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# Effect of Lemon Leaf Extracts on Bacterial Count and Keeping Quality of Minced Meat

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**Abstract**

Minced meat is among the most popular and widespread meat products. Despite its high nutritive value, the product is subjected to contamination with many kinds of microorganisms. Therefore, the aim of this work was to improve the keeping quality and safety of chilled minced meat by using lemon oil extracted from fresh lemon leaves. Lemon leaf oil (LLO) used at three concentrations (0.5, 1, and 1.5%) to assess their effect on the sensory attributes, chemical parameters (pH, total volatile nitrogen, and thiobarbituric acid), and bacteriological status including total bacterial count, Enterobacteriaceae count, staphylococcal count, coliform count, and *Escherichia coli* count of minced meat stored at 4°C for 12 days. The results revealed that the addition of different concentrations of LLO improved the sensory attributes of treated minced meat samples compared with control samples. Also, LLO improved the chemical quality of chilled minced meat as it decreased pH, TVN, and TBA values compared to control group. Furthermore, LLO reduced the different bacterial counts significantly. The concentration of 1.5% of LLO had the optimal significant effect on improving sensory, chemical, and bacterial quality compared to the concentrations 0.5 and 1%. The study concluded that when lemon oil is used as a natural antioxidant and antibacterial preservative for minced meat stored at refrigerated temperature, the shelf-life of the meat can be extended up to 12 days, compared to the control group which was completely spoiled at 6 days.

***Keywords***: Lemon Oil-Minced meat; Sensory attributes; Chemical Quality; Bacterial count

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## 1.Introduction

Minced meat is typically sold at chilled temperatures (2-5°C). Retailers, consumers, and public health officials are all concerned about the microbiological quality and safety of frozen minced beef. To improve shelf life and consumer safety, it is essential to reduce contamination and inhibit the growth of spoilage and pathogenic organisms in the product. However, refrigeration of minced meat at temperatures between 2-5°C can lead to many undesirable changes in the product due to microbial growth, resulting in quality reduction, meat spoilage, and economic loss **(Elabbasy et al., 2014)**.

Many people fall ill from consuming contaminated meat and meat products, resulting in a large number of deaths annually in the world (**Bisholo et al., 2018)**. Scientists tries to reduce contamination in meat products by applying measures such as radiation and inorganic chemicals, but chemical preservatives have many carcinogenic and teratogenic consequences, as well as residual toxicity (**Costa et al., 2019**).

Today, most people look for plant extracts as natural preservatives for food products especially with increasing resistance of many bacterial strains to antimicrobial agents **(Raven, 2019).** Plant essential oils (EOs) and extracts are used as preservation and flavoring agents in the food industry. Plant extracts contain a high concentration of phytochemical compounds and secondary metabolites that inhibit pathogenic agents **(Morshdy et al., 2022)**. Citrus fruits are an important source of bioactive compounds in particular, flavonoids and vitamin C. The main flavonoids found in citrus species are hesperidine, narirutin, naringin, and eriocitrin (**Schieber et al., 2001).** One of the most promising citrus fruit extracts is lemon leaves extract, which is used to maintain the quality, increase the shelf life, and reduce risk of spoilage of minced meat (**Cantini et al., 2011; Abdel-Naeem et al. 2022)**. Lemon leaf extract could control bacterial and fungal pollutants in food such as *S. aureus, E. coli,* and *B. subtilis*, also the antioxidant activity of the plant essential oil has been proven **(Tizianna and Giuseppe, 1998)**. So, this work was planned to detect the effect of lemon leaf oil (LLO) on the quality and safety of chilled minced meat by sensory evaluation (color, odour, and taste), chemical indices (pH, TBA, and TVN) and bacteriological examination including total bacterial count, Enterobacteriaceae, staphylococcal count, coliform count, and *Escherichia coli* count during storage at 4°C throughout 12 days of storage.

## 2.Materials and methods

*2.1. Extraction of lemon leaf oil according to Reyes-Jurado et al. (2015):*

After being thoroughly cleaned with distilled water, lemon leaves were dried in a hot air oven at 50°C and then ground into a fine powder using a blender. Extraction of oil made by hydro distillation,100 grams of lemon leaves were put inside Clevenger apparatus then increase the temperature to 80°C for 3-4 hours, after that, oil fall into separating funnel. Each 100 grams of lemon leaves gives 1.5 to 2 ml of oil. Lemon essential oil stored in dark vials at 4°C. The oil was prepared at three concentrations (0.5, 1, and 1.5%) and applied according to meat weight. The extraction process was done in Faculty of Agriculture, Damanhour University, Egypt.

*2.2. Preparation of minced meat samples according to Barbosa et al. (2009):*

A total of 85 fresh minced beef samples (100 grams of each) were randomly collected from local markets in different centers of El-Behira government, Egypt. The samples were rapidly transferred in a separate sterile and labeled plastic bags within an ice box to the post graduate laboratory, Food Hygiene Department, Animal Health Research Institute, El- Behira government without undue delay under complete aseptic conditions. The minced beef samples were split into two groups: a control group and a treatment group. The treatment group were divided into three groups (15 samples /group, 1.5 kg), which were then thoroughly blended with lemon leaf oil at various concentrations (0.5, 1, and 1.5%). The treatment and control samples were kept at 4°C and frequently tested for sensory, chemical, and bacteriological parameters every three days at (day 0), day 3, day 6, day 9, and day 12.

*2.3. Sensory evaluation.*

According to **Lawless and Heymann (2010),** Twenty adults who were untrained and unaware of the experimental approach were given 100 ±10 grams of minced meat for each concentration. The samples were coded with a specific number and the panelists were asked to rate the overall acceptance (color, odor, and texture) while the samples were fresh (uncooked). After that, the samples were cooked without any additives and presented to the panelists to evaluate their sensory qualities. The panelists drank warm water between each sample and used a ten-point descriptive scale; 7-10 indicated ‘‘very good’ quality, 4.0- 6.9 indicated ‘‘good’ quality, and 1.0-3.9 indicated “spoiled”. This was used for assessing appearance, smell, texture, taste, and overall acceptability. The sensory evaluation was repeated after each treatment.

*2.4. Chemical indices of treated minced meat samples.*

*2.4.1. Potential of hydrogen ion concentration (pH) measurement.*

Ten ml of neutralized distilled water and 10 grammes of minced beef samples were blended. After 10 minutes of constant shaking at room temperature, the mixture was set aside. A pH electrical meter was used to calculate the pH value (Bye model 6020, USA). The pH meter was calibrated using two buffer solutions with precisely defined pH values (alkaline pH 7.01, acidic pH 4.01). To clean the pH electrode, neutralized water was used, and it was introduced to the homogenizer after the temperature correction system was adjusted according to **EOS, (63-11/2020).**

*2.4.2.Determination of total volatile nitrogen "TVN".*

According to **EOS: 63-9/ (2006)**, In a clean distillation flask, 300 ml of distilled water and 10 grammes of minced meat samples were combined and carefully mixed. The prior mixture was supplemented with two grammes of magnesium oxide and an anti-foaming agent. 25 ml of 2% boric acid and a few drops of indicator were put to a 500 ml receiving flask. The receiver tube of the receiving flask was positioned so that it dropped below the boric acid solution. Within 10 minutes, the distillation flask reached boiling temperature. Distillation followed for another 25 minutes. Then titration of TVN against H2So4 M 0.1 was performed until pink color was appeared, TVN was calculated according to the following formula:

$$\text{TVN/l00 grams} = (\text{mls } H_2So_4 \text{ n } 0.1 \text{ for sample} - \text{ml } H_2So_4 \text{ n } 0.1 \text{ for Blank}) \times 14$$

*2.4.3.Determination of thiobarbituric acid number "TBA".*

According to **EOS: 63- 10/(2006)**, The test depends on determination of malonaldehyde (MDA) as a product of lipid peroxidation. Briefly, 50 ml of distilled water were mixed with ten grams of prepared minced meat samples and transferred to a distillation flask, then antifoaming agent and 50 ml of diluted hydrochloric acid were added to flask. The distillation flask was heated for distillation of 50 ml of diluted hydrochloric within 10 minutes from the beginning of boiling. Accordingly, 5 ml of a distilled solution was put in a tube with cover, then 5 ml of prepared thiobarbituric acid, the tube was covered and put on water bath and boiled for 35 minutes, then cooled by water for 10 minutes. By using Spectrophotometer (UNICAM969AA Spectronic, USA), the absorbance of sample was measured under wavelength 538 n $m^3$.
TBA value= absorbance of sample x 7.8 (malonaldehyde (mg) /Kg).

*2.5.Bacteriological examination of minced meat treated with different concentrations of LLO.*

Twenty-five grams of minced beef samples were weighed under aseptic condition and homogenized for 1 min in a laboratory blender containing 225 ml of 0.1 % a sterile peptone water **(**Oxide CM0009**)** for prepared of an original dilution of 1: 10. Ten-fold serial dilutions up to $10^6$ were prepared to cover the expected range of samples contamination, using plate count agar for estimation of the total aerobic bacterial count, plates were incubated at 37°C for 48 hours according to **APHA (2001).**
According to **ISO 21528-2 (2004)** *Enterobacteriaceae* were quantified using violet, red bile glucose agar medium and plates were incubated at 37°C for 24 hours. According to **FDA (2001)** the staphylococcal count was performed using the Baird Parker agar medium supplemented with egg yolk tellurite emulsion and incubated at 37°C for 48 hours. The coliform count was calculated using violet, red bile agar medium (VRBG), and plates were incubated at 37°C for 48 hours. The Enteropathogenic *Escherichia coli* was estimated using Eosin Methylene Blue (EMB) with incubation at 37°C for 24 hours according to **ICMSF (1996)**.

*2.6.Statistical Analysis*

The statistical analysis system (**SAS, 2014**), Cary, USA, Version 9.3) software was used to statistically analyze the data. The mean and standard deviation ("SD") of the organoleptic, chemical, and bacteriological parameters were displayed. Tukey's Studentized Range (HSD) post-hoc test (p0.05) and a nested procedural model ($P < 0.05$) were used to compare significant means.

**3.Results and Discussion**

*3.1.Overall acceptability of treated minced meat with Lemon leave oil (LLO)*

The control minced meat sample was completely spoiled after the sixth day of storage at 4 °C which was observed during overall acceptance of minced meat. The addition of LLO at 0.5, 1, and 1.5% significantly improved the overall acceptability for sensory properties of minced meat. Samples treated with 0.5% LLO maintained their overall acceptability until the ninth day, while 1% and 1.5% LLO kept their overall acceptability until the $12^{th}$ day. Moreover, samples containing 1.5% LLO had the highest acceptability, while those with 0.5% LLO had the least enhancement as shown in table (1).

Our findings revealed that LLO improved the overall acceptability of minced meat, and results were concentration dependent, these results agree with **Hamada et al., (2023)** found that treated minced meat with 2% lemon oil showed improved overall acceptability up to day 7 of storage compared with control samples, which was only acceptable up to day 5. In addition, **Hamma et al., (2020)** reported that the meat samples treated with lemon oils and pulp extract at 3 levels (1, 2, and 3%) showed an improvement in sensory traits such as flavor and aroma score which increased with increasing concentration.

*3.2.Chemical analysis of minced meat treated with Lemon leaf oil (LLO)*

*3.2.1.Effect of LLO on pH of treated minced meat.*

The recorded data in Table 2 demonstrate that, during different periods of experiment, the treated minced meat samples with different levels of LLO had lower pH values than the control samples. Additionally, LLO showed the greatest impact on lowering pH values of minced meat through 12 days of storage especially when concentration increased to 1.5%.

Different concentration of LLO (0.5, 1, and 1.5%) decreased the pH values as compared with control samples which making minced meat

undesirable for growth of most bacteria during chilled storage and thereby LLO has antimicrobial effect. The pH increases in control minced meat indicates the degree of meat spoilage because of protein breakdown into free amino acids, which results in the generation of NH3 and amines, which are alkaline reaction chemicals (**Karabagias et al., 2011**).

Our observations were corroborated by **Hamada et al. (2023)** who observed that treated minced meat with lemon essential oil showed significant reduction in pH values at day 8 of storage in comparison with untreated minced meat. This might be due to lemon oil containing organic acid which helps in decreasing pH values making meat undesirable media for bacterial growth during storage **(Braddock, 1995)**. In addition, **Ben Hsouna et al., (2017)** mentioned that pH values were lower in minced meat treated with citrus limon essential oil (0.06 and 0.312%) during storage period.

*3.2.2.Effect of LLO on total volatile nitrogen (TVN) content of minced meat*

The amount of nitrogen that is released as a result of protein decomposition caused by microorganisms and/or tissue proteolytic enzymes during storage can be measured by the Total Volatile Nitrogen (TVN) content **(Gibriel et al., 2007).** TVB-N is frequently used to estimate the rate of deterioration and shelf life of different types of meat (**Morshdy et al., 2021)**. TVN mean values of control minced samples was increased as shown in table (3) and were exceed the permissible limits established by **EOS-1694 (2005) (**TVN should not exceed 20 mg/100 grams) by the sixth day, this might be quick growth of spoilage bacteria which caused protein breakdown and the production of free amines such trimethylamine and dimethylamine as well as ammonia (**Rukchon et al., 2011**). Treated minced meat with different concentration of LLO showed significantly decrease in TVN values as compared with control samples. additionally, compared to lower concentrations of the LLO concentration (0.5 and 1%) the higher concentration of LLO (1.5%) was more successful in lowering TVN value, especially on day 9$^{th}$ of chilled storage.

Our findings showed that the addition of LLO decreases TVN values until day 12 of storage period. Our findings were in consistent with **Hamada et al., (2023),** who reported that TVN values of treated mined meat with lemon grass essential oil 2% significantly reduced to 16.10±0.02 mg/100 gram on day 8 compared with the TVN in untreated minced meat on the same day (34.75± 0.02 mg/100 gram). According to **Salem et al. (2010),** 1.5% lemon grass oil significantly decreased the TVB-N levels in minced beef.

*3.2.3. Effect of LLO on thiobarbituric Acid (TBA) content of minced meat*

TBA is a lipid oxidation indicator (**Abd El-Khalek and Zahran, 2013).** TBARS values are used to measure the concentration of secondary lipid oxidation products, such as aldehydes and carbonyls of hydrocarbons, which can cause off-aromas in meat (**Safa et al., 2015**). In our study, TBA values of control minced meat exceeded the permissible limits established by **EOS-1694 (2005)** (not more 0.9 mg MDA/kg) by day 6$^{th}$ during storage but treated minced meat with different concentration of LLO does not exceed the same permissible limit during different periods of storage. Treated minced meat samples with LLO specifically 1 and 1.5% continued to have normal flavor until the end of storage at 12 days without any rancidity while, rancid flavor in the control samples began to develop on the sixth day of storage.

**Salem et al. (2010)** demonstrated the efficiency of essential oils in delaying lipid oxidation in minced beef, and **Ben Hsouna et al. (2017)** reported that TBA values were lower in minced meat treated with citrus lemon essential oil at 0.06 and 0.312% during the storage period, this means that lemon essential oils have strong antioxidant properties, **Mustafa (2015)** confirms that phenolic contents in lemon leaves may be the cause of lemon essential oils' antioxidant effect. In addition, **Hamma et al. (2020)** observed a decrease of TBA in ram muscles treated with lemon peel extracts stored at refrigeration temperature for 8 days due to polyphenol components of lemon peel extracts have antioxidant properties and able to be a donor of hydrogen atoms or electrons and to capture free radicals. Also, **Disha et al. (2020)** concluded that lemon extract at 1% has potent antioxidative properties so it has been added as a natural antioxidant to chicken meat balls to increase their shelf life during frozen storage.

**Table 1.** Overall acceptance means values of treated minced meat with different concentrations of lemon leaf oil during refrigeration at 4°C

| | **Control** | **Lemon leaves extract concentrations** | | |
|---|---|---|---|---|
| | | **0.5%** | **1%** | **1.5%** |
| | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** |
| **0 day** | 9.00 ± 0.00$^{Aa}$ | 9.00 ± 0.00$^{Aa}$ | 9.00 ± 0.00$^{Aa}$ | **8.56 ± 0.25$^{Ba}$** |
| **3$^{rd}$ day** | 6.51 ± 0.16$^{Db}$ | 8.40± 0.20$^{Cb}$ | 8.85 ±0.15$^{Ab}$ | **8.55 ± 0.23$^{Ba}$** |
| **6$^{th}$ day** | 3.66± 0.59$^{Dc}$ | 6.97± 0.32$^{Cc}$ | 7.65 ±0.25$^{Bc}$ | **8.0 ± 0.10$^{Ab}$** |
| **9$^{th}$ day** | Decomposed | 4.76 ±0.13$^{Cd}$ | 6.56 ± 0.37$^{Bd}$ | **6.78 ± 0.42$^{Ac}$** |
| **12$^{th}$ day** | **Decomposed** | **Decomposed** | **4.53 ±0.27$^{Be}$** | **5.21 ± 0.07$^{Ad}$** |

SD= Standard deviation, Min.= Minimum, Max.= Maximum

* Means carrying different superscript capital letter on the same row are significantly different ($P < 0.05$).

**Means carrying different superscript small letter on the same column are significantly different ($P < 0.05$).

**Table 2.** Mean pH values of treated minced meat with different concentrations of lemon leaf oil during refrigeration at 4°C

| | **Control** | **Lemon leaves extract concentrations** | | |
|---|---|---|---|---|
| | | **0.5%** | **1%** | **1.5%** |
| | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** |
| **0 day** | 5.61 ± 0.03$^{Ac}$ | 5.59 ± 0.03$^{Bd}$ | 5.57 ± 0.02$^{Cd}$ | **5.57 ± 0.02$^{Ce}$** |
| **3$^{rd}$ day** | 6.19 ± 0.06$^{Ab}$ | 5.85 ± 0.03$^{Bc}$ | 5.78 ± 0.02$^{Cc}$ | **5.73 ± 0.02$^{Dd}$** |
| **6$^{th}$ day** | 6.92 ± 0.09$^{Aa}$ | 6.18 ± 0.10$^{Bb}$ | 6.01 ± 0.06$^{Cb}$ | **5.88 ± 0.04$^{Dc}$** |
| **9$^{th}$ day** | Decomposed | 6.50 ± 0.12$^{Aa}$ | 6.33 ± 0.06$^{Ba}$ | **6.14 ± 0.03$^{Cb}$** |
| **12$^{th}$ day** | **Decomposed** | **Decomposed** | **6.41 ± 0.02$^{Aa}$** | **6.15 ± 0.03$^{Ba}$** |

* Means carrying different superscript capital letter on the same row are significantly different ($P < 0.05$).

**Means carrying different superscript small letter on the same column are significantly different *(*$P < 0.05$).

**Table 3.** TVN (mg%) means values of treated minced meat with different concentrations of LLO during refrigeration at 4°C.

| | **Control** | **Lemon leaves extract concentrations** | | |
|---|---|---|---|---|
| | | **0.5%** | **1%** | **1.5%** |
| | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** |
| **0 day** | $1.93 \pm 0.06^{Ac}$ | $1.84 \pm 0.05^{Bd}$ | $1.79 \pm 0.04^{Ce}$ | $1.77 \pm 0.03^{Ce}$ |
| **3rd day** | $13.68 \pm 0.09^{Ab}$ | $6.05 \pm 0.08^{Bc}$ | $5.42 \pm 0.08^{Cd}$ | $5.21 \pm 0.07^{Dc}$ |
| **6th day** | $24.01 \pm 0.13^{Aa}$ | $11.85 \pm 0.12^{Bb}$ | $11.11 \pm 0.08^{Cc}$ | $9.94 \pm 0.09^{Db}$ |
| **9th day** | Decomposed | $19.34 \pm 0.23^{Aa}$ | $17.76 \pm 0.12^{Bb}$ | $16.33 \pm 0.10^{Ca}$ |
| **12th day** | **Decomposed** | **Decomposed** | **$17.85 \pm 0.08^{Aa}$** | **$16.36 \pm 0.09^{Ba}$** |

* Means carrying different superscript capital letter on the same row are significantly *different* ($P < 0.05$).
**Means carrying different superscript small letter on the same column are significantly different ($P < 0.05$).

**Table 4.** TBA (mg/kg) mean values of treated minced meat with different concentrationsof lemon leaf oil during refrigeration at 4°C

| | **Control** | **Lemon leaves extract concentrations** | | |
|---|---|---|---|---|
| | | **0.5%** | **1%** | **1.5%** |
| | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** |
| **Zero day** | $0.056 \pm 0.01^{Ac}$ | $0.053 \pm 0.01^{Ad}$ | $0.056 \pm 0.01^{Ae}$ | **$0.046 \pm 0.01^{Ce}$** |
| **3rd day** | $0.60 \pm 0.03^{Ab}$ | $0.33 \pm 0.02^{Bc}$ | $0.27 \pm 0.02^{Cd}$ | **$0.21 \pm 0.02^{Dc}$** |
| **6th day** | $1.26 \pm 0.07^{Aa}$ | $0.66 \pm 0.03^{Bb}$ | $0.47 \pm 0.03^{Cc}$ | **$0.34 \pm 0.02^{Db}$** |
| **9th day** | Decomposed | $0.84 \pm 0.02^{Aa}$ | $0.68 \pm 0.03^{Bb}$ | **$0.54 \pm 0.04^{Ca}$** |
| **12th day** | **Decomposed** | **Decomposed** | **$0.69 \pm 0.02^{Aa}$** | **$0.56 \pm 0.03^{Ba}$** |

* Means carrying different superscript Capital letter on the same row are significantly different ($P < 0.05$).
**Means carrying different superscript small letter on the same column are significantly different ($P < 0.05$).

*3.3.Effect of lemon leaves oil "LLO" on microbiological quality of minced meat*

*3.3.1.Effect of LLO on total bacterial counts of minced meat*

The Aerobic Plate Count (APC) is a measure of the bacterial population in meat products; a higher APC usually indicates lower quality and shorter shelf life (**Kim and Yim, 2016**).

Results observed in Table (5), explain the effect of lemon oil concentration on minced meat aerobic bacterial count, the obtained results cleared that, the aerobic bacterial count in meat differ significantly ($P < 0.05$) among different concentrations of lemon oil at different period of experiment. The values of aerobic bacterial count of control minced meat samples were higher than treated minced meat with different concentration of LLO till sixth day of storage. Mean values of aerobic bacterial count in treated minced meat with LLO at concentration 1.5% were lower than treated meat with LLO at 0.5 and 1%. Treated minced meat with LLO 0.5 % started to decompose after day 9 of storage while treated minced meat with LLO 1 and 1.5% still fit for consumption till the end of experiment. The higher concentrations of LLO (1 and 1.5%) were more effective in decreasing bacterial count than lower concentration (0.5%). Control minced meat samples started to decompose after 6th days of storage, this in accordance with **EOS-1694 (2005)** which established that the total bacterial count of minced meat shall not be higher than $10^6$ cfu/g.

Our results indicate that LLO has antimicrobial properties, this was supported by **Shi et al. (2016)** who found that lemon essential oil contains citral at concentrations of approximately 65-85% w/w which has anti-microbial properties; **Mancuso et al. (2019)** observed that lemon essential oil have high antibacterial activity against gram-negative bacteria and gram-positive. **Ibrahim et al. (2021)** reported that lemon juice 0.2% decreased aerobic bacterial counts in chickenmeat stored at 4 ºC for 9 days.

*3.3.2.Effect of LLO on Enterobacteriaceae count of treated minced meat.*

Results observed in Table 6 shows that Enterobacteriaceae count in control minced meat samples were higher than treated minced meat with different concentration of LLO till sixth day of chilled storage and control samples started to decompose after six days of storage. Mean values of Enterobacteriaceae count in treated minced meat of LLO at concentration 1.5% were lower than treated minced meat with LLO at 0.5 and 1%, respectively. Treated minced meat with LLO 0.5% started to decompose after ninth day of storage while treated with 1 and 1.5% LLO still fit for consumption till end of experiment. This indicates that when oil concentration increases, the count of Enterobacteriaceae decreases. Moreover, the treated samples with highest concentration of lemon oil contain lower Enterobacteriaceae count than the control samples or samples with lowerconcentration of lemon oil.

Our findings were confirmed by **Ibrahim et al. (2021)** who observed that lemon juice 0.2% decreased Enterobacteriaceae counts in chicken meat stored at 4ºC for 9 days with reduction percent reached 100% started by third day of storage. In addition, **Abu-Salem and Abou Arab (2010**) came to the conclusion that lemon grass oils with a 1.5% concentration had the strongest impact on the growth of Enterobacteriaceae, with the effect diminishing as the oil concentration increased. **Singh et al. (2011)** proved that lemon grass oil has a good activity against Enterobacteriaceae isolated from food samples.

*3.3.3.Effect of LLO on total staphylococcal count of minced meat*

Results observed in Table 7 demonstrate that staphylococcal mean values of control minced meat samples were higher than treated minced meat with different concentrations of LLO till the sixth day of storage and then decompose. Mean values of staphylococcal count in treated minced meat with 1.5% of LLO were lower than treated minced meat with LLO at 0.5 and 1%. Treated minced meat with 0.5% started to decompose after ninth day of storage, while treated with 1 and 1.5% still fit till the end of experiment. This indicates that higher concentration of LLO (1 and 1.5%) more successful at lowering the countof staphylococci than lower ones (0.5%).

The results elucidated that the treated minced meat samples with higher concentration of lemon oil had lower *Staphylococcus aureus* count, but the samples that treated with lower concentration of lemon oil showed a higher *S. aureus* count level. Our results were confirmed by **Ibrahim et al. (2021)** observed that lemon juice 0.2% decreased staphylococcal counts during refrigeration of chicken meat at 4ºC for 9 days with 100% reduction percent (staphylococci could not be isolated) started by third day of storage. In addition, the activity of lemon grass oil against staphylococci was reported by **Elizabeth et al. (2019)**.

According to **Roy et al. (2012)** *S. aureus* was significantly inhibited by lemon peel essential oil *in vitro*.

*3.3.4.Effect of LLO on coliform count of minced meat*

The total aerobic and coliform count are key indicators of overall microbial contamination, and the presence of these bacteria in high concentrations indicates a higher risk of meat degradation and the presence of pathogens **(De Oliveira et al., 2021)**.

The data in table 8 shows that the coliforms count of minced meat increased gradually from second day to 6th day of storage in control groups and then samples started to decompose, while the coliforms count of minced meat decreased in its level with the higher concentration of lemon oil 1.5%, but the samples that treated with lower concentration of lemon oil 0.5% showed a higher coliforms count level compared with concentrations 1 and 1.5%.

The present findings were supported by **Hamada et al. (2023)** who found that coliform count of treated mined meat with lemon grass essential oil 2% significantly reduced to 3.18± 0.02 $\log_{10}$ cfu/ g on day 8 compared with the coliform count in untreated minced meat on the same day (4.33± 0.03 $\log_{10}$ Cfu/g). In addition, **Abu-Salem and Abou Arab (2010**) proved that 1.5% lemon grass oil had the strongest impact on the development of coliforms as the oil concentration increased.

*3.3.5. Effect of LLO on E.coli count of minced meat*

Coliform and *E. coli* count are used to estimate the level of fecal pollution and poor hygiene during the processing of meat, as their presence in high amounts is associated with a higher risk of foodborne pathogens (**Kim and Yim, 2016**). Results observed in Table (9) showed that *E. coli* count in treated minced meat differ significantly ($P < 0.05$) among different concentration of lemon oil during different periodof experiment.

According to data, the treated minced samples with different concentrations of LLO had lower *E. coli* count than control samples particularly on the sixth day of storage. Also, higher concentrations of LLO (1 and 1.5%) decrease the count of *E. coli* significantly than lower concentration 0.5%. The *E. coli* count of minced meat increased gradually from zero day to 12th day of storage and the *E. coli* count of minced meat decreased in its level with the higher concentration of lemon oil, but the samples that treated with lowerconcentration of lemon oil showed a higher *E.coli* count level.

Our findings were confirmed by **Tyagi and Malik (2012)** who proved that structural damage of *E. coli* was due to the antimicrobial properties of lemon grass which was observed by a scanning electron microscope. Additionally, According to **Hamada et al. (2023),** the highest concentrations of lemon grass oil (1.5% and 2%) consistently showed antibacterial activity against *E. coli*, while the lowest concentrations of lemon grass oil (1%), only slightly inhibited the growth of *E. coli*.

## 4.Conclusion

The study concluded that during the storage period, lemon leaf oil (LLO) may enhance the sensory qualities, chemical quality, and bacteriological quality of minced beef. LLO had significant antibacterial properties against Enterobacteriaceae, staphylococcus, coliform, and *Escherichia coli*. The observed results were concentration dependent with increasing LLO concentration (1% and 1.5%). The more significant effect on the sensory, chemical, and bacteriological parameters compared to (0.5 %). Therefore, Lemon leaves oil could be used as a natural preservative in food against different pathogenic bacteria instead of synthetic preservatives.

**Table 5.** Mean values of aerobic bacterial count (log10 cfu/g) of treated minced meat with different concentrations of Lemon leaf extract during refrigeration at 4°C

| | **Control** | **Lemon leaves extract concentrations** | | |
|---|---|---|---|---|
| | | **0.5%** | **1%** | **1.5%** |
| | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** |
| **0 day** | $4.58 \pm 0.66^{Ac}$ | $4.04 \pm 0.04^{Bb}$ | $3.99 \pm 0.05^{Cc}$ | $\mathbf{3.91 \pm 0.07^{Dc}}$ |
| **3rd day** | $4.61 \pm 0.07^{Ab}$ | $4.02 \pm 0.04^{Bb}$ | $3.95 \pm 0.06^{Cd}$ | $\mathbf{3.85 \pm 0.11^{Dd}}$ |
| **6th day** | $4.72 \pm 0.13^{Aa}$ | $3.98 \pm 0.04^{Bc}$ | $3.91 \pm 0.07^{Ce}$ | $\mathbf{3.77 \pm 0.16^{De}}$ |
| **9th day** | Decomposed | $4.09 \pm 0.13^{Aa}$ | $4.01 \pm 0.09^{Bb}$ | $\mathbf{3.93 \pm 0.11^{Cb}}$ |
| **12th day** | **Decomposed** | **Decomposed** | $\mathbf{4.04 \pm 0.08^{Aa}}$ | $\mathbf{3.96 \pm 0.12^{Ba}}$ |

* Means carrying different superscript capital letter on the same row are significantly different ($P < 0.05$).
**Means carrying different superscript small letter on the same column are significantly different ($P < 0.05$).

**Table 6.** Mean values of enterobacteriaceae count (log10 cfu/g) of treated minced meat with different concentrations of lemon leaf extract during refrigeration at 4°C.

| | **Control** | **Lemon leaves extract concentrations** | | |
|---|---|---|---|---|
| | | **0.5%** | **1%** | **1.5%** |
| | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** |
| **0 day** | $3.93 \pm 0.23^{Ac}$ | $3.83 \pm 0.28^{Bb}$ | $3.78 \pm 0.29^{Ca}$ | $\mathbf{3.71 \pm 0.31^{Da}}$ |
| **3rd day** | $4.03 \pm 0.21^{Ab}$ | $3.80 \pm 0.27^{Bc}$ | $3.71 \pm 0.30^{Cb}$ | $\mathbf{3.64 \pm 0.31^{Db}}$ |
| **6th day** | $4.23 \pm 0.07^{Aa}$ | $3.75 \pm 0.29^{Bd}$ | $3.66 \pm 0.32^{Cc}$ | $\mathbf{3.57 \pm 0.36^{Dd}}$ |
| **9th day** | Decomposed | $3.86 \pm 0.28^{Aa}$ | $3.68 \pm 0.29^{Bc}$ | $\mathbf{3.60 \pm 0.32^{Cc}}$ |
| **12th day** | **Decomposed** | **Decomposed** | $\mathbf{3.71 \pm 0.26^{Ab}}$ | $\mathbf{3.64 \pm 0.29^{Bb}}$ |

* Means carrying different superscript Capital letter on the same row are significantly different ($P < 0.05$).
**Means carrying different superscript small letter on the same column are significantly different ($P < 0.05$).

**Table 7.** Mean values of Staphylococcal count ($log_{10}$ cfu/g) of treated minced meat with different concentrations of lemon leaf oil during chilling refrigeration at 4°C

| | **Control** | **Lemon leaves extract concentrations** | | |
|---|---|---|---|---|
| | | **0.5%** | **1%** | **1.5%** |
| | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** |
| **0 day** | $3.91 \pm 0.14^{Ac}$ | $3.65 \pm 0.29^{Bb}$ | $3.57 \pm 0.28^{Ca}$ | **$3.51 \pm 0.25^{Da}$** |
| **$3^{rd}$ day** | $3.99 \pm 0.12^{Ab}$ | $3.60 \pm 0.27^{Bc}$ | $3.50 \pm 0.26^{Cb}$ | **$3.43 \pm 0.24^{Dc}$** |
| **$6^{th}$ day** | $4.33 \pm 0.57^{Aa}$ | $3.55 \pm 0.26^{Bd}$ | $3.42 \pm 0.27^{Cd}$ | **$3.37 \pm 0.24^{Dd}$** |
| **$9^{th}$ day** | Decomposed | $3.67 \pm 0.26^{Aa}$ | $3.47 \pm 0.23^{Ac}$ | **$3.42 \pm 0.21^{Cc}$** |
| **$12^{th}$ day** | **Decomposed** | **Decomposed** | **$3.49 \pm 0.24^{Ac}$** | **$3.45 \pm 0.20^{Bb}$** |

*Means carrying different superscript Capital letter on the same row are significantly different ($P < 0.05$).
**Means carrying different superscript small letter on the same column are significantly different ($P < 0.05$).

**Table 8.** Mean values of coliforms (log10 cfu/g) of treated minced meat with different concentrations of lemon leaf extract during refrigeration at 4°C

| | **Control** | **Lemon leaves extract concentrations** | | |
|---|---|---|---|---|
| | | **0.5%** | **1%** | **1.5%** |
| | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** |
| **0 day** | $3.91 \pm 0.15^{Ac}$ | $3.83 \pm 0.15^{Ba}$ | $3.79 \pm 0.15^{Ca}$ | **$3.73 \pm 0.12^{Da}$** |
| **$3^{rd}$ day** | $4.00 \pm 0.09^{Ab}$ | $3.75 \pm 0.13^{Bb}$ | $3.74 \pm 0.13^{Cb}$ | **$3.65 \pm 0.14^{Dc}$** |
| **$6^{th}$ day** | $4.10 \pm 0.05^{Aa}$ | $3.84 \pm 0.14^{Ba}$ | $3.67 \pm 0.12^{Cd}$ | **$3.59 \pm 0.15^{De}$** |
| **$9^{th}$ day** | Decomposed | $3.76 \pm 0.13^{Ab}$ | $3.70 \pm 0.13^{Bc}$ | **$3.63 \pm 0.15^{Cd}$** |
| **$12^{th}$ day** | **Decomposed** | **Decomposed** | **$3.73 \pm 0.14^{Ab}$** | **$3.68 \pm 0.13^{Bb}$** |

* Means carrying different superscript capital letter on the same row are significantly different ($P < 0.05$).
**Means carrying different superscript small letter on the same column are significantly different ($P < 0.05$).

**Table 9.** Mean values of *E. coli* (log10 cfu/g) of treated minced meat with different concentrations of Lemon leaf oil during chilling storage period at 4°C

| | **Control** | **Lemon leaves extract concentrations** | | |
|---|---|---|---|---|
| | | **0.5%** | **1%** | **1.5%** |
| | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** |
| **0 day** | $3.67 \pm 0.02^{Ac}$ | $3.62 \pm 0.12^{Ba}$ | $3.56 \pm 0.18^{Ca}$ | **$3.45 \pm 0.01^{Da}$** |
| **$3^{rd}$ day** | $3.75 \pm 0.04^{Ab}$ | $3.55 \pm 0.04^{Bb}$ | $3.47 \pm 0.01^{Cc}$ | **$3.34 \pm 0.04^{Cb}$** |
| **$6^{th}$ day** | $3.94 \pm 0.16^{Aa}$ | $3.46 \pm 0.02^{Bd}$ | $3.39 \pm 0.06^{Ce}$ | **$3.26 \pm 0.01^{Dd}$** |
| **$9^{th}$ day** | Decomposed | $3.49 \pm 0.03^{Ac}$ | $3.43 \pm 0.03^{Bd}$ | **$3.32 \pm 0.01^{Cb}$** |
| **$12^{th}$ day** | **Decomposed** | **Decomposed** | **$3.50 \pm 0.02^{Ab}$** | **$3.42 \pm 0.01^{Bb}$** |

* Means carrying different superscript capital letter on the same row are significantly different ($P < 0.05$).
**Means carrying different superscript small letter on the same column are significantly different ($P < 0.05$).

**5.References**


**Abd El-Khalek, H.H., & Zahran, D.A. (2013).** Utilization of fruit by-product in ground meat preservation. International Journal of Food Science and Nutrition, 2(6), 24-30.

**Abdel-Naeem, H.H.S., Elshebrawy, H.A, Imre, K., Morar, A., Herman, V., Paşcalău, R., & Sallam K.I. (2022).** Antioxidant and antibacterial effect of fruit peel powders in chicken patties. Foods. 11(3),301.

**Abu-Salem, F.M., & Abou Arab, E.A. (2010)**. Chemical Properties, Microbiological Quality and Sensory Evaluation of Chicken and Duck Liver Paste (foie gras). Grasasy Aceites, 61,126-135.

analytical manual .8th Ed. Chapter12. Academic Press, Gaithersburg, UK.

**APHA (American Public Health Association), (2001).** Compendium of methods for the microbiological examination of foods, 4a ed., Washington, D.C.: APHA.

**Barbosa, L., Rall, V., Fernades, A., Ushimaru, P., De Silva, I., & Fernandes, T. (2009).** Essential oils against food borne pathogens and spoilage bacteria in minced meat. Foodborne Pathogens and Disease, 6, 725-728.

**Ben Hsouna, A., Ben Halima, N., Smaoui, S., & Hamdi, N. (2017).** Citrus lemon essential oil: chemical composition, antioxidant, and antimicrobial activities with its preservative effect against Listeria monocytogenes inoculated in minced beef meat. Lipids in Health and Disease, 16(1):146,11pages.

**Bisholo, K.Z., Ghuman, S., & Haffejee, F. (2018).** Food-borne disease prevalence in rural villages in the Eastern Cape, South Africa. African Journal of Primary Health Care & Family Medicine, 10,1796.

**Braddock, R. J. (1995).** By-products of citrus fruit. Food Technol., 49, 74-77.

**Cantini, C., Katsi K. M.G., Neill, F.T., O'Sullivan, M., Dowling, D.P., & Monahan, F.J. (2011).** Development of active packaging containing natural antioxidants. Procedia food science 1, 224-228.

**Costa, K.A.D., Moura, R., & Millez, A.F. (2019).** Antimicrobial and antibiofilm activity of Cymbopogon flexuosus essential oil microemulsions. RevistaCeres Viçosa, 66, 372- 379.

**De Oliveira, M.J., Boué, G., Prévost, H., Maillet, A., Jaffres, E., Maignien, T., Arnich, N., Sanaa, M., & Federighi, M. (2021).** Environmental monitoring program to support food microbiological safety and quality in food industries: A scoping review of the research and guidelines. Food Control, 130, 108283.

**Disha, M.N.A., Hossain, M.A., Kamal, M.T. Rahman, M.M., & Hashem, M.A. (2020).** Effect of different levels of lemon extract on quality and shelf life of chicken meat balls during frozen storage. SAARC Journal of Agriculture, 18(2), 139-156.

**Elabbasy, M.T., Eldesoky, K. I., & Morshdy, A. E. (2014).** Improvement of the shelf life of minced beef. Life Science Journal, 11(9), 185-190.

**Elizabeth, B.D., Otusemade, G.O., Elizabeth, O., Agboola, O., Oyeniyi, E., & Deborah, A.K. (2019).** Antimicrobial activity and phytochemical screening of neem leaves and lemon grass essential oil extracts. International Journal of mechanical Engineering and Technology, 10(3), 882-889.

**EOS " Egyptian Organization for Standardization " 1694/ 2005.** Reports related to No 1694/2005 for frozen minced meat. Egyptian Standards, Ministry of Industry,Egypt.

**EOS "Egyptian Organization for Standardization" 63/ 10" 2006.** Methods of analysis and testing for meat. Part 10: determination of thiobarbituric acid (TBA).

**EOS "Egyptian Organization for Standardization" 63/9" (2006).** Methods of analysis and testing for meat. Part 9: determination of total volatile nitrogen (TVN).

**EOS "Egyptian Organization for Standardization" 63/11'' 2020.** Egyptian Organization for Standardization and quality control. Egyptian Standards for poultry meat products treated with heat. Methods of analysis and testing for meat and meat products Part: 11 Measurement of pH.

**FDA " Food and Drug Administration" 2001.** *Staphylococcus aureus.* Bacteriological

**Gibriel, A.Y., Ebeid, H.M., Khalil, H.I. & Abd ElFattah, A.A. (2007).** Application of Monascuspurpureus pigments produced by using some food industry wastes in beef sausage manufacture. Egyptian Journal of Food Science, 35, 27-45.

**Hamada, M.A., Ahmed M.S., & Hoda H. El-Hendawy. (2023).**Seasonal variations of bacterial populations in refrigerated minced meat and the role of different essential oils in extending shelf life. Egyptian Journal of Botany 63 (1), 45-56.

**Hamma, A., Dahir, H. G., Khidhir, Z. K., Mohamma d, M.M., Ahmmed, D.J., Ahmmed A.K., & Kaki, S.O. (2020).** Effect of lemon peel and pulp extract on some local Kurdish ram meat qualities and its shelf life. Anbar Journal of Agricultural Sciences. 18 (1),141-153.

**Ibrahim, H.M., Mohamed, A.H., Reham, A.A., & Alaa, K.A. (2021).** Effects of lemon and kiwi juice in reduction of some pathogens contaminating chicken breast meat. Benha Veterinary Medical Journal 40, 169-173.

**ICMSF " International commission of Microbiological Specification for Foods "1996.** Microorganisms in Food. I. Their Significance and methods of enumeration. 3rd Ed. Univ. of Toronto, Canada.

**ISO 21528-2:2004.** Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count method

**Karabagias, I., Badeka, A., & Kontominas, M.G. (2011)**. Shelf-life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. Meat Sci., 88,109-116.

**Kim, J.H., & Yim, D.G. (2016).** Assessment of the microbial level for livestock products in retail meat shops implementing HACCP system. Korean Journal for Food Science of Animal Resources, 36, 594-600.

**Lawless, H.T. & Heymann, H. (2010).** Sensory evaluation of food: principles andpractices. Springer Pub., New York, NY, USA.

**Mancuso, M., Catalfamo, M., Laganà, P., Rappazzo, A.C., Raymo, V., Zampino, D., & Zaccone, R. (2019).** Screening of antimicrobial activity of citrus essential oils against pathogenic bacteria and candida strains. Flavour Fragr. J., 34,187-200.

**Morshdy, A. E. M., El-Tahlawy, A. S., Qari, S. H., Qumsani, A. T., Bay, D. H., Sami, R., Althubaiti, E.H., Mansour, A.M., Aljahani, A.H., Hafez, A.E., Mahmoud, A.F., El Bayomi, R., and Hussein, M. A. (2022).** Anti-Biofilms' Activity of Garlic and Thyme Essential Oils against Salmonella *Typhimurium*. Molecules, 27(7), 2182.

**Morshdy, A.M., Al Ashkar, A.T., & Mahmoud, A.F.A. (2021).** Improving the quality and shelf life of rabbit meat during chilled storage using lemongrass and black seed oils. J. Anim. Health Prod. 9(s1), 56-61.

**Mustafa N.E.M. (2015).** Citrus essential oils: Current and prospective uses in the food industry. Recent Patents Food Nutr Agric.,7,115-127.

**Raven, P. (2019).** Saving plants, saving ourselves. Plants, People, Planet, 1,8-13.

**Reyes-Jurado, F., Franco-Vega, A., Ramírez-Corona, N., Palou, E., & López-Malo, A. (2015).** Essential oils: Antimicrobial activities, extraction methods, and their modeling. Food Engineering Reviews, 7, 275–297.

**Roy, S., Bania, R., Chakraborty, J., Goswami, R., Laila, R., & Ahmed, S.A. (2012).** Pharmacognostic, phytochemical, physicochemical property and antimicrobial activity studies of lemon peel oil. Journal of Natural Product and Plant Resources. 2, 431- 435.

**Rukchon, C.H., Trevanich S., Jinkarn T., & Suppakul P. (2011).** Volatile compounds as quality indicators of fresh chicken and possible application in intelligent packaging. In 12th Asian Food Science Conference, Bangkok, Thailand, pp: 287- 294.

**Safa, H., Gatellier, P., Lebert, A., Picgirard, L., & Mirade, P.S. (2015).** Effect of combined salt and animal fat reductions on physicochemical and biochemical changes during the manufacture of dry-fermented sausages. Food Bioprocess Technol. 8,2109-122.

**Salem, A.M., Amin, R.A., & Afifi, G.S.A. (2010).** Studies on antimicrobial and antioxidant efficiency of some essential oils in minced Beef. J. Am. Sci. 6: 691-700.

**SAS, (2014).** Statistical user's Guide. Statistical analysis system. INT., Cary, NC. USA.

**Schieber, A., Stintzing F.C., & Carle, R. (2001).** By-products of plant food processing as a source of functional compounds-recent developments. TrendsFood Science and Technology, 12:401-413.

**Shi, C., Song, K., Zhang, X., Sun, Y., Sui, Y., Chen, Y., Jia, Z., Sun, H., Sun, Z., & Xia, X. (2016).** Antimicrobial Activity and Possible Mechanism of Action of Citral against Cronobacter sakazakii. PLoS ONE, 11, e0159006.

**Singh, B.R., Singh V., Singh R.K., & Ebibeni, N. (2011).** Antimicrobial activity of lemongrass (Cymbopogon citratus) oil against microbes of environmental, clinical and food origin. Int. Res. J. Pharm. Pharmacol. 1 (9),228-236.

**Tizianna, B.M., & Giuseppe, R. (1998).** Antimicrobial and antioxidant properties of some commercial essential oils. Flavour and Fragrance, 13,235-44.

**Tyagi, A.K. & Malik, A. (2012)**. Morphostructural Damage in Food-Spoiling Bacteria due to the Lemon Grass Oil and Its Vapour: SEM, TEM, and AFM Investigations. Evidence-Based Complementary and Alternative Medicine, vol 2012,1-12.