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# Sensory and Antibacterial Impacts of Some Added Essential Oils to Raw Chicken Meat

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***Abstract***
***Background****: Improving the physical and bacteriological properties of foods by safe additives is necessary for wholesome and quality of foods. Because of the reported hazards of chemical additives, many studies were conducted to replace it with natural additives, especially herbal oils that not only have antimicrobial activity, but also enhance flavor and other sensory characters of foods.*
***Materials and Methods****: The current study was designed to investigate the impact of clove (Syzygiumaromaticum); thyme (Thymus vulgaris) and rosemary (Rosmarinusofficinalis) oils adding (in vivo) at a concentrationof 0.5%on the shelf life of raw chilled chicken meat collected from University hospital, as well as their inhibitory effects on foodborne Escherichia coli(E. coli) and Staphylococcus aureus(Staph. aureus) during along nine days of cold storage at 4°C.*
***Results****: Referring to the obtained results, treated groups showed better findings than control untreated groups, where decreasing values of Staph. aureusand E. colicounts and extending acceptable sensory properties to the 9th day of storage was reported. It is worth noting thatthe rosemary oil-treated group showed the best results in the experiment, followed by thyme and clove oils, respectively.*
***Conclusion****: From the obtained results, it concludes that the tested essential oils of 0.5% concentration had a significant role in improving the wholesome properties of raw chicken meat and recommended to be used commercially.*
***Key Words:*** *Essential oil; Chicken meat; Bacteriological quality; Sensory quality.*

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## I. Introduction

Poultry meats, including chicken meat,are known to be a common vehicle of some foodborne pathogenic bacteria like*E. coli,* Salmonellae, and coagulase-positive *Staph. aureus*that considered the most important cause of food poisoning outbreaks worldwide[1].

It was reported that *E. coli* of poultry meat origin had extra-intestinal pathogenicity, where some studies recorded that the extra-intestinal pathogenic avian *E. coli* (ExPEC) strains could belong to the same clones as human entero-pathogenic strains which can infect human-beings through consumption of contaminated food causing a variety of serious diseases[2, 3].Moreover, *Staph. aureus*is considered one of the major foodborne intoxication causes[4]through significantproduction of heat-resistant entero-toxins,proteolytic and lipolyticenzymes causing food spoilage[5].

Therefore, many studies were conducted to eliminateor reduce foodborne bacterial hazards by extending the shelf-life of meat products by replacing traditional preservation ways such as drying, smoking, brining, refrigeration with new techniques such as chemical additives, bio-preservation[6], and non-thermal techniques[7].

Some extracted essential oils (EOs) of plant origins have characteristic antimicrobial activities and stated to be excellent natural preservatives instead of chemical ones,where healthier additives and better aroma demands have been achieved[8, 9]. Many previous studies recorded significant microbial reductions and improving sensory properties of meat products after the addition of clove oil[10], thyme oil[11], and rosemary oils[12]which have been stated to possess bactericidal or bacteriostatic properties whichare mostly attributed to its phenolic compounds and to hydrocarbons which depending on their effective concentration.Therefore, the present work was carried out to evaluate theeffect of clove, thyme, and rosemary oils addition on *E. coli*and *Staph. aureus*in chicken meat, besides the enhancement of its shelf life along nine days of cold storage.

## II. Material and methods

**1. Fresh chicken meat**

A total of 2400g of the fresh chicken breast meat slices was collected from University hospital kitchen located in Qalubiya Governorate, Egypt.Before the experiment, the meat was surface treated with ultraviolet light (UV) for 15 min to minimize background microflora[13].

**2. Essential oils (EOs)**

Commercially prepared ready-to-useessential oils of clove (*Syzygiumaromaticum*), thyme (*Thymus vulgaris*), and rosemary (*Rosmarinusofficinalis*) were purchased from Green Field Oil Factory in Amman, Jordan. Hydro-distillation is usually used in this factory to extract the EOs from their sources with a purity of 99%. The EOs were kept in dry sealed dark glass vials at 4 °C until use.

**3. Bacterial strains**

*Escherichia coli* and *Staph.aureus*isolates, of food origin, were obtained from Food Analysis Center, Faculty of Veterinary Medicine; Benha University, Egypt.

**3.1.Preparation of bacterial strains was performed according to [14]**

**A-*Escherichia coli* strain**

Four to five colonies of *E. coli* strain were inoculated into the tube of sterile peptone water 0.1% (5ml) and incubated at 37˚C/24hrs. From this culture, serial dilutions up to $10^{-10}$ were plated on Eosin Methylene Blue (EMB) agar to determine the cell concentration. The cell count was adjusted to $10^6$cfu/ml for *E. coli* with tube dilution methods and considered as an infective dose.

**B- *Staphylococcus aureus* strain**

Four to five colonies of *Staph. aureus*strain were picked by sterile inoculating loop and inoculated into thetubeof sterile peptone water 0.1% (5 ml) and was then incubated at 37°C/24 hrs. From this culture, dilutions up to $10^{-10}$ were plated on Baird-Parker agar to determine the cell concentration. The cell count was adjusted to $10^6$ cfu/ml for *E. coli* with tube dilution methods and considered as an infective dose.

The tested strains were inoculated on fresh chicken meat samples by pouring andswabbing over the chicken meat surfaces following[15].

**4. Experiment design**

The meat samples were grouped into two primary groups (1200g weight), where $10^6$ cfu/ml of *E. coli* and *Staph. aureus* was injected in each group; after which, each group was sub-divided to four secondary groups (300g weight for each) represented as follow:

**Group 1**: untreated control group.

**Group 2:** treated with clove oil (0.5%).

**Group 3:** treated with thyme oil (0.5%).

**Group 4:** treated with rosemary oil (0.5%).

Treated groups were inoculated by EOs and kept all refrigerated at $4^O$c until sensory and bacteriological examination at day zero (within 2 hours after treatment) then periodically every 3 days (0, 3, 6 and 9) days of cold storage.

**4.1. Bacteriological examination**

Following[16],twenty five grams of meat samples of each group were stomached with 225ml of 0.1% peptone water for making decimal serial dilution, from which 0.1 ml was inoculated on Tryptone Bile X-glucuronide (TBX) and Baird-Parker (BP) agars forcounting of *E. coli* and *Staph. aureus*following[17] and[18], respectively.

The experiment was performed in triplicate.

**4.2. Sensory evaluation of examined meat samples**

Meat samples were assessed for their sensory characters following[19] in scores (1 to 5), where 1- represented the worst while 5- represented the excellent mark.

**Statistical analysis**

Data was analyzed using SPSS version 20 (SPSS Inc., Chicago, IL). ANOVA analysis was used to ascertain the significance of differences between mean values of the examined groups. The level $P < 0.05$ was considered as significance.

## III. Result

**3.1. Sensory evaluation**

Results in Table (1)demonstrated the promoting effects of EOs (clove,thyme, and rosemary0.5% concentration)on the sensory characteristics and the overall acceptability of the *E. coli* and *Staph. aureus*artificially inoculated chicken meat samples with *E. coli* and*S. aureus*. Rosemary (0.5%) andthyme (0.5%)oils showed overall acceptability extend to $9^{th}$ days, while for clove(0.5%) oilextends to $6^{th}$ day, but control untreated sub-groupsshowed spoilage characters after the $3^{rd}$ day of cold storage.

**Table no 1:**The effects of 0.5% essential oils (clove, thyme and rosemary) on overall acceptability of chicken meat samples stored at 4ºC

| Group | Storage time | Color (5) | odor (5) | Appearance (5) | Consistency (5) | Overall (5) | Grade |
|---|---|---|---|---|---|---|---|
| Control | Zero time | 5 | 5 | 5 | 5 | 5 | Very good |
| | 3 days | 3.2 | 2.6 | 3.0 | 3.2 | 3.0 | Acceptable |
| | 6 days | 1.4 | 1.2 | S | 1.6 | 1.3 | Bad |
| | 9 days | S | S | 5 | S | S | Spoiled |
| Clove oil | Zero time | 5 | 5 | 5 | 5 | 5 | Very good |
| | 3 days | 4.0 | 3.8 | 4.4 | 4.2 | 4.1 | Good |
| | 6 days | 3.2 | 2.8 | 3.2 | 3.0 | 3.1 | Acceptable |
| | 9 days | 2.6 | 2.6 | 2.8 | 2.4 | 2.6 | Unacceptable |
| Thyme oil | Zero time | 5 | 5 | 5 | 5 | 5 | Very good |
| | 3 days | 4.2 | 4.0 | 4.2 | 4.4 | 4.2 | Good |
| | 6 days | 3.8 | 3.2 | 3.4 | 3.6 | 3.5 | Acceptable |
| | 9 days | 3.2 | 3.0 | 3.0 | 2.8 | 3.0 | Acceptable |
| Rosemary oil | Zero time | 5 | 5 | 5 | 5 | 5 | Very good |
| | 3 days | 4.6 | 4.4 | 4.4 | 4.6 | 4.5 | Good |
| | 6 days | 4.2 | 3.8 | 3.8 | 4.4 | 4.1 | Good |
| | 9 days | 3.2 | 3.0 | 3.4 | 3.2 | 3.2 | Acceptable |
| | 5: Very good | 4: Good | | 3: Acceptable | 2: Unacceptable | 1: Bad | S: Spoiled |

### 3.2. The antibacterial activity

3.2.1. The antibacterial activity ofEOs on the viability of *Staph. aureus*strains

As shown in Table no 2,the mean initial counts of *Staph. aureus*in clove, thyme, and rosemary treated groups were $1.97\times10^6\pm 0.33\times10^6$, $1.96\times10^6\pm 0.35\times10^6$, and $1.94\times10^6\pm 0.38\times10^6$ cfu/g, respectively. Results revealed that the rosemary-treated group had the highest reduction percent after the 9th day of cold storage (60.9%) in comparison with the thyme-treated group (46.9%) and the clove oil-treated group (38.5%) to indicate that rosemary oil was the most effective against *Staph. aureus*. Moreover, the mean count of *Staph.aureus*in the control untreated group was $2.87\times10^6\pm 0.42\times10^6$ cfu/g on the 9th day of the experiment.

It is worth noting that significant differences when ($p \geq 0.05$) were recorded between all treated groups.

**Table no 2:**The antibacterial effects and Reduction % of different essential oils (0.5%) on *S. aureus* counts (cfu/g) inoculated into chicken meat

| Storage time | Control | Clove oil | | Thyme oil | | Rosmary oil | |
|---|---|---|---|---|---|---|---|
| | Average count | Average count | R% | Average count | R% | Average count | R% |
| Zero time | $1.99\times10^6\pm 0.36\times10^{6aB}$ | $1.97\times10^6\pm 0.33\times10^{6aA}$ | ------ | $1.96\times10^6\pm 0.35\times10^{6aA}$ | ------ | $1.94\times10^6\pm 0.38\times10^{6aA}$ | ------ |
| 3rd day | $2.12\times10^6\pm 0.27\times10^{6aB}$ | $1.64\times10^6\pm 0.32\times10^{6aA}$ | 16.8 | $1.50\times10^6\pm 0.24\times10^{6bAB}$ | 23.5 | $1.43\times10^6\pm 0.29\times10^{6bB}$ | 26.3 |
| 6th day | $2.45\times10^6\pm 0.33\times10^{6aA}$ | $1.39\times10^6\pm 0.20\times10^{6bAB}$ | 29.4 | $1.18\times10^6\pm 0.13\times10^{6abB}$ | 39.7 | $9.90\times10^5\pm 1.67\times10^{5bC}$ | 48.9 |
| 9th day | $2.87\times10^6\pm 0.42\times10^{6aA}$ | $1.21\times10^6\pm 0.15\times10^{6bB}$ | 38.5 | $1.04\times10^6\pm 0.11\times10^{6cC}$ | 46.9 | $7.58\times10^5\pm 1.05\times10^{5dD}$ | 60.9 |

R %= Reduction percent

Results are expressed as mean ± S.E.M.

a, b & c: There is no significant difference (P>0.05) between any two means, within the same row (of each group) have the same superscript letter.

A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same column have the same superscript letter.

3.2.2. The antibacterial activity of EOs on the viability of *E. coli* strains

As shown in Table no 3, the mean initial counts of *E. coli* in the clove, thyme and rosemary oil treated groups were $1.99\times10^6\pm 0.32\times10^6$, $1.98\times10^6\pm 0.35\times10^6$ and $1.98\times10^6\pm 0.34\times10^6$ cfu/g, respectively. Results revealed that the rosemary treated-group had the highest reduction percent after the 9th day of cold storage (50.4%) in comparison with the thyme treated group (36.8%) and the clove oil treated group (32.7%) to indicate that the rosemary oil was the most effective against *E. coli*. Moreover, the mean count of *E. coli* in the control untreated group was $2.87\times10^6\pm 0.46\times10^6$ cfu/gon the 9th day of the experiment.

**Table no 3:**The antibacterial effects and Reduction % of different essential oils (0.5%) on *E. coli* counts (cfu/g) inoculated into chicken meat samples.

| Storage time | Control | Clove oil | | Thyme oil | | Rosmary oil | |
|---|---|---|---|---|---|---|---|
| | Average count | Average count | R % | Average count | R % | Average count | R % |
| Zero time | $2.0\times10^{6}\pm 0.37\times10^{6aB}$ | $1.99\times10^{6}\pm 0.32\times10^{6aA}$ | ------ | $1.98\times10^{6}\pm 0.35\times10^{6aA}$ | ------ | $1.98\times10^{6}\pm 0.34\times10^{6aA}$ | ------ |
| 3rd day | $2.19\times10^{6}\pm 0.31\times10^{6aB}$ | $1.76\times10^{6}\pm 0.29\times10^{6aA}$ | 11.6 | $1.63\times10^{6}\pm 0.25\times10^{6bA}$ | 17.7 | $1.54\times10^{6}\pm 0.22\times10^{6bAB}$ | 22.2 |
| 6th day | $2.57\times10^{6}\pm 0.40\times10^{6aA}$ | $1.53\times10^{6}\pm 0.24\times10^{6bAB}$ | 23.1 | $1.38\times10^{6}\pm 0.19\times10^{6bB}$ | 30.3 | $1.19\times10^{6}\pm 0.15\times10^{6cB}$ | 39.9 |
| 9th day | $2.87\times10^{6}\pm 0.46\times10^{6aA}$ | $1.34\times10^{6}\pm 0.18\times10^{6bB}$ | 32.7 | $1.25\times10^{6}\pm 0.14\times10^{6bB}$ | 36.8 | $9.82\times10^{5}\pm 1.37\times10^{5cB}$ | 50.4 |

R%= Reduction percent

Results are expressed as mean ± S.E.M.

a, b & c: There is no significant difference (P>0.05) between any two means, within the same row (of each group) have the same superscript letter.

A, B & C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same column have the same superscript letter.

## IV. Discussion

The recorded results in this study cleared that, the sensory properties of differently treated chicken meat samples during cold storage (4°C) were improved by using rosemary, thyme, and clove oils(0.5% concentration) in comparingwith the control samples after 3rd, 6th and 9th day of the storage period. The treated samples with rosemary oil 0.5% revealed the highest improvement of sensory attributes, followed by thyme oil; while the samples treated with clove oildemonstrated the lowest one when compared with control samples (Table, 1). Moreover, essential oils prolonged the shelf life of the treated samples (as samples remain without organoleptic changes till the 9th day for rosemary and thyme oils0.5% treatment, while clove oil 0.5% treated samples still accepted till the 6th day, butin the control, it remain without organoleptic changes till 3rd day). These results are in harmony with those recorded by [11, 20, 21] who recorded significant organolepticimprovement after adding the examined EOs in comparing withthe untreated groups. The obtained result may be referred to the many antioxidant components that herbs and spices contain that improve both color and aromaof meat as reported by [22].

Results of the antibacterial effects of the used EOs on*Staph.aureus*and *E. coli*counts in artificially inoculated chicken meat samples as illustrated in Tables(2 and 3, respectively) showed a significant inhibitory effect on*Staph. aureus*and *E.coli* growth, where rosemary oil had the highest inhibitory effects followed by thymeoil and clove oils; therefore, the used EOs can be chosen for use as potential antibacterial food additives that were in agreement with findings of[11, 20, 21]who reported that these essential oils possess a good potential to act as a natural preservative against *Staph. aureus* and *E. coli*because of rosemary α-pinenecontent, 1,8–cineole, camphene, β- myrcene, and borneol, which have antimicrobial activity throughmoving through bacterial cell membranes results in disrupting their structure and selective permeability[23].

On the other hand, thyme oil (*thymus vulgaris*) showmicrobial inhibitory action because of their content of thymol which is considered highly efficient herbal antibacterial agent by binding to cell membrane proteins through hydrogen bonds leading to changing the membrane permeability[24].

The obtained results proved the reports of[8, 10] who demonstrated that rosemary, thyme, and cloveEOs were more effective on Gram-positive (*Staph. aureus*) than Gram-negativebacteria (*E. coli*) which may be attributed to its volatile action of EOs and absence of lipopolysaccharide layer in Gram-positive bacteria which consider a good barrier against any external biomolecule. Also, it may be due to that EOs can perfectly prevent bacterial respiration and raise the plasma membrane permeability,this resulted in bacterial cell death.

## V. Conclusion

Finally, the present study concluded thatrosemary, thyme, and clove oilsof 0.5%concentration havesignificantinhibitory effects onfoodborne*Staph. aureus*and *E. coli* and enhanced the safety and shelf life of raw chilled chicken meat. So, their use in the food industry can help to reduce the addition of chemical preservatives and can be an alternative to satisfy the increasing consumer demands for safe, meat, and their products.

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