



#### Faculty of Veterinary Medicine Department of Food Hygiene

# Essential Oils as Antimicrobial Agents in Minced Meat

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# **Meat Hygiene**

By

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#### 7. SUMMARY

The meat is regarded as one of the major sources of food-borne diseases; its evaluation is able to comprise a valuable source of information that can be used in the design of the supportive prophylaxis programs in public health. Therefore, the present study was conducted to evaluate the safety of minced meat at Al- Bohiera governorate and evaluation of efficacy of natural essential oils as antimicrobial agents and food preservatives.

A total of 60 random samples of minced meat (fresh and imported) were collected from local butcher shops and supermarkets in Al- Bohiera governorate. The collected samples were transferred directly to the laboratory of food hygiene, Animal Health Research Institute, Damanhour Branch, in an isolated ice box under complete aseptic conditions with undue delay to be subjected to microbial examination; in addition to, evaluation of natural preservative effect of essential oil.

#### **Our results revealed that:**

#### 7.1. Microbiological examination:

- ✓ Mesophilic bacteria count: the mean values of mesophilic bacteria count (cfu/g) of examined fresh and imported minced meat samples were 8.3x10<sup>5a</sup> ±5.6x10<sup>5</sup> and 7.7×10<sup>5b</sup> ±4.3×10<sup>5</sup>, respectively, and the percentages of Mesophilic bacteria incidence in examined fresh and imported minced meat samples were 100% and 93.33%, respectively.
- ✓ Enterobacteriaceae count: the mean value of *Enterobacteriaceae* count (cfu/g) of examined fresh and imported minced meat samples were  $6.4 \times 10^{5a} \pm 2.4 \times 10^{5}$  and  $2.0 \times 10^{5b} \pm 0.9 \times 10^{5}$ , respectively, and the

percentages of *Enterobacteriaceae* incidence in examined fresh and imported minced meat samples were 96.67% and 73.33%, respectively.

- ✓ Psychorophilic bacteria count: the mean value of the Psychorophilic count (cfu/g) of examined fresh and imported minced meat samples were 5.9x10<sup>4a</sup> ± 2.7x10<sup>4</sup> and 4.9x10<sup>4b</sup> ±1.7x104, respectively, and the percentages of Psychorophilic bacteria incidence in examined fresh and imported minced meat samples were 76.67% and 80%, respectively.
- ✓ **Mould count:** the mean value of mould count (cfu/g) of examined fresh and imported meat samples were  $6.7 \times 10^{2a} \pm 1.6 \times 10^{2}$  and  $3.3 \times 10^{2b} \pm 1.4 \times 10^{2}$ , respectively, and the percentages of mould incidence in examined fresh and imported minced meat samples were 60% and 63.33%, respectively.
- ✓ Yeast count: the mean value of yeast count (cfu/g) of examined fresh and imported meat samples were  $5.6 \times 10^{3a} \pm 2.6 \times 10^{3}$  and  $7.1 \times 10^{2b} \pm 2.8 \times 10^{2}$ , respectively, and the percentages of yeast incidence in examined fresh and imported minced meat samples, were 70% and 43.33% respectively.
- ✓ Salmonella spp. incidence: the incidence of the identified Salmonella spp. isolated from examined minced meat samples (fresh and imported) was 4 and 1 with percentage of 13.33 % and 3.33, respectively.
- ✓ Staphylococcus aureus incidence: the incidence of staphylococcus aureus Coagulase positiveisolated from examined fresh and imported minced meat samples were 4 and 6 with percentage of 13.33% and 20%, respectively.

#### 7.2. Experimental work:

Regarding to studying the antibacterial activity of examined essential oils (Thyme, Clove and Cumin) with different concentration (0.3, 0.5 and 1) % on viability of *Staphylococcus aureus* and Salmonella *Typhimurium*,the results revealed the count of tested bacteria in examined minced meat samples were as follow:

- In control samples, the Mean ± SD of *Staphylococcus aureus* counts % log CFU / g. of examined minced meat at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> days) which were 5.59±0.21<sup>a</sup>, 5.55±0.49<sup>a</sup>, 5.56±0.64<sup>a</sup>, 5.25±0.67<sup>a</sup> and 5.46±0.58<sup>a</sup>, respectively. While the Mean ± SD of Salmonella *Typhimurium* counts % log CFU / g. of examined minced meat were 5.14±0.29<sup>a</sup>, 5.27±0.40<sup>a</sup>, 5.59±0.21<sup>a</sup>, 6.74±0.34<sup>a</sup> and 6.84±0.38<sup>a</sup>, respectively at the storage period.
- Concerning to Thyme essential oil effect on *Staphylococcus aureus*, the counts of Mean ± SD of *Staphylococcus aureus* % log CFU / g. in treated minced meat samples with **Thyme 0.3%** at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> days) were 3.43±2.97<sup>e</sup>, 2.46±2.13<sup>ef</sup>, 2.17±1.88<sup>de</sup>, 0.00±0.00<sup>e</sup> and 0.00±0.00e, respectively, with a reduction percent of 38.69, 55.61, 60.98, 100.00 and 100.00 %, respectively. While with **Thyme 0.5%** at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> days) the counts were 1.67±2.89<sup>g</sup>, 2.33±2.02<sup>f</sup>, 1.93±1.72<sup>e</sup>, 0.00±0.00<sup>e</sup> and 0.00±0.00<sup>e</sup>, respectively, with a reduction percent of 70.19, 58.04, 65.34, 100.00 and 100.00 %, respectively. **Thyme 1%** at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> days) the counts were 0.93±1.60<sup>h</sup>, 0.00±0.00<sup>h</sup>, 0.00±0.00<sup>g</sup>, 0.00±0.00<sup>e</sup> and 0.00±0.00<sup>e</sup> and 0.00±0.00<sup>e</sup> and 0.00±0.00<sup>e</sup> and 0.00±0.00<sup>e</sup> and 0.00±0.00<sup>e</sup> and 0.00±0.00<sup>e</sup>.

- Mean while Thyme essential oil effect on Salmonella *Typhimurium*, the counts of Mean ± SD of Salmonella *Typhimurium* % log CFU / g. in treated minced meat samples with **Thyme 0.3%** at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> days) were 4.91±0.31<sup>a</sup>, 4.77±0.24<sup>b</sup>, 4.65±0.12<sup>b</sup>, 4.90±0.93<sup>b</sup> and 5.20±0.41<sup>c</sup> respectively, with a reduction percent of 4.63, 9.55, 16.91, 27.31 and 24.02 % respectively. With **Thyme 0.5%** the Mean ± SD counts of S. *Typhimurium* at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> days) were 2.30±2.00<sup>b</sup>, 1.04±1.80<sup>e</sup>, 1.00±1.73<sup>f</sup>, 0.97±1.68<sup>f</sup> and 0.87±1.50<sup>e</sup>, respectively, with a reduction percent of 55.26, 80.30, 82.11, 85.64 and 87.33%, respectively. While **Thyme 1%** the Mean ± SD counts of S. *Typhimurium* at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> days) were not detected from the first day of treatment with thyme 1% with a reduction percent 100 % through the four days of experiment.
- Concerning to Clove essential oil effect on *Staphylococcus aureus*, the counts of Mean ± SD of *Staphylococcus aureus* % log CFU / g. in treated minced meat samples with Clove 0.3% at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> days) were 4.62±0.11<sup>c</sup>, 4.46±0.23<sup>cd</sup>, 4.24±0.34<sup>b</sup>, 2.63±2.27<sup>c</sup> and 1.19±2.05<sup>d</sup>, respectively, with a reduction percent of 17.44, 19.57, 23.69, 50.01 and 78.29%, respectively. With Clove 0.5% at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> days) were 4.34±0.07<sup>cd</sup>, 4.23±0.19<sup>d</sup>, 4.12±0.14<sup>b</sup>, 2.51±2.18<sup>c</sup> and 0.95±1.64<sup>d</sup>, respectively, with a reduction percent of 22.39, 23.69, 25.87, 52.18 and 82.63 %, respectively. While with Clove 1% at storage period (3hrs, 1st, 2nd, 3rd, 4th days) were 2.26±1.97<sup>f</sup>, 2.10±1.83<sup>f</sup>, 0.83±1.43<sup>f</sup>, 0.67±1.15<sup>d</sup> and 0.00±0.00<sup>e</sup>, respectively, with a reduction percent of 59.59, 62.13, 85.14, 87.30 and 100.00 %, respectively.

- $\downarrow$  While the effect of Clove oil on S. Typhimurium, the counts of Mean  $\pm$ SD of S. Typhimurium % log CFU / g. in treated minced meat samples with Clove 0.3% at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> days) were  $0.83\pm1.43^{\text{e}}$ ,  $1.10\pm1.91^{\text{f}}$ ,  $4.37\pm0.42^{\text{c}}$ and  $0.97 \pm 1.68^{d}$ , 5.09±0.20° respectively with a reduction percent of 81.19, 84.33, 80.32, 35.21 and 25.70% respectively. With Clove 0.5% the Mean  $\pm$  SD counts of S. Typhimurium at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> days) were  $0.83\pm1.43^{d}$  after 3 hrs of treatment, and then not detected from the first day till 4<sup>th</sup> day of experiment with a reduction percent 83.95% after 3hrs of treatment and 100% in the remain days of experiment. While with Clove 1% the Mean ± SD counts of S. Typhimuriumat storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> days) were not detected from the first day of experiment with a reduction percent 100%.
- Concerning to Cumin essential oil effect on *Staphylococcus aureus*, the counts of Mean ± SD of *Staphylococcus aureus* % log CFU / g. in treated minced meat samples with **Cumin 0.3%** at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> days) were 5.12±0.63<sup>b</sup>, 5.20±0.37<sup>b</sup>, 2.60±2.43<sup>c</sup>, 5.14±0.72<sup>a</sup> and 5.42±0.59<sup>a</sup>, respectively, with a reduction percent of 8.40, 6.17, 53.13, 2.06 and 0.79 %, respectively. With **Cumin 0.5%** at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> days) the counts were 5.15±0.47<sup>b</sup>, 4.91±0.21<sup>b</sup>, 2.54±2.26<sup>c</sup>, 5.14±0.29<sup>a</sup> and 5.34±0.74<sup>a</sup>, respectively, with a reduction percent of 7.80, 11.55, 54.27, 2.05 and 2.14 %, respectively. While with **Cumin 1%** at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> days) the counts were 5.19±0.27<sup>b</sup>, 4.24±0.20<sup>cd</sup>, 2.37±2.07<sup>cd</sup>, 4.48±0.46<sup>b</sup> and 4.88±0.13<sup>b</sup>, respectively, with a reduction percent of 7.16, 23.48, 57.29, 14.59 and 10.69 %, respectively.

- While the effect of Cumin oil on S. *Typhimurium*, the counts of Mean ± SD of S. *Typhimurium* % log CFU / g. in treated minced meat samples with Cumin 0.3% at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> days) were5.07±0.43<sup>a</sup>, 4.68±0.24<sup>b</sup>, 3.27±2.85<sup>d</sup>, 4.75±0.68<sup>b</sup> and 5.79±0.57<sup>b</sup> respectively, with a reduction percent of 1.49, 11.27, 41.57, 29.51 and 15.42 % respectively. With cumin 0.5% at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> days) the Mean ± SD counts were 5.08±0.26<sup>a</sup>, 4.63±0.24<sup>b</sup>, 4.40±0.11<sup>bc</sup>, 4.26±0.95<sup>c</sup> and 5.13±0.29<sup>c</sup>, respectively, with a reduction percent of 1.29, 12.06, 21.39, 36.76 and 25.09 %, respectively. While Cumin 1% at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> days) the Mean ± SD counts were 4.83±0.28<sup>a</sup>, 4.17±0.40<sup>c</sup>, 1.49±2.57<sup>e</sup>, 1.61±2.78<sup>e</sup> and 1.65±2.86<sup>d</sup>, respectively, with a reduction percent of 6.05, 20.88, 73.41, 76.15 and 75.86%, respectively.
- Concerning to mixture of the three tested oils by concentration 0.3% in the experiment, the Mean ± SD counts of Staphylococcus aureus with mix of the 3 oils 0.3% at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> days) were 4.24±0.25<sup>d</sup>, 3.94±0.07<sup>e</sup>, 2.43±2.11<sup>cd</sup>, 2.37±2.05<sup>c</sup> and 2.04±1.77<sup>c</sup> respectively with a reduction percent 24.18, 29.02, 56.27, 54.86 and 62.67 %, respectively.
- While the Mean ± SD counts of Salmonella Typhimurium with mix of the 3 oils 0.3% at storage period (3hrs, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> days) were 2.64±2.31<sup>b</sup>, 1.40±2.42<sup>d</sup>, 4.15±0.19<sup>c</sup>, 3.18±2.76<sup>d</sup> and 5.03±0.49<sup>c</sup> respectively with a reduction percent of 48.58, 73.49, 25.70, 52.75 and 26.55 %, respectively.
  - From the obtained results, it was revealed that the sensory properties of the samples were enhanced by addition of essential oils (Thyme,

Clove and Cumin) and the most improvement in sensory properties of minced meat occurred with addition of Thyme 1%.

• Finally, we found that addition of essential oils leads to extension of shelf life of minced meat samples at storage period at 4 <sup>0</sup>C.

The public health significance of isolated microorganisms and sources of contamination of minced meat and meat products with these organisms are suggestive hygienic measures to improve the quality of meat and control food borne infection.