

Anti-Salmonella Potential of *Salvia sclarea* Essential Oil in Chicken Meat

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Abstract

The group of pathogens that most commonly cause food poisoning includes pathogens of the genus *Salmonella*. The growth of *Salmonella enterica* subs. *enterica* Enteritidis CCM 3807 in chicken meat was examined, as well as the *Salvia sclarea* essential oil alone and in combination, as well as their ability to prevent spoilage at two storage temperatures. In food model studies, the impact of accompanying microbiota on fresh meat was examined, and the anti-salmonella activity of artificially inoculated raw meat was assessed. The experiments were conducted in two storage environments: +4 °C (the temperature at which meat is typically stored) and +18 °C (room temperature, which favors the spoilage of investigated meat samples and development of food pathogens). The microbiological quality of the chicken meat samples was assessed, including the total bacteria count, coliform bacteria, and *Salmonella* with the classical microbiological method. A significant effect of essential oil against microbial meat spoilage was indicated by changes in the normal microbiota, where various groups were impacted by various treatments. Additionally, compared to the control samples, the treatment with *S. sclarea* essential oil decreased the quantity of salmonella cells.

Keywords: chicken meat, *Salvia sclarea*, anti-salmonella effect, essential oil, total count of bacteria, coliform bacteria

1. Introduction

Meat and meat products must be preserved with additives since they are perishable goods that can become contaminated with dangerous bacteria, and foodborne pathogens. The upper limit of *Salmonella* infections has been severely exceeded over the previous few years. Although has a low infection incidence, *Salmonella* has become a problem for the food sector due to its widespread diffusion globally [1–3]. *Salmonella* bacteria not only cause minor to severe infections but also infections that can be lethal [4]. The need for products free of artificial preservatives has

prompted researchers to look for other sources of antibacterial chemicals. These objectives are met by essential oils (EOs), which have been utilized since the dawn of time and have proven to be efficient antibacterial agents in meat and meat products [5] Recent years have seen a rise in the search for new natural alternatives due to the negative consequences of synthetic preservatives [6, 7]. Terpenoids and phenylpropanoids are the major compounds that constitute their composition, although they are also accompanied by fatty acids, oxides, and derivatives of sulfur. Since many plant's EOs have been discovered to exhibit antibacterial activity, extending the shelf life of meat and meat products is now a viable option. The use of EOs enables the reduction of a number of harmful bacteria and spoilage

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microorganisms that synthetic chemicals may occasionally be unable to eliminate [8].

However, there hasn't been much research on the use of EOs as antimicrobial agents in meat and meat products because additional research is required to fully understand their technical and organoleptic effects.

The aromatic plant *Salvia sclarea*, also known as "clary sage," is used to treat a variety of illnesses, and clary sage oil is considered as a safe oil with no side effects [9]. *S. sclarea*, is found all over the world, from southern Europe to the middle east. The term "clary" is derived from "sclarea," which has the base "clarus," which meaning clear [10].

The aim of this research was to determine how *Salvia sclarea* inhibit *Salmonella enterica* subsp. *Enterica* in raw and thermally processed chicken meat.

2. Materials and methods

2.1 Essential oil

Commercial *Salvia sclarea* essential oil (SSEO) was purchased from a local herbal producer (Hanus, Nitra, Slovakia). The company reported that the EO was extracted by steam distillation of fresh flowering cloves and leaves. The major compounds of SSEO were linalyl acetate 56-78 %, linalool 6-24 %, D-germacrene 1-12 %, and sclareol 2.6 %.

2.2 Microorganisms tested

The reference strain used in the analysis of sage essential oil antimicrobial activity was *Salmonella enterica* subsp. *enterica* serovar Enteritidis CCM 3807 (Czech Collection of Microorganisms, Brno, Czech Republic).

2.3 Experiment with meat

The experiments were created to study the individual short-term preservative effects of SSEO and their combination (four incubation periods: 0 h, 24 h, 48 h, and 72 h). Two distinct trials were used in the testing. The first one investigated the effect of SSEO to the changes in accompanying bacterial meat microbiota; the second experiment studied the effect of the SSEO to salmonella-inoculated raw meat, while the third experiment explored their effect to salmonella on thermally processed (sous vide) meat inoculated with salmonella. After being purchased from a local butcher shop, raw chicken meat (breasts) was

delivered to the lab within 30 minutes. The breast fillets were then aseptically cut into 5.0 g pieces, put in sterile bags, and used right away in experiments. The meat samples were placed in sterile bags and kept at 4 °C or at an ambient temperature of 18 °C for the duration of the 15 minutes treatment with the SSEO (2 %) solution. The rapeseed oil (RO) was used for preparing 2 % solution of SSEO. Throughout the entire incubation time of 72 hours, the samples were examined at 0, 24, 48, and 72 h.

Each piece was removed after the incubation time, homogenized in 30 mL of physiological saline, and then prepared in tenfold serial dilutions in sterile saline. By plating 100 mL aliquots of the suitable dilution on the corresponding agar media, bacteria were counted. After 48 hours of incubation at 30 °C with inoculated plates, total viable counts were made on Plate count agar (PCA). After 24 hours of incubation at 37 °C coliform bacteria on Violet red bile agar with lactose (VRBL). The amount of CFU/g (colony forming units) was calculated. The untreated (control) samples were used in all experiments, which were conducted independently in triplicate. The findings are shown as the mean values and standard deviations.

2.4 Anti-Salmonella inhibitory effect

The experimental set of meat pieces, raw meat, was used to assess the inhibitory effects of the tested oils against *S. enterica* growth in chicken meat (surface-sterilized by 30 min of UV radiation). Then, meat pieces were inoculated with salmonella by dipping them for 15 minutes into the bacterial inoculum that had been made (the final concentration was 10⁶ CFU/g). The treated samples were incubated at two temps (4 °C and 18 °C) for 0 h, 24 h, 48 h, and 72 h. Samples in triplicate were homogenized in physiological saline following each incubation period, serially diluted, and inoculated into Xylose Lysine Deoxycholate agar (XLD) for salmonella counting.

3. Results and discussion

It is well known that certain coliform bacteria play a significant role in the spoilage of meat. Depending on the hygienic handling of meat samples, the total viable count, and the number of coliform bacteria indicate significant indicators of

general microbial contamination. As a result, the prevalence of these groups suggests that there is a greater risk of meat spoilage and the existence of pathogens. Temperature and storage circumstances, such as the availability of oxygen, are crucial factors in the spoilage process [11].

The total viable count of raw meat treated with SSEO and RO in 4 °C showed Figure 1. The lower number of total viable count were found in all groups in 0. hour. The highest total viable count was found in 72 hours in control group. The addition of SSEO reduced number of total viable count in each evaluated hours.

Previous research has given sage preparations antimicrobial activity a lot of attention and suggested that it may be possible to use them in food as a substitute for synthetic preservatives to improve safety. Strong antimicrobial activity can be attributed to its abundance in phenolic acids, monoterpenes, and diterpenes [12–16].

Coliform bacteria of raw meat were treated with SSEO and RO at 4 °C showed Figure 2. The lower number of coliform bacteria were found in all groups in 0. hour. The highest numbers of coliform bacteria were found in 72 hours in the control group. The addition of SSEO reduced the number of coliform bacteria in each evaluated hour.

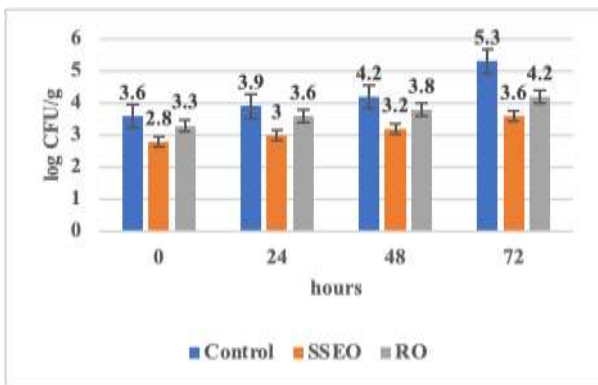


Figure 1. Microbiological quality of raw meat treated with SSEO and RO at 4 °C temperature: Total count of bacteria

At room temperature, the findings revealed variable inhibition of various bacterial groups, with SSEO showing significant reductions (Figure 3, 4). In contrast to those obtained at the storage temperature of 18 °C, the results for the treated meat samples stored at +4 °C revealed very different effects of oils. The combined impact of the oils was similarly effective and bacterial growth-inhibiting at this temperature.

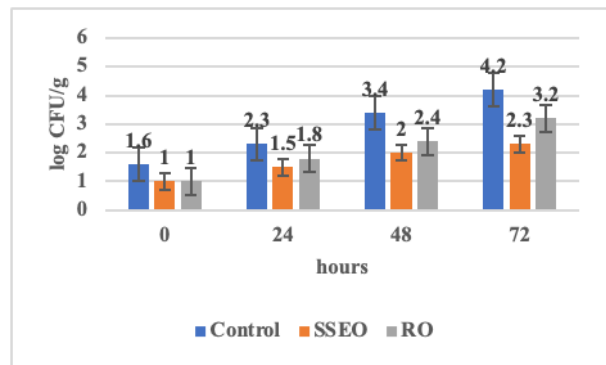


Figure 2. Microbiological quality of raw meat treated with SSEO and RO at 4 °C temperature: Coliform bacteria

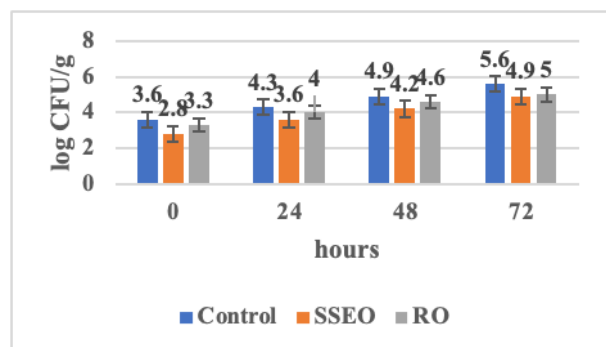


Figure 3. Microbiological quality of raw meat treated with SSEO and RO at 18 °C temperature: Total count of bacteria

Variable findings were found in earlier research on the topic of microbial flora changes in chicken meat in the presence of plant essential oils [17, 18]. Recent research examined the effectiveness of different plant metabolites against *Salmonella* species in food models, and promising results for carvacrol [19] was discovered, and essential oil of *Salvia officinalis*, *Schinus molle* [14], *Origanum vulgare* [20], *Ocimum basilicum* [21] and *Rosmarinus officinalis* [22].

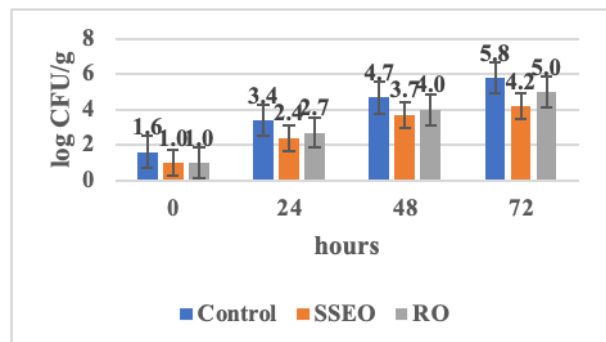


Figure 4. Microbiological quality of raw meat treated with SSEO and RO at 18 °C temperature: Coliform bacteria

Along with oregano, cumin, and eucalyptus essential oils, rosemary and basil have also been found to be helpful in reducing the overall bacterial load [23–25]. The obtained inhibitory impact against salmonella cells varies significantly between the two meat models, as observed. It is abundantly obvious that SSEO has the greatest impact when used on fresh meat (Figure 4, 5).

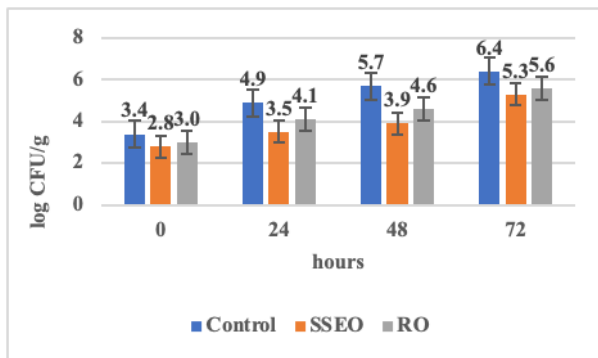


Figure 5. Anti-Salmonella effect of raw meat treated with SSEO and RO at 4 °C

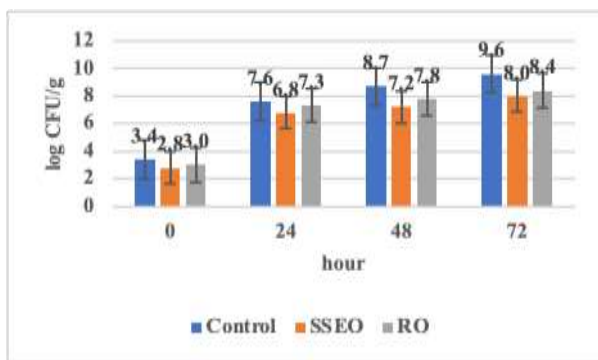


Figure 6. Anti-Salmonella effect of raw meat treated with SSEO and RO at 18 °C

According to reports, SSEO show a preserving effect in various food models against various bacterial species, which is in line with our findings. Previous studies on the use of essential oils to reduce *Salmonella* spp. in poultry were few in number. It was discovered that carvacrol, an essential oil component, can decrease or completely eradicate *S. Enteritidis* in raw chicken meat that has been artificially inoculated [19]. According to Hayouni et al. [14] peppertree (*Schinus molle*) and sage (*Salvia officinalis*) essential oils showed a decrease of *S. Enteritidis* (clinical strain) in minced beef meat that had been kept at 4 °C for 15 days. During 12 days of storage at 4 °C and 10 °C, oregano oil demonstrated high efficacy at 0.9 % in lowering *S. Enteritidis* concentration in minced sheep flesh

[20]. In a variety of meat model experiments, SSEO has thus far demonstrated a decrease in the target bacterial cell number.

4. Conclusions

The current research examined storage conditions and the effectiveness of sage essential oil against salmonella. According to the findings of the current research, very brief contact with SSEO has the greatest impact on reducing the mixed bacteria and is more effective at preventing *Salmonella* Enteritidis in meat. The fact that all of the treatments could inhibit the development of different bacterial spoilage groups, as well as pathogenic. *S. Enteritidis* in the meat model, and that they could do so at both storage temperatures, suggests that they have a high potential for protecting this kind of food against this bacterium.

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