Antibacterial Efficacy of Essential Oils and Sodium Nitrite in Vacuum processed Beef Fillet

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Abstract

Background and Objective: Minimizing the exposure to nitrate and nitrite and therefore reducing the level of potentially carcinogenic nitrosamines is desired due to the strong public demand and political controversy around this issue in many countries. The present study was designed to investigate antibacterial activity of five different types of essential oils alone or in combination with different concentrations of sodium nitrite.

Material and Methods: Five types of essential oils Zataria multiflora Boiss, Satureja bachtiarica Bunge, Rosmarinus officinalis L., Mentha pulegium, and Origanum vulgare L. were used in the experiments. NaNO₂ in concentrations of 0, 100, and 200 mg kg⁻¹ were used to study the growth inhibition of Clostridium spp. inoculated in vacuum processed beef fillet. Essential oils were analyzed by gas chromatography-mass spectrometry. Antimicrobial activity against vegetative cells of Clostridium perfringens and Clostridium sporogenes were primarily done by disc diffusion method and minimum inhibitory concentration of essential oils against vegetative cells were determined by broth macro dilution. Sensory evaluation of the uninoculated cooked vacuum processed beef fillet samples with three essential oils with higher antibacterial activity against the more resistant Clostridium spp. was done

Results and Conclusion: Among the examined Essential oils Satureja bachtiarica Bunge showed the most inhibition effect on Clostridium perfringens (4.1 mg ml⁻¹) and Clostridium sporogenes (5.5 mg ml⁻¹) followed by Zataria multiflora Boiss, Origanum vulgare L., Mentha pulegium and Rosmarinus officinalis L. The antimicrobial activity of essential oils against Clostridium spp. was increased in combination with sodium nitrite. It can therefore be assumed that the combination of these two additives could have significant repercussion in the control of Clostridium perfringens and Clostridium sporogenes in vacuum processed beef fillet samples without compromising the organoleptic properties. The best result was achieved by the combination of 100 mg kg⁻¹ sodium nitrite and 1.1 %v w⁻¹ Satureja Bachtiarica Bunge which could inhibit the growth of Clostridium species. The results of the current study showed that the essential oils of interest have had drastic effects on clostridium inhibition and could be used in the meat industry especially for sausages due to their impact on technological, microbiological and sensory properties.

Conflict of interest: The authors declare no conflict of interest.

1. Introduction

Clostridium species as gram-positive, rod-shaped, spore-forming anaerobic bacteria that can be found in some environments such as soil as well as the intestinal tract of humans and animals. Clostridium species especially Clostridium (C.) perfringens and C. botulinum can be considered as main reasons for fatal food infections [1]. C.
botulinum produces seven different types of toxins (A-G). Foodborne botulism is associated with both proteolytic and non-proteolytic C. botulinum.

Chronic diseases that afflict humans caused by C. perfringens are gas gangrene and food poisoning. Temperature abuse during cooling or reheating of cooked products is a major reason in C. perfringens spoilage [2].

Meat and meat products are the favored medium for proliferation and toxin production of Clostridium species. Vacuum processed beef fillet (VPBF) stored at abusive temperature could pose the high risk as result of growing and producing toxin of Clostridium species.

Nitrate and nitrite salts are widely used in processed meat for the inhibition of spoilage as a result of pathogen bacteria, especially Clostridium spp. [3]. Reducing the incorporation of nitrate and nitrite in food formulation is recommended by the European Food Safety Authority (EFSA) due to the high potential carcinogenicity of nitrosamines [4].

Essential oils (EOs) are volatile, natural and complex compounds of an aromatic plant’s secondary metabolism [3]. EOs are active against a wide variety of microorganisms, including gram-positive bacteria such as Clostridium spp. depending on their concentration and presence of terpenoid and phenolic compounds [5,6]. Origanum (O.) vulgare belongs to the Lamiaceae family and has been used for a long time as a flavoring agent in meat products [7]. The potent antibacterial activity of O. vulgare L and Satureia (S.) Montana against C. perfringens was reported by Kačániová et al. [6]. S. bacthiarica Bunge is an endemic species of the genus Satureja in Iran. It is an aromatic medicinal plant which belongs to the Lamiaceae family [8]. Zataria (Z.) multiflora Boiss grows only in Iran, Pakistan, and Afghanistan and belongs to the family of Lamiaceae. The extracted EO can be used as a flavoring agent in different foods and demonstrated the antimicrobial and anti-inflammatory effects [8]. Rosemary (Rosmarinus (R.) officinalis L.), a member of the Lamiaceae family, is used as a medicinal agent, functional food and additive in traditional Mediterranean cuisine [9]. Mentha pulegium is an aromatic and medicinal plant that belongs to Labiate family and is used as an herbal tea and flavoring agent [10].

The C. sporogenes is used as a non-toxicogenic surrogate of C. botulinum (due difficulties of working with C. botulinum as a highly potent food poisoning organism). In this context, C. sporogenes is widely used in studies on controlling the growth of C. botulinum [11]. No alternative with the same effects of nitrate has been introduced in the meat industry. Therefore, finding a good substitute to avoid adverse side effects and prevent the resulting chronic illnesses of nitrosamine is already a major need for both manufacturers and consumers.

Therefore, this research aimed to investigate the antimicrobial activity of five essential oils including Z. multiflora Boiss, S. bacthiarica Bunge, R. officinalis L., M. pulegium, and O. vulgare L. on C. perfringens and C. sporogenes in vitro. Also, different levels of sodium nitrite in combination with the essential oils for the inhibition of C. perfringens and C. sporogenes growth in vacuum processed beef fillet after cooling and during storage at abusive temperature were evaluated.

2. Materials and Methods

2.1. Bacterial strains and spore preparation

The C. perfringens (ATCC 13124) and C. sporogenes (ATCC 3584) strains were provided by Austrian Agency for Health and Food Safety. C. perfringens spore preparation was done according to described method by Redondo-Solano using modified Duncan-Strong medium [2]. Spore of C. sporogenes was produced in cooked meat medium (OXOID, UK) according to the proposed procedure by Khanipour et al. [11]. The anaerobic atmosphere for growing Clostridia was prepared using self-contained gas generating system AnaeroGen™ (Oxoid, UK). The spores were collected by centrifugation at 7012×g for 20 min at 4°C (GS-15R, Beckman, Palo Alto, USA). The pelleted spores were washed with 100 ml sterile distilled water and centrifuged at 2000×g (10 min). The washing process was done according to Brunt et al, and repeated three times [12]. The spore suspension was mixed with sterile phosphate-buffered saline (Sigma, UK) and heated at 80°C for 15 min to destroy the vegetative cells. Spores were suspended in sterile distilled water and stored frozen at -80°C until the day of the experiment.

2.2. Plant essential oils

Hydro-distilled EO from Z. multiflora Boiss, S. bacthiarica Bunge, R. officinalis L., M. pulegium, and O. vulgare L were supplied by Barij Essence Pharmaceutical Co., Kashan, Iran and stored at 4°C in the brown sealed glass.

2.2.1. Essential oil analysis

The composition of EOs were determined by gas chromatography-mass spectrometry (GC-MS) analysis using Agilent HP-6890 gas chromatography (Agilent Technologies, Palo Alto, CA, USA) with HP-5MS capillary column (30mx0.25 mmx0.25 mm) coupled with a HP 5973 mass spectrometer (Agilent Technologies) with electron impact ionization 115 (70 eV).

Two μl of a hexane solution of essential oil was injected with the following conditions: The initial temperature: 50°C, final temperature: 250°C, and program rate: 15°C per min. Helium was set as the carrier gas with a flow rate of 0.8 ml min⁻¹ according to the recommended procedure by Moshayedi et al. [13].
2.3. Antimicrobial activity assay

2.3.1. Agar disk diffusion method

Antimicrobial activity of essential oils against vegetative cells of *C. perfringens* and *C. sporogenes* were primarily determined by disc diffusion method. Briefly, Mueller Hinton Agar plates were inoculated with approximately 0.1 ml of 10^6 CFU ml^-1 vegetative cells of *C. sporogenes* and *C. perfringens*, separately. Sterile blank disks (6 mm diameter) were soaked with 15 µl of each essential oil and placed on the surface of the agar plates. Plates were incubated anaerobically using self-contained gas generating system AnaeroGen™ (Oxoid, UK) at 37°C for 24 h. Microbial inhibition was determined by measuring the diameter of inhibition zones (mm) by digital caliper [6].

2.3.2. Broth macro dilution

Minimum inhibitory concentration (MIC) of essential oils against vegetative cells of *C. perfringens* and *C. sporogenes* were determined by broth macro dilution. EOs were dissolved in Tween 80 (concentration of 0.5 %v w^-1) (Sigma, UK) and different concentrations of essential oils (1-50 mg ml^-1) were prepared. *C. sporogenes* and *C. perfringens* were cultured separately in Mueller-Hinton Broth (MHB) (Oxoid, UK) to reach a final density of approximately 10^8 CFU ml^-1. The tubes were incubated anaerobically using self-contained gas generating system AnaeroGen™ (Oxoid, UK) at 37°C for 24 h. Three essential oils with the higher antibacterial activity against *C. perfringens* and *C. sporogenes* were selected for further investigation in VPBF samples.

2.4. Sensory evaluation

Sensory evaluation of the treated VPBF samples with three EOs with higher antibacterial activity against the more resistant *Clostridium* spp. at MIC/2, MIC, 2 MIC, 3 MIC levels (*S. bachtiarica* Bunge at 0.275, 0.55, 1.1 and 1.65 %v w^-1 and *Z. multiflora* Boiss at 0.35 0.71 , 1.42 and 2.13 %v w^-1 as well as *O. vulgare* L. at 0.39, 0.79, 1.58 and 2.37%v w^-1) was done on un-inoculated cooked VPBF samples in the sensory laboratory at the National Nutrition and Food Technology Research Institute using a selected trained panel of ten judges (5 females and 5 males) according to ISO 13299, 2003 “General guidance for establishing a sensory profile” [7]. The evaluation was carried out by each panelist in private booths under white fluorescent lights. Drinking water was provided for the panelist to clean the palate between samples. The VPBF samples were served warm in dishes coded with a 3-digit random number. The panelists used a 9-point hedonic scale for scoring the odor and taste attribute that 1=unacceptable, 9=very acceptable while samples with scores below 5 were presumed unacceptable [8].

2.5. Preparation of vacuum processed beef fillet

Beef fillets were obtained from a slaughterhouse in Tehran. Outer surface of meat were removed with sterile knives and the inner part cut into 100 g pieces and were cured by injection of a solution [sodium nitrite (0, 100 and 200 mg kg^-1), sodium polyphosphate (0.3 %w w^-1), sugar (0.5 %w w^-1), sodium chloride (1.85 %w w^-1), ascorbic acid (0.05 %w w^-1) and various concentrations of selected essential oils based on MIC against the more resistant Clostridium spp. and sensory evaluation test (*S. bachtiarica Bunge* at (0.275, 0.55and 1.1 %w^-1) , *Z. multiflora* Boiss at (0.355 and 0.71 %v w^-1) and *O. vulgare* L. at (0.395 and 0.79%v w^-1)]. Then, injected meat pieces were transferred into the tumbler Tumbling takes place at temperatures of ≤4°C for 45 min tumbling and 2 hours resting periods.

One hundred gram portions of the processed beef fillet from each treatment were placed in vacuum bags made of laminate polyvinylidene chloride with 16µm thickness and aseptically inoculated with *C. sporogenes* and *C. perfringens* spores separately to reach spore population of ca. 3 log CFUg^-1 of processed meat [2]. Inoculated prepared beef fillets were vacuum packaged, massaged manually and flattened according to ascribed method Redondo-Solano et al. [2]. VPBF samples were heated in water baths at 75°C for 20 min. Chilling were done in a refrigerated bath with water circulation capabilities from 54.4°C to 26.7°C within 5 h and then from 26.7°C to 7.2°C for 10 h according to recommended procedure by the United States Department of Agriculture Food Safety and Inspection Service [2] and stored at room temperature and analyzed for 30 days.

2.6. Enumeration of *Clostridium* spp. in VPBF

For count of *C. perfringens* and *C. sporogenes*, 10 g of the VPBF samples were weighed and transferred to a sterile stomaching bag, and then 90 ml of sterile peptone water (Oxoid, UK) was added to the bags and stomached for 2 min. Serial dilutions of stomached slurries were prepared with peptone water (0.1% w v^-1), and aliquots (100 µl) were inoculated in tryptose sulfitc cycloserine agar (Oxoid, UK) supplemented with 200 mg of D-cycloserine and egg yolk emulsion according to Oliveira et al, and tryptone peptone glucose yeast extract agar (Sigma, UK) according to Young Byun et al. [3,15]. Plates were incubated for 36 h at 37°C using self-contained gas generating system AnaeroGen™ (OXOID, UK) and the colonies were counted.

2.7. Statistical analysis

The obtained results were presented as the means± standard deviation of each treatment. Analysis of variance was done to determine the significant differences. Differences between means were tested by Duncan and values of p≤0.05 were considered as significantly different. Friedman test was used to analyze the sensory preference ranking. All statistical tests were conducted using SPSS 10.0 software (SPSS Inc, Chicago, IL, USA).
3. Results and Discussion

The antimicrobial activity of essential oils and the possibility of using EOs in combination with sodium nitrite for inhibition of inoculated Clostridium spp. in VPBF after cooling and during storage at abusive temperature were evaluated.

The recorded values for the diameter of inhibition zone which primary determined the antibacterial activities of EOs against C. perfringens and C. sporogenes by discs diffusion method were shown in Table 1. The Satureja bachtiarica Bunge EO had the highest and R. officinalis L. EO had the lowest antibacterial activity. C. perfringens showed higher susceptibility (4.92±0.38 mm) while C. sporogenes showed less sensitivity (4.12±0.43 mm) toward all tested EOs.

The antibacterial potency of M pulegium mostly has been associated with the presence of pulegone and menthone. The antibacterial activity of R. officinalis L. EOs has been related to the presence of α-Pinene, 1, 8-Cineole and β-pinene. The antibacterial potency of S. bachtiarica Bunge, Z. multiflora Boiss., and O. vulgare L. EOs can be attributed to the carvacrol and thymol [9]. The antibacterial activities of pinene-type monoterpenic hydrocarbons are due to membrane disruption by the lipophilic compounds while carvacrol and thymol could bind to proteins of the cell membrane with hydrogen bonding and change the permeability, resulting in the release of cellular contents, as well as disruption of electron transport, nucleic acid synthesis and nutrient uptake [16]. Carvacrol and thymol are phenolic monoterpenes with similar structures that both contain a hydroxyl group on the phenolic ring. The difference between carvacrol and thymol is the position of the hydroxyl group in their phenolic ring [17]. A hydrophilic feature of functional groups and lipophilic feature of hydrocarbon skeleton are important in the antibacterial activity of essential oil components. So, phenols have the highest antibacterial activity followed by aldehydes, ketones, alcohols, ethers and hydrocarbons [18]. Thymol and carvacrol have more antibacterial activity compared to 1, 8-Cineole which belongs to the ether group and it may result in a weaker antibacterial activity of R. officinalis. In the current study, the amount of carvacrol and thymol in S. bachtiarica Bunge were the highest, 46 and 28.5 %v w⁻¹ respectively which is in agreement with the results of Moshayedi et al. [19]. The quantity of carvacrol and thymol in O. vulgare L. in the present study were reported as 29 and 16 %v w⁻¹ while Moshayedi et al. reported 11.7 and 9.4 %v w⁻¹ [14]. In this study, the amount of carvacrol and thymol in Z. multiflora Boiss were recorded as 35 and 22 %v w⁻¹ while Mahboubi and Bidgoli reported the amount of carvacrol 15 %v w⁻¹ and for thymol 38.7 %v w⁻¹ [20]. These differences correlate with a number of factors including the geographical origins, the level of macronutrients, micronutrients, hydration, the harvesting seasons, the genotype, the climate, the drying and extraction method and the distilled part of the plant.

Sensory evaluation revealed that the organoleptic properties of VPBF samples treated with essential oils of S. bachtiarica Bunge at 0.275, 0.55 and 1.1 %v w⁻¹ and Z. multiflora Boiss at 0.355 and 0.71 %v w⁻¹ and O. vulgare L. at 0.395 and 0.79 %v w⁻¹ were acceptable by the panelists and attribute scores of odor and taste were above 6 (p<0.05) but the odor and taste of VPBF treated with EOs at 3 MIC levels against C. sporogenes were unacceptable (Fig. 1). In general, VPBF samples treated with S. bachtiarica Bunge obtained the highest scores of odor and taste in comparison with other examined essential oils and were significantly preferred (p<0.05).

![Figure 1](image1.png)

Figure 1. Sensory evaluation odor (A) and taste (B) of vacuum processed beef fillet treated with Z. multiflora Boiss EO (ZMB) and O. vulgare L EO (OV) S. bachtiarica Bunge EO (SBB) at MIC/2, MIC, 2MIC and 3MIC levels against C. sporogenes (values with the same letter are not significantly different at p<0.05)
Acceptability of VPBF treated with 1.1 %v w⁻¹ S. bachtiarica Bunge in meat does not mean that this EO does not influence the organoleptic properties, but the sensorial effect of VPBF formulated with 1.1 %v w⁻¹ S. bachtiarica is acceptable and could be suitable for commercial use. Van Haute et al. reported that addition of EOs to meat could positively, neutrally or negatively change the sensorial properties. As cooking improves the odor of meat, possibly due to the combination of EO odors and cooked meat odor compounds and volatilization of EO compounds [21]. The MIC values of the five EOs against C. perfringens and C. sporogenes were determined between 4.1 and 24 µg ml⁻¹ (data were shown in Table 1). S. bachtiarica Bunge showed the highest inhibition effects (lowest MIC values) against C. perfringens (4.1 mg ml⁻¹) and C. sporogenes (5.5 mg ml⁻¹) followed by Z. multiflora Boiss, O. vulgare L., M. pulegium and R. officinalis L. S. bachtiarica Bunge, Z. multiflora Boiss and O. vulgare L. were selected for investigation of antimicrobial activity against C. perfringens and C. sporogenes in VPBF.

Major chemical constituents of the EOs of Z. multiflora Boiss, O. vulgare L., M. pulegium, S. bachtiarica Bunge, and R. officinalis L. were demonstrated in Table 2.

The antibacterial effects of the S. bachtiarica Bunge, Z. multiflora Boiss, and O. vulgare L. on C. perfringens and C. sporogenes were significantly lower in VPBF in compare to in vitro (Fig. 2). For example, adding S. bachtiarica Bunge, at the MIC level against C. sporogenes to meat reduced C. sporogenes population by 0.84 log CFU g⁻¹ compared to the control sample after one-week storage. It could be due to the protection of the bacteria by meat components such as lipids and proteins. Cui et al. suggested nutrients in food matrix promote the repair of the bacterial cell and reduce the efficiency of antibacterial agents. Moreover, phenolic compounds may bind to amino acids, and proteins with hydrogen bonding resulted in reduced antibacterial effect in beef medium [22]. VPBF can be considered as a suitable medium for the growth of Clostridium bacteria due to the high amount of protein and minimum required moisture as well as anaerobic conditions. Although heat processing and sanitizing treatment could destroy the vegetative cells of Clostridium spp., their spores can survive and grow in prepared and stored food at room temperature. Temperature abuse during distribution, handling, and storage of food products not only leads to a reduction in shelf-life and decreasing the quality but also allows for the spore germination and rapid growth of bacteria such as Clostridium spp. and consequently, foods spoilage and increasing the risk of food poisoning. The optimal temperature for preparation, storing and transportation of fresh meat and meat products is between +2°C to +7°C depending on the type of meat [22]. In our study preparation and packaging of VPBF was done at ≤4°C but the used heat treatment was appropriated for activation of Clostridium spp. to investigate temperature abuse due to processing disruption. VPBF then cooled in 15h to 7.2°C and stored at room temperature.

**Table 1. Antimicrobial activity of essential oils against C. perfringens and C. sporogenes.**

<table>
<thead>
<tr>
<th>Plant species</th>
<th>C. perfringens</th>
<th>C. sporogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agar disc diffusion (mm)</td>
<td>MIC (mg ml⁻¹)</td>
</tr>
<tr>
<td>Zataria multiflora Boiss</td>
<td>6.50±0.50 a</td>
<td>5.50±0.50 A</td>
</tr>
<tr>
<td>Oreganum vulgare L</td>
<td>6.00±0.70 a</td>
<td>6.20±0.20 A</td>
</tr>
<tr>
<td>Mentha pulegium</td>
<td>3.10±0.12 b</td>
<td>15.00±0.50 B</td>
</tr>
<tr>
<td>Satureja bachtiarica Bunge</td>
<td>7.00±0.42 c</td>
<td>14.10±0.10 C</td>
</tr>
<tr>
<td>Rosmarinus officinalis L.</td>
<td>2.00±0.20 d</td>
<td>20.00±0.60 D</td>
</tr>
</tbody>
</table>

*Data in the same columns followed by different letters are statistically different (p≤0.05).*

**Table 2. Major chemical constituents of the essential oils determined by GC-MS**

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Composition (% v w⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>Z. multiflora Boiss</td>
<td>Carvacrol (35.5%), Thymol (22%), p-Cymene (13.02%), γ-Terpine (6.32%), α-Pinene (4%)</td>
</tr>
<tr>
<td>O. vulgare L</td>
<td>Carvacrol (29%), γ-Terpine (20%), Thymol (16%), β-Pinene (7%), p-Cymene (5.3%)</td>
</tr>
<tr>
<td>M. pulegium</td>
<td>Pulegone (50%), Menthone (21%), Piperitenone (11%)</td>
</tr>
<tr>
<td>S. bachtiarica Bunge</td>
<td>Carvacrol (46%); thymol (28.5%); p-cymene (4.4%); γ-terpine (4.0%)</td>
</tr>
<tr>
<td>R. officinalis L.</td>
<td>α-Pinene (26%), 1, 8-Cineole (21%), b-pinene (19%) Camphor (13%), limonene (8%)</td>
</tr>
</tbody>
</table>
Figure 2a. Number of \textit{C. perfringens} in vacuum processed beef fillet formulated with different levels of \textit{S. bachiariaca Bunge} EO (SB), \textit{Z. multiflora Boiss} EO (ZMB), \textit{O. vulgare L.} EO (OV) and without sodium nitrite during storage at room temperature for 30 days.

Figure 2b. Number of \textit{C. perfringens} in vacuum processed beef fillet formulated with different levels of \textit{S. bachiariaca Bunge} EO, \textit{Z. multiflora Boiss} EO, \textit{O. vulgare L.} EO and (100 mg kg$^{-1}$) sodium nitrite during storage at room temperature for 30 days.

Figure 2c. Number of \textit{C. perfringens} in vacuum processed beef fillet formulated with different levels of \textit{S. bachiariaca Bunge} EO, \textit{Z. multiflora Boiss} EO, \textit{O. vulgare L.} EO and (200 mg kg$^{-1}$) sodium nitrite during storage at room temperature for 30 days.
Figure 2.d. Number of C. sporogenes in vacuum processed beef fillet formulated with different levels of S. banchtiarica Bunge EO, Z. multiflora Boiss EO, O. vulgare L. EO and without sodium nitrite during storage at room temperature for 30 days.

Figure 2.e. Number of C. sporogenes in vacuum processed beef fillet formulated with different levels of S. banchtiarica Bunge EO, Z. multiflora Boiss EO, O. vulgare L. EO and (100 mg kg⁻¹) sodium nitrite during storage at room temperature for 30 days.

Figure 2f. Number of C. sporogenes in vacuum processed beef fillet formulated with different levels of S. banchtiarica Bunge EO, Z. multiflora Boiss EO, O. vulgare L. EO and (200 mg kg⁻¹) sodium nitrite during storage at room temperature for 30 days.
Although vacuum packing techniques are used to increase the shelf-life of VPBF by removing air and limits the growth of aerobic bacteria but Clostridium bacteria including C. perfringens and C. botulinum are still able to grow and proliferate and produce toxins. Curing meat by nitrate and nitrite salts provide protection against pathogenic bacteria, especially C. botulinum [5]. Our result show that C. perfringens is more sensitive than C. sporogenes to the sodium nitrite and the antibacterial activity of sodium nitrite against C. perfringens and C. sporogenes inoculated in VPBF was increased by using higher levels of nitrite. Antibacterial potency of sodium nitrite against Clostridium spp. can be attributed to the reaction of nitrite and nitrous acid with SH-groups in bacterial cells and the interference in enzymatic activities, DNA and gene expression and membrane and cell wall damage as the primary effect of nitrite [3].

The combined addition of tested EOs at the concentration of MIC/2 and MIC with sodium nitrite at 100 mg kg\(^{-1}\) or 200 mg kg\(^{-1}\) in VPBF resulted in better antimicrobial activity against C. perfringens and C. sporogenes inoculated in VPBF during cooling and storage. Our result is in agreement with the results of Cui et al. that observed synergy effect between plant extracts and NaNO\(_2\) on C. botulinum resulting in growth inhibition or inactivation [21]. De Oliveira et al. reported the synergetic effect between S. montana L. EOs and reduced sodium nitrite against C. perfringens in mortadella-type sausages [3]. In our study, the use of S. bachtiarica Bunge at the level of 1.1 % v w\(^{-1}\) with 100 mg kg\(^{-1}\) NaNO\(_2\) showed the similar antibacterial effect against Clostridium spp. compared to using 200 mg kg\(^{-1}\) NaNO\(_2\) alone, and according to our result, C. perfringens sensitivity was more than C. sporogenes in treatments containing EOs and nitrite. Since the permitted added levels of nitrite in meat formulations vary from 120 to 200 mg kg\(^{-1}\) in different standards; Iran maximum 120 mg kg\(^{-1}\) [24] and USA 200 mg kg\(^{-1}\) [25], this treatment could be used in different countries to reduce the amount of nitrite and control the outgrowth of Clostridium bacteria in vacuum cured meat with acceptable sensory properties and using temperature management systems could help to monitor and control the temperature and avoid temperature abuse. There are some reports for reducing the level of nitrite in meat products by using essential oils. Doolaeg et al. reported adding rosemary extract to liver pate, could reduce the amount of nitrite from 120 to 80 mg kg\(^{-1}\) [26]. Moearefian et al. reported the amount of nitrite in sausage could be decreased to 50 % by adding Cinnamomum zeylanicum essential oil [27,28].

Producing VPBF with 1.1 % v w\(^{-1}\) S. bachtiarica Bunge essential oils in combination with reduced sodium nitrite by 100 mg kg\(^{-1}\) will increase the cost of products slightly, but it would be negligible because the product meets the demands of the consumers and food authorities for using natural additives and reducing the level of nitrite in meat products.

4. Conclusion

Ensuring the safety of VPBF is an important issue due to high risk of the growing and producing toxin of Clostridium species. The results of this study support the synergistic effects between EOs and NaNO\(_2\) on C. perfringens and C. sporogenes. Curing the fillet with 1.1 % v w\(^{-1}\) S. bachtiarica Bunge essential oils in combination with reduced sodium nitrite by 100 mg kg\(^{-1}\) could significantly decrease the risk of Clostridium spp. Outgrowth. To develop and produce safe food with acceptable organoleptic properties is among food safety goals and the proposed method appears to be feasible in financial and technological terms. Further studies are needed to demonstrate the possibility of using this treatment for controlling psychrotolerant clostridia which could cause plowed pack spoilage in vacuum-packed meat resulting in high amounts of drip, gas and unpleasant odor as well as appearance of discoloration and tender meat.

5. Acknowledgements

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6. Conflict of Interest

The authors declare that there is no conflict of interest.

References


7. ISO 13299:2003, Sensory analysis, methodology, general guidance for establishing a sensory profile


Antibacterial efficacies EOs beef fillet
کارایی ضد باکتریایی اساسن‌های روغنی و سدیم نیتریت در فیله گاو فرآیند شده تحت خلاء

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چکیده

مواد و روش‌ها: پنج نوع اساسن روغنی در این تحقیق مورد استفاده قرار گرفت. اسانس های روغنی در برابر گونه کلستریدیوم روزانه در 200 mg kg⁻¹ از لحی جوزه ویکنر به تنهایی یا در ترکیب با سدیم نیتریت مورد آزمون، کنترل، و مورد خالی قرار گرفتند. نتایج نشان دادند که اسانس های روغنی در برابر گونه کلستریدیوم روزانه اثر قابل توجهی داشتند. بهترین نتیجه در ترکیب اسانس های روغنی در برابر سلول کلستریدیوم پرفرنجنس در ولتاژ 3 را داشتند.

واژگان کلیدی
Satureja bachtiarica Bunge Zataria multiflora Boiss Rosmarinus officinalis L. Mentha pulegium L. Satureja hortensis Bunge

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