Antibacterial effect of Rosemary and Clove oil on *Staph. aureus* in minced meat

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A B S T R A C T

The present study was designed to investigate antimicrobial and antioxidant activity of clove oil and rosemary oil for their effects on the growth and survival of *Staphylococcus aureus* artificially inoculated into minced beef. Fresh minced beef samples were inoculated with (~ 10^6 CFU/ml) (6 log CFU/g) of *Staph. aureus* Initial counts of *Staph. aureus* in minced beef samples immediately after inoculation were **(**10.86±5.18 log CFU/g**).** Essential oils of clove (Syzygium aromaticum) and rosemary (Rosmarinus officinalis) (%v/g) were added to the minced beef samples to achieve final concentrations of 1, 1.5 and2%. Sensory (color, odor and texture) and bacteriological (*Staph. aureus* counts) analyses were conducted after 1, 2, 3, 4.5.6. 24 hrs, 2nd, 3rd and 6th day during cold storage at 4°C. clove oil (2%) group give the best effectiveness with a significant advantage in extend shelf-life of refrigerated minced meat to 5 days compared to all groups specially control ones (3 days).

Key words: Antimicrobial, Antioxidant, Rosemary, clove.

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1. INTRODUCTION

Meat and meat products are ideal for many organisms to grow because they are high in moisture, rich in nitrogenous compounds (amino acids, peptides, and proteins) and plentifully supplied with minerals and accessory growth factors. Furthermore, they have some fermentable carbohydrates, usually glycogen and keep favorable pH for growth of most microorganisms. (Gálvez et al., 2010). When the meat is cut into pieces according to the technological procedure used in the preparation of minced meat with its surface area enlarged, the microorganisms forming on the surface, micro flora, spread all over it and grow under convenient conditions; thus they shorten the shelf-life and cause a potential risk to consumer health (Baskaya et al., 2004). *Staphylococcus. aureus* has a huge impact on animals' health and welfare and causes major economic losses in livestock's production (Peton and Le Loir, 2014).So the uses of natural antioxidants from plant extracts have experienced growing interest due to some human health professionals and consumer's concern about the safety of synthetic antioxidants in foods (Sun and Ho, 2005). Essential oils are regarded as natural alternative of chemical preservatives and their use in food meets the demands of consumer for mildly processed or natural product. (Burt, 2004).Clove oil can be used in food as a flavoring agent, in folk medicine as an antiepileptic remedy, as a fragrance in personal care products, in aromatherapy, transdermal drug delivery systems, pharmacy, perfumery and cosmetics.(Daniel et al., 2009). On the other hand, *Rosmarinus officinalis* has several applications especially in the food processing and preserving industry because of its natural antioxidant and antimicrobial effect (Abdel-Massih and Abraham, 2014).

Therefore, the main goal of the present work was carried out to evaluate the efficacy of clove and rosemary essential oils as antimicrobial agents in vivo and to determine their minimal inhibitory and bactericidal activities against *Staph. aureus.*

2. MATERIAL AND METHODS

2.1. Samples:

About 2500 gm. of minced meat samples were collected from different butcher shops from Tanta city. Samples were kept in a separate plastic bag and transferred directly with a minimum of delay to the laboratory in an insulating refrigerated container under complete aseptic condition to avoid any changes in the quality of the sample.

2.2. Preparation of bacterial strains:

Staphylococcus. aureus strain obtained from Media Unite, Food Hygiene Department, Animal Health Research Institute, Dokki, Giza, Egypt. The cell count was adjusted to 10^6cfu/ml. (Stewart et al., 2003).

2.3. Essential oils:

The ready-made herbal oils of clove (Syzygium aromaticum) and rosemary (Rosmarinus officinalis) were purchased from Elkabtin Co., Cairo, Egypt. All the used chemicals were of analytical reagent grade. These oils were stored in amber colored bottles at 4^0C until use.

2.4. Preparation of minced meat:

Samples were incubated by activated selected culture of *Staph. aureus* (10^6 CFU/g) and divided into seven equal groups (100 gm. each). The initial count was made 30 min after inoculation, the other were treated with 1%, 1.5% and 2% of rosemary and clove, respectively. Untreated samples served as control. All samples were packed in polyethylene bags, labeled and stored at 4ºC and then examined for Sensory (color odor and texture) and bacteriological (*Staph. aureus* count) at 1, 2, 3, 4, 5, 6, 24 hours, 2nd day, 3rd, 6th day, respectively. Using the serial dilutions and spread plate technique (Jay, 1992). PBS was used as control.
-All experiments were conducted in triplicate.

2.5. Sensory analysis:

The color, odor and overall acceptability were determined for each sample of meat and were recommended by(Pearson and Tauber, 1984).

2.6. Bacteriological analyses:

Bacterial counts were applied using standard methods (FDA et al., 2001). *Staphylococcus aureus* count was determined on Baird Parker agar (Oxoid). Plates were incubated at 37ºC/48 hours and black shiny colonies with narrow white margins surrounded by a clear halo zone extending into the opaque medium were enumerated.

2.7. Statistical analysis:

The data was statistically treated by one way ANOVA using SPSS program for windows (Version 16) (SPSS Inc. Chicago, IL and USA) and Duncan's post hoc test with $p < 0.05$ considered to be statistically significant(Steel and Torrie, 1980).

3. RESULTS

It is obvious from results obtained in table (1) that the sensory attributes of different treated minced beef samples during cold storage (4ºC) were improved by using different concentrations of clove and rosemary oils, compared to the control samples during the storage period. Generally, the samples treated with clove oils revealed the higher improvement of sensory attributes, while the samples treated with rosemary oil demonstrated the lower one.

Table (2) showed *Staph. aureus* count in minced beef samples treated with different concentrations of clove and rosemary oils. The present data exhibited the Importance of plant essential oils as natural food preservatives against *Staph. aureus* in minced beef. The initial counts of *Staph. aureus* in minced beef samples after inoculation was (6.90±0.05, 7.078±0.036, 7.44±0.015, 8.5±0.01, 8.57±0.011, 8.9±0.005, 8.95±0.004, 9.57±0.01) and (10.4±0.039) log CFU/g, respectively. All results showed significant growth inhibition of *Staph. aureus* in the minced beef samples.

Initial counts of *staph. aureus* in minced beef samples decreased following treatment with rosemary oil at a concentration of (1%), to (6.84±0.06, 6.84±0.06, 6.76±0.07, 6.66±0.009, 6.44±0.015, 6.30±0.021, 6.25±0.024, 6.14±0.031) and (5.39±0.03) log CFU/g, at rosemary oil (1.5)%, to (6.79±0.007, 6.74±0.007, 6.62±0.010, 6.57 ±0.011, 6.25±0.02, 6.22±0.25, 6.20±0.02, 5.27±0.027) and (5.17±0.029) log CFU/g and rosemary oil (2%), to (6.74±0.007, 6.62±0.01, 6.55±0.012, 6.38±0.018, 6.20±0.027, 5.77±0.073, 5.25±0.024, 5.07±0.036) and (4.9±0.054) log CFU/g after 1hr, 2hrs, 3hrs, 4hrs, 5 hrs, 6 hrs, 24 hrs, 2 days and 3 days of inoculation, respectively.

While using clove oil at a concentration of (1%), to (6.77±0.073, 6.66±0.009, 6.57±0.01, 6.44±0.015, 6.41±0.016, 6.25±0.024, 6.20±0.027, 5.90±0.054) and (5.07±0.036) log CFU/g, clove oil (1.5%), to (6.69±0.08, 6.59±0.01, 6.38±0.018, 6.26±0.24, 6.11±0.03, 5.77±0.073, 5.20±0.027, 5.20±0.027) and (4.77±0.073) log CFU/g and. At concentration of clove oil (2%), to (6.64±0.009, 6.55±0.012, 6.34±0.019, 6.25±0.024, 6.07±0.036, 5.59±0.11, 5.07±0.03, 4.9±0.05) and (4.5±0.11) log CFU/g after 1hr, 2hrs, 3hrs, 4hrs, 5hrs, 6hrs, 24 hrs, 2 days and 3days of inoculation, respectively. Additionally, the present data indicated that clove oil showed maximum activity followed by rosemary oil. The inhibition of *Staph. aureus* is related to the concentration of the studied essential oils, since they declined and even inhibited completely, when increasing the concentration of the studied essential oils.

Table (2): Antimicrobial effect of different concentration of rosemary and clove oils against staph. aureus (log CFU/g) experimentally inoculated in minced beef during cold storage at 4^0C.

	1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour	24 hour	2nd day	3rd day
Control	6.9008±.05461^{a}	7.0782±.03629^{a}	7.4470±.01552^{a}	8.5050±.01358^{a}	8.5797±.01143^{a}	8.9008±.05461^{a}	8.9008±.05461^{a}	9.5797±.01143^{a}	10.0402±.03960^{a}
Rosemary1%	6.7924±.00701 ^{abc}	6.7481±.00776^{c}	6.6232±.01034^{bc}	6.5797±.01143^{c}	6.4148±.01671^{b}	6.2548±.02416^{b}	6.2548±.02416^{b}	6.1454±.03108^{b}	6.1454±.03108^{b}
Rosemary 1.5%	6.7741±.07315^{bc}	6.6627±.00944^{cd}	6.5797±.01143^{c}	6.4470±.01552^{d}	6.2548±.02416^{c}	6.2036±.02718^{b}	6.2036±.02718^{b}	5.2784±.02288^{d}	5.1754±.02900^{d}
Rosemary 2%	6.6931±.08819^{bd}	6.6232±.01034de	6.2594±.24127^{d}	6.2548±.02416^{f}	6.2036±.02718^{d}	5.5927±.11122^{d}	5.2548±.02416^{c}	5.0782±.03629^{f}	4.9008±.05461^{f}
Clove 1%	6.8421±.06252 ^{ab}	6.8421±.06252^{b}	6.7634±.00749^{b}	6.6627±.00944^{b}	6.4470±.01552^{b}	6.2548±.02416^{b}	6.2036±.02718^{b}	5.9008±.05461^{c}	5.3977±.01738^{c}
Clove 1.5%	6.7741±.07315 ^{bc}	6.5927±.11122de	6.5562±.01207^{c}	6.3421±.01976^{e}	6.0782±.03629^{e}	5.7741±.07315^{c}	5.2036±.02718^{c}	5.2036±.02718^{e}	5.0782±.03629^{e}
Clove 2	6.6434±.00987 ^{d}	6.5562±.01207^{e}	6.2548±.02416^{d}	6.2548±.02416^{f}	6.0782±.03629^{e}	5.5927±.11122^{d}	5.0782±.03629^{d}	4.9008±.05461^{g}	4.7741±.07315^{g}

Initial load of *staph. aureus* at zero hour = 10.86± 5.18 log CFU/g. Different letters within the same column represent significant differences ($P< 0.05$).

Table (3) Reduction percentage of *Staph. aureus* growth with different concentrations of rosemary and clove oils.

Concentration / Time	1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour	1st day	2nd day	3rd day
Rosemary 1%	0.87 %	3.25%	9.14%	21.6%	24.9%	29%	29.7%	35.9%	46%
Rosemary 1.5%	1.59%	4.66%	11%	22.7%	27%	30%	30%	44.9%	48%
Rosemary 2%	2.3%	6.365	11.96%	24.9%	27.7%	35%	41%	47%	51%
Clove 1%	1.88%	5.79%	11.69%	24%	25%	29.7%	30%	38%	49%
Clove 1.5%	3.04%	6.78%	14.2%	26.3%	28.7%	35%	41.5%	45.7%	52%
Clove 2%	3.7%	7.35%	14.87%	26.4%	29%	37%	43%	48.8%	54%

Table (1) Sensory evaluation of untreated and treated minced beef samples with clove and rosemary oils that inoculated with *Staph. aureus* during cold storage at 4ºC.

Oil / Time	1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour	24 hours	2nd day	3rd day	6th day
Control	8	7	6	6	5	4	4	2	1	1
Rosemary 1%	8	8	6	6	5	4	4	4	2	1
Rosemary 1.5%	8	8	7	7	6	6	5	5	3	1
Rosemary 2%	9	9	8	8	7	7	6	5	4	1
Clove 1%	7	7	7	6	6	6	5	4	3	1
Clove 1.5%	8	8	8	7	7	7	6	5	4	1
Clove 2%	9	9	9	8	8	8	7	6	5	1

Score System for Sensory Evaluation **(**Pearson and Tauber, 1984): 9: Excellent, 6: Good, 3: Poor, 8: Very very good, 5: Medium, 2: Very poor, 7: Very good, 4: Fair, 1: Very very poor.

4. DISSUCSION

Staphylococcus. aurous is pathogen causes wide variety of diseases of particular relevance to the food processing industry is the ability of some *Staph. Aureus* stains to produce heat stable enterotoxins whish cause food poisoning (Dinges et al., 2000). In the recent years, the essential oils deriving from plants are being thoroughly investigated worldwide to seek their ability to replace the synthetic antimicrobial agents that are facing increasing resistance. Big number of studies published in the last few years has had the aim of finding reasonable answers(Jeyakumar et al., 2011; Milosevic et al., 2007; Upadhyay et al., 2010). α- pinene is reported as the major component of rosemary essential oil, followed by 1, 8 – cineole, camphene, β- myrcene, camphor and borneole. It was determined that rosemary essential oil exhibits antimicrobial activity by passing through the cell wall and cytoplasm membranes and disrupting their structure as a typical lipophilic substance (Stojanović-Radić et al., 2010). Eugenol (2-methoxy-4-allyl phenol) with a small addition of cariophyllene and humulene is the cause of clove oil antibacterial action .The antimicrobial action of clove oil is related to its ability to inactivate microbial adhesion, enzymes and cell envelope proteins(Marí et al., 2004) .

The results in table (1) showed that Organoleptic analysis, control (untreated groups) remain accepted until 2nd day while treated groups with rosemary oil (1%) remain accepted until 3rd day. Moreover (1.5%) rosemary oil remains accepted until 4th day & rosemary oil (2%) remain accepted until 4th day. The obtained results indicated that the best sensory quality was obtained at highest concentration of rosemary oil (2%), while slight improvement quality was noticed in samples treated with rosemary oil (1%). These results were nearly similar to results obtained by Hafez et al. (2011); Helmy (2012); Reham (2013).

While treated groups with clove oil (1%) remain accepted until 3rd day, moreover (1.5%) clove oil remains accepted until 4th day & clove oil (2%) remain accepted until 5th day.

The obtained results indicated that the best sensory quality was obtained at highest concentration of clove oil (2%), while slight improvement quality was noticed in samples treated with clove oil (1%). These results were nearly similar to results obtained by Cervenka et al. (2006); Helmy (2012); Hoque et al. (2008); Reham (2013) and Abd El Fattah- Hend, (2016).

Result in table (2) revealed that the initial count of *Staph. aureus* in minced beef sample after inoculation was 10.86 log CFU/g. the inhibition of *Staph. aureus* is related to the concentration of rosemary oil, since they decline and even inhibited completely, when increasing the concentration of oil.in case of using rosemary oil at concentration of (2%) give better reduction in growth of *staph. aurus* than 1.5% and 1%, respectively. after 1hr, 2hrs, 3hrs, 4hrs, 5hrs 6hrs,24hrs,2days and 3days of inoculation, respectively. This result was come in agree with Burt (2004); Hafez et al. (2011); Reham (2013); Stojanović-Radić et al. (2010). while using clove oil 2% it gives better reduction in growth of *staph. aurus* the clove oil 1.5% and then clove 1% after 1hr, 2hrs, 3hrs, 4hrs, 5hrs 6hrs, 24hrs, 2days and 3days of inoculation, respectively. These finding come in agreement with those of Babu et al. (2011); Helmy (2012); Reham (2013); Zengin and Baysal (2015) and Abd El Fattah- Hend, (2016).

In conclusion, the information given by the obtained results revealed that using Eos in minced beef could improve its quality by enhancing sensory characters and due to their antibacterial effect against Gram-positive bacteria. Moreover, it was found that clove oil was more effective than rosemary against *Staph. aureus*.

5. REFRANCES

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