending on the season, geographical location of the plants, and the methodology used for plant extraction (13).

In the past decades, the consumer demand for fresh food products has risen. Negative perceptions of consumers toward chemical food preservatives have caused natural preservation techniques and natural additives to receive great attention from the food industry (8). Non-phytotoxic extracts are considered to be safe food additives and have been certified as "Generally Recognized as Safe" (GRAS), which has resulted in higher consumer acceptability (11).

Oregano is recognized as a remarkably effective herbal extract/essential oil owing to its antibacterial capability (14). Several studies have used oregano or its constituents (e.g., carvacrol and thymol) as antibacterial agents against spoilage and pathogenic bacteria in fresh meat and meat products (14, 15), vegetables (16), and fresh fish and cephalopods (17, 18).

The present study aimed to evaluate the effects of oregano extract (OE) at the concentrations of 0.1%, 0.2%, and 0.5% against *L. monocytogenes*, *Salmonella typhimurium*, and *S. aureus* in raw beef meat during chilled storage ($4\pm 1^\circ\text{C}$) for 10 days.

Material and methods

Experimental Materials

In the present study, oregano was obtained from Kermanshah, Iran. The species of the plant was authenticated by Dr. Seyyed Ali Masoumi (plant taxonomist) at Razi University in Kermanshah. The chemical solutions and culture media were of analytical grade and purchased from Sigma (United Kingdom) and Merck (Germany), respectively.

Extraction Procedure

The oregano plant was rinsed with tap water, air-dried for two weeks at ambient temperature, and powdered using a Moulinex food processor. Initially, five grams of the ground plant was extracted on a magnetic stirrer (IKA, Germany) using pure methanol (10 ml) as the solvent for 24 hours in the dark at ambient conditions ($25\pm 1^\circ\text{C}$). The extract was passed through a Whatman grade 4 filter paper, and the residue was extracted again and evaporated under pressure below the temperature of 40°C in order to remove the solvent. Afterwards, it was preserved under chilled storage ($4\pm 1^\circ\text{C}$) before further use (19).

Microorganisms and Growth Conditions

The examined strains in this study were *L. monocytogenes* (ATCC 19118), *S. typhimurium* (ATCC 14028), and *S. aureus* (ATCC 6538). All the microorganisms were preserved at the temperature of -20°C in Tryptic soy broth containing 30% (v/v) glycerol and subcultured twice ($7 \log \text{CFU/g}$) before use in the assays (20).

Preparation of Raw Beef Meat

The semimembranosus muscle was cut from the beef carcass within 24 hours after slaughter at a local slaughterhouse in Kermanshah (located in the west of Iran), and the sample was transferred to the food hygiene laboratory under chilled storage ($4\pm 1^\circ\text{C}$) within 30 minutes. Before the experiments, each of the meat samples was sterilized for one hour using ultraviolet light in a laminar flow hood, which was divided into four batches of control (without OE) and three batches that were treated with the OE concentrations of 0.1% v/w, 0.2% v/w, and 0.5% v/w. Selecting 0.1% as the lowest concentration of OE in the current research was based on our preliminary study. Accordingly, lower OE concentrations than 0.1% had no significant effect on the shelf life extension of the samples.

At the next stage, the samples were dipped in the OE solutions (0.1%, 0.2%, and 0.5%) for five minutes at ambient conditions through agitation with a shaker in order to ensure the complete distribution of the herbal extract. Following that, the samples were air-dried for 15 minutes to allow extract attachment onto the samples (21).

The samples were subdivided into four batches, including batch one (without inoculated pathogenic bacteria), batch two (inoculated with *S. aureus*), batch three (inoculated with *S. typhimurium*), and batch four (inoculated with *L. monocytogenes*). Each batch composed of six batches (100 g), each of which had three portions.

For the inoculation of the pathogenic bacteria, 1000 µl of each bacterial suspension (containing 7 log CFU/ml) was incorporated into two samples of raw beef meat (100 g) separately and completely mixed by a stomacher to ensure the uniform distribution of the pathogenic microorganisms. Non-inoculated and inoculated samples were packed separately in stomacher bags, followed by storage at the temperature of 4°C for 10 days (8).

Microbiological Analysis

At this stage, 10 grams of raw beef meat were incorporated into 90 milliliters of sterile buffered peptone water and homogenized in a stomacher (Interscience BagMixer 400, Nom la Bretèche, France) for two minutes. In addition, sufficient decimal dilutions were prepared in buffered peptone water for microbial enumeration, including *S. typhimurium* on Salmonella Shigella (SS) agar incubated at 37°C for 24 hours, *L. monocytogenes* on PALCAM listeria selective agar incubated at 30°C for 48 hours, and *S. aureus* on Baird-Parker agar

incubated at 37°C for 48 hours (3). All the analyses were performed on days zero, two, four, six, eight, and ten in triplicate.

Sensory Analysis

A panel including 12 judges within the age range of 23-35 years old was involved in the sensory analysis. Prior to the analysis, the panelists were trained on the sensory properties of raw beef meat. Sensory parameters (odor, color, and overall acceptability) of the samples were evaluated using based on a 10-point descriptive scale within a score range of 1-10 (1=Extremely Dislike, 10=Extremely Like). All the analyses were performed on days zero, two, four, six, eight, and ten in triplicate (9).

Statistical Analysis

Data analysis was performed in SPSS version 16 using the analysis of variance to assess the effects of various OE concentrations and time of storage on pathogens. Moreover, Duncan's multiple range test was used to compare the mean values. In all the statistical analyses, P-value of less than 0.05 was considered significant.

Results&Discussion

Figures 1-3 respectively depict the counts of *S. aureus*, *S. typhimurium*, and *L. monocytogenes* in the raw beef meat samples during 10 days of

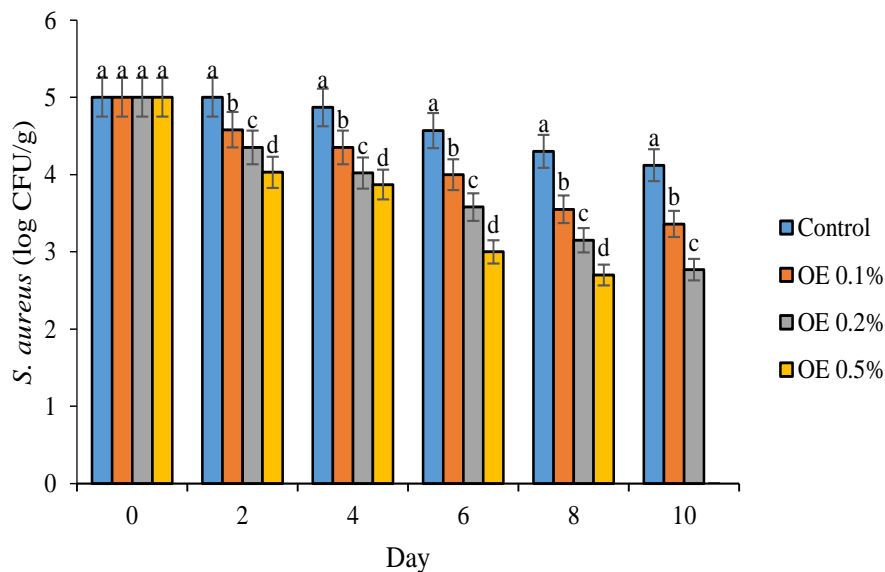


Figure 1. Effect of Oregano Extract (OE) on Survival of *Staphylococcus aureus* in Raw Beef Meat during Refrigerated Storage

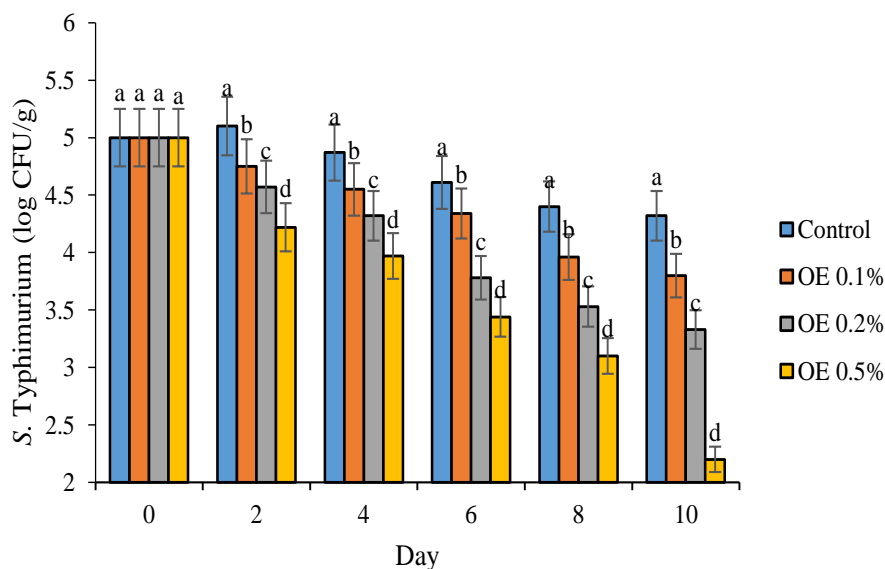


Figure 2. Effect of OE on Survival of *Salmonella typhimurium* in Raw Beef Meat during Refrigerated Storage

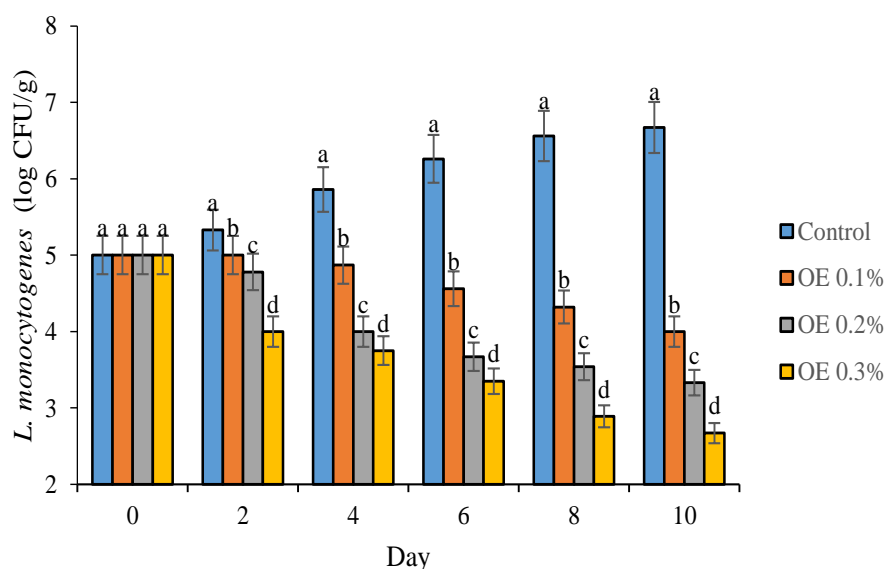


Figure 3. Effect of OE on Survival of *Listeria monocytogenes* in Raw Beef Meat during Refrigerated Storage (Each sample was analyzed in triplicate. On each sampling day, lowercase letters a-d indicate significant differences.) ($P < 0.05$).

refrigerated storage with and without OE (0.1%, 0.2%, and 0.5% concentrations). In all the experiments, no *L. monocytogenes*, *S. typhimurium*, and *S. aureus* growth was observed in the non-inoculated control samples (data not shown). Furthermore, higher concentrations of OE were associated with increased antibacterial activity ($P < 0.05$). In general, the addition of OE to raw beef meat decreased the microbial counts of the tested pathogens during refrigerated storage

($P < 0.05$).

The activity of various concentrations of OE against *S. aureus* is shown in Figure 1. The inhibitory effect of OE at the concentration of 0.5% was observed at the beginning of storage, with the reduction of 1 log CFU/g. However, at lower concentrations, the reduction was only significantly visualized at the end of storage, with smaller logarithmic reduction compared to the control group (1-2 log CFU/g) ($P < 0.05$). In a study in this regard, Pesavento et al. (22)

reported the reduction of 1.5 and 2.5 log CFU/g for *S. aureus* in minced meat at the OE concentrations of 0.5% and 2%, respectively after 14 days of storage. On the other hand, only the higher concentrations of the extract were effective for *S. aureus*. Regarding the low concentrations of OE, our findings were consistent with the study by García-Diez et al. (23). In the mentioned research, no *S. aureus* inhibition was denoted in chouriço (a traditional pork sausage) with 0.05% and 0.005% of OE and basil and tarragon extracts. Moreover, the previous studies in this regard have indicated that the application of various OEs could ensure the reduction of 2-3 log CFU/g in the count of *S. aureus* in various food models, which is in line with the results of the present study (7, 8, 24, 25).

Our findings regarding *S. typhimurium* are illustrated in Figure 2-A. Accordingly, *S. typhimurium* gradually decreased over time at all the OE concentrations. At the concentration of 0.5%, an immediate reduction was observed after 48 hours, which was estimated at 0.8, 1, and 2 log CUF/g on days four and eight, respectively. After 10 days, *S. typhimurium* reached 2.2 log CFU/g. As for the other concentrations, the reduction of *S. typhimurium* was verified to be more continuous over time, and a reduction of ~1-2 log CFU/g was also obtained. After 10 days, a reduction of 2 and 2.5 log CFU/g was observed at the OE concentrations of 0.1% and 0.2%, respectively ($P < 0.05$). The survival of *S. typhimurium* was clearly affected by the addition of OE; at all the investigated concentrations of the extract, the survivors decreased through the storage period ($P < 0.05$). Similarly, the findings of Barbosa et al. (26) demonstrated that the 0.9% concentration of OE resulted in the reduction of 1 log CFU/g for *S. enteritidis*. In contrast, García-Diez et al. (23) reported no effects against *S. enteritidis* at the 0.005% concentration of OE in fermented meat sausage.

For *L. monocytogenes* (Figure 3), our findings indicated the inhibitory activity of the higher concentration of OE (0.5%) at the outset of storage, with a logarithmic reduction of ~1 log CFU/g, whereas at lower concentrations, the effect took longer to be visualized on day four of storage. Several studies have exposed *L. monocytogenes* to various concentrations of OE,

reporting reductions in microorganism counts. For instance, Dussault et al. (27) applied the 0.05% concentration of OE in ham, reporting a reduction of 1.5 log CFU/g after 20 days. In addition, Tsigarida et al. (28) used the concentration of 0.8% in meat, observing a reduction of 2-3 log CFU/g after 14 days of refrigerated storage at the temperatures of 2 and 10°C. In another study, Pesavento et al. (22) used the concentrations of 0.5% and 2% in minced meat, and achieved 1.5 and 2.5 log CFU/g reductions after 14 days, which is in congruence with the findings of the current research.

Previous studies have investigated the antibacterial activity of OE against some food-borne pathogenic bacteria *in-vitro* (23, 27, 29), and a growing number of studies are being performed on its inhibitory effects *in-vivo* on various foodstuffs, including raw meat and meat products (23, 30). According to the literature, the main constituents of OE are phenolic compounds, thymol, carvacrol, p-cymene, linalool, γ -terpinene, β -pinene, terpinen, limonene, and borneol (11, 23, 31). The mechanisms of the combined activities of antibacterial compounds in different essential oils have not been thoroughly studied. In recent years, a number of researchers have examined the mechanism of the antibacterial effects of herbal essential oils, concluding that the lipophilic nature of these compositions results in important morphological damages, disturbing the phospholipid bilayer membrane structure and increasing the permeability of the cell membrane, which in turn lead to the leakage of intracellular proteins and electrolytes (11).

Sensory Evaluation

Figure 4 shows the sensory attributes of the treated and untreated raw beef meat samples, including odor, color, and overall acceptability, after 10 days of storage. Accordingly, the control samples had significantly lower sensory attributes compared to the treated samples ($P < 0.05$), which could be due to high microbial growth and chemical changes (e.g., lipid oxidation) (12). Therefore, it could be concluded that the incorporation of OE (0.1% and 0.2%) significantly increased the sensory properties of the raw beef meat samples compared to the controls ($P < 0.05$). The high odor attribute in the

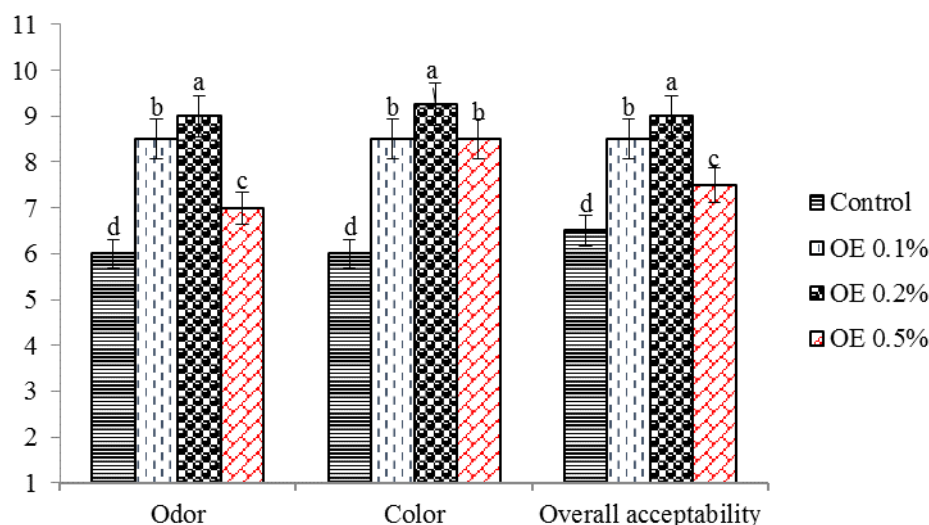


Figure 4. Effect of OE on Sensory Properties (odor, color, and overall acceptability) of Raw Beef Meat during Refrigerated Storage (Each sample was analyzed in triplicate. For each attribute, lowercase letters a-d indicate significant differences.) ($P < 0.05$)

treated samples was not only due to lower microbial growth but also associated with the flavor-contributing compounds (e.g., phenols and flavone) (17).

Conclusion

According to the results, utilization of OE in raw beef meat could result in an interesting method to assure safety against *L. monocytogenes*, *S. typhimurium*, and *S. aureus*. However, sensory limitations may not allow its use at high concentrations, which are more effective in pathogen inhibition. In the present study, it was concluded that the high concentration of OE (0.5%) decreased the counts of *L. monocytogenes*, *S. typhimurium*, and *S. aureus* in raw beef meat. At lower concentrations (0.1% and 0.2%), although the reduction was lower, it was considered significant for *L. monocytogenes*, *S. typhimurium*, and *S. aureus*. The present study may be considered a starting point for further investigations focusing on methods to “mask” the unpleasant sensorial consequences caused by OE in raw meat and meat products. Moreover, such investigations could assess the combination of the lower concentrations of essential oils with other technologies so as to achieve balance between the microbial safety

and sensory acceptability of raw beef meat.

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Conflict of interest

None declared.

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