



Title

Effect of some natural preservatives on *Staph.aureus* and *E.coli*

- 1 Hindya, Amany Adel adameisa45@yahoo.com BSc Other 01010538299 Menoufia University Faculty of Veterinary Medicine Food Hygiene and Control Department
- 2 Elbagoury, Abd Elrahman elbagory200@yahoo.com Ph.D. Professor 0100544 6473 Milk Hygiene and Control Department, Faculty of Veterinary Medicine –Menuofia University
- 3 Edres, Abobakr drabobakr2012@yahoo.com Ph.D. Professor 01005060472 Meat Hygiene , Faculty of Veterinary Medicine, Benha University

ABSTRACT

Key words:

M. olifera extract ,
Cinnamon oil , Cumin oil ,
Staph.aureus and *E.coli*.

*Correspondence to

adameisa45@yahoo.com

Article History

Received: 27 May 2024.

Accepted: 21 Jun 2024

Natural preservatives are effective in extending the shelf life of meat without health risks. This study investigated the antibacterial effect of Moringa olifera extract, Cinnamon and Cumin oils against *Staph.aureus* and *E. coli*. A total of 2000 g minced beef was divided into three equal groups, first and second groups were inoculated with *Staph. aureus* and *E. coli* (10^6), the last group was control negative. Each inoculated group was subdivided into four groups, one group was control positive and the other three groups were treated with M.olifera extract, Cinnamon and Cumin oils (1.5% for each), then stored at 4°C for 18 days. Sensory examination, *Staph. aureus* and *E. coli* counts were done every 3 days. The results revealed that control group spoiled at 9th day, meanwhile Cumin, Cinnamon oils and M.olifera extract showed signs of deterioration at 12th, 15th and 18th day of storage, respectively. The bacterial count of control +ve groups significantly increased from $5.0 \times 10^6 \pm 0.3 \times 10^6$ to $9.5 \times 10^6 \pm 0.8 \times 10^6$ and from $2.0 \times 10^6 \pm 0.1 \times 10^6$ to $7.9 \times 10^6 \pm 0.6 \times 10^6$ for *Staph. aureus* and *E. coli*, respectively, however treated samples showed slight increase in the count to $7.1 \times 10^6 \pm 0.6 \times 10^6$, $6.4 \times 10^6 \pm 0.6 \times 10^6$ and $5.8 \times 10^6 \pm 0.5 \times 10^6$ cfu/g with reduction percent 25.2% , 32.6% and 38.9 % & $4.8 \times 10^6 \pm 0.4 \times 10^6$, $4.1 \times 10^6 \pm 0.3 \times 10^6$ and $3.7 \times 10^6 \pm 0.3 \times 10^6$ with reduction percent 39.2% , 48.1% and 53.2% for *Staph. aureus* and *E. coli*, respectively, at 6th day of storage period for Cumin, Cinnamon oils and M.olifera extract, respectively.

1. Introduction

Meat has the advantage of high quality protein that contains the essential amino acids, as well as some important minerals such as iron, copper, tin, manganese, zinc, and other trace elements which

play an important role in the process of body metabolism and the production of antioxidant enzymes (1).

On the other side, meat is considered as a perfect medium for growth of several organisms because of the high moisture content, the high percentage of nitrogenous compounds, fermentable

carbohydrates (glycogen) and ideal pH for most microorganisms **(2)**. Microbial growth and lipid oxidation have unfavorable effects on the nutritional quality which lead to meat spoilage and tremendous economic losses **(3)**. Additionally, the microbial growth might also have negative impact on the sensory criteria of meat due to the development of adverse reactions that involve bad odor, color and textural changes in meat **(4)**. Consequently, preservation of meat by using antioxidants and antimicrobial agents is very important, resulting in consumers getting safe and high-quality of meat and meat products **(5)**. Recently, the use of natural preservatives are preferable than the chemical synthetic additives that may cause health problems to consumers **(6)**.

Natural food additives such as essential oils and plant extracts derived from spices and herbs have become very common in meat technology to prolong the shelf-life of meat and meat products and preventing the organoleptic and nutritional losses induced by microbiological or enzymatic reactions (4).

Moringa oleifera (MO) is a fast-growing, drought-resistant tree belongs to the family Moringaceae, usually known as drumstick tree (7). The leaves have rich sources of minerals such as calcium, magnesium, potassium, iron, zinc and copper (8). These leaves are utilised to extend the shelf life of foods due to the presence of antioxidants such as phenolics, flavonoids, carotenoids and ascorbic acid (9). The leaves and seeds of *Moringa olifera* have significant medicinal properties that include antibacterial, antioxidant, and antifungal activities and have been evaluated as natural preservative for different types of meat products (10,11). In addition to its nutritional properties, it provides food with favorable taste and aroma (5).

The Cinnamon bark and leaves are frequently used as spices and their distilled essential oils are used as flavoring materials in the food industry (12). The main component in the essential oil of *Cinnamomum zeylanicum* is cinnamaldehyde which was reported to inhibit ATPase enzyme and disrupt the outer cell membrane of microorganisms. (13).

Cumin (*Cuminum cyminum*) is an herbaceous plant used as a constituent in many foods processing not only for its seasoning and flavoring effect, but also as natural antimicrobial preservative due to its inhibitory effect against many microorganisms (14).

The antimicrobial activity of Cumin essential oil is attributed to the high level of Cumin-aldehyde and other minority compounds that may contribute to the antimicrobial activity such as β -pinene, limonene and α -pinene (15).

Control of food borne pathogens remain as an important issue for specialists of food industry. Therefore the main target of the present work was carried out to evaluate the efficacy of some natural preservatives against two serious pathogenic bacteria such as *Staph.aureus* and *E. coli* in minced beef stored at 4°C.

2. Material and methods:

2.1 Bacterial strains:

Staph.aureus (ATCC 25923) and *E. coli* (O78 ATCC 700928) strains were obtained from Food analysis center, Food Hygiene Department,

Faculty of Veterinary Medicine, Benha University, with recommended dose 2.0×10^6 cfu/ml for *E.coli* and 5.0×10^6 cfu/ml for *Staph.aureus*.

2.2. Natural essential oils:

Cinnamon and cumin essential oil (1.5% for each) were purchased from Food analysis center, Food Hygiene Department, Faculty of Veterinary Medicine, Benha University. These materials were of analytical reagent grade. The oils were stored in refrigerator until be used.

2.3 Collection and Preparation of *Moringa olifera* leaves (MOL):

Fresh *M. oleifera* leaves (MOL) were purchased from a local herbal store in Menofia Governorate, Egypt. MOL was prepared according to the method reported by (16). The leaves were washed well in order to get rid of any dirt and contaminants, then dried in a hot air oven at 60 °C and crushed into a fine powder. The powder was passed through sieve (No. 60) and extracted by soaking 400 g of dried powder in 2 L of boiled water at room temperature for 1 h, with frequent stirring. The obtained aqueous extract of *M. oleifera* leaves was filtered by Whatman No. (1) filter paper, and the residue was re-extracted again with 1 L distilled water. Both filtrates were mixed, kept in a sterile glass container and stored in refrigerator until be used.

2.4 Preparation of samples according to: Barbosa et al..(17)

2000 g of fresh minced beef were bought from a butcher shop in Shbien –Elkom city, Menofia governorate then labeled in an ice box to Animal health research institute –Shbien El-kom branch. Minced meat was divided into three equal groups (650 g each) then spreaded to very thin sheets and exposed to ultraviolet light to decrease bacterial count.

After that 1st group was inoculated with *Staph. aureus* strain (10^6 cfu/g), the 2nd group was inoculated with *E.coli* strain (10^6 cfu /g) and the last group was served as control –ve.

The inoculation was done by pouring and swabbing over the minced meat surface (18). The inoculated samples were kept for 30 minutes to allow attachment and absorption of inoculated bacteria. Each inoculated group was subdivided into 4 minor groups (160 g each) one group was served as control +ve and the other three groups were treated with *M.olifera* extract, Cinnamon and Cumin oils with the same concentration 1.5%,

then mixed thoroughly by squeezing over the bags for 10 minutes . Samples were packaged ,4labeled and stored at 4 °c .

Sensory evaluation , *Staph. aureus* and *E. coli* counts were conducted from day 0 and every 3 days until the end of the experiment using serial dilutions and spread plate techniques.

2.5 Sensory evaluation:

The color ,odor ,appearance, consistency and overall acceptability were determined according to (19)

2.6 Determination of *Staph. aureus* count :

Staph. aureus count was determined according to (20)

2.7 Determination of *E. coli* count :

E. coli count was determined according to (21)

2.8 Statistical analysis

All data statistically analysed by using Two – way analysis of variance (ANOVA) according to (22)

3. Results:

Table (1) sensory characteristic scores of control untreated minced meat samples during chilling at 4 °c (n=3)

| Trait Storage time | Color | Odor | Appearance | Consistency | Over all acceptability | Grade |
|-----------------------|---------|---------|------------|-------------|------------------------|------------|
| Zero time | 4.8±0.3 | 4.8±0.1 | 4.8±0.1 | 4.8±0.2 | 4.8±0.1 | Very good |
| 3 rd day | 3.4±0.2 | 3.2±0.1 | 3.2±0.2 | 3.0±0.1 | 3.2±0.1 | Acceptable |
| 6 th day | 1.6±0.1 | 1.4±0.1 | 1.6±0.1 | 1.4±0.1 | 1.5±0.1 | Bad |
| 9 th day | S | S | S | S | S | Spoiled |
| 12 th day | S | S | S | S | S | Spoiled |
| 15 th day | S | S | S | S | S | Spoiled |
| 18 th day | S | S | S | S | S | Spoiled |

4.5-5 :Very good 4-4.5 : Good 3-4: Acceptable 2-3: Un acceptable 1-2:Bad S:Spoiled

Table (2) Sensory characteristic scores of minced meat treated with 1.5% cumin oil during chilling at 4 °c (n=3)

| Trait Storage time | Color | Odor | Appearance | Consistency | Over all acceptability | Grade |
|-----------------------|---------|---------|------------|-------------|------------------------|---------------|
| Zero time | 4.8±0.3 | 4.8±0.1 | 4.8±0.1 | 4.8±0.2 | 4.8±0.1 | Very good |
| 3 rd day | 4.0±0.1 | 4.0±0.2 | 3.8±0.2 | 4.0±0.1 | 4.0±0.1 | good |
| 6 th day | 3.6±0.2 | 3.4±0.2 | 3.6±0.1 | 3.8±0.1 | 3.6±0.2 | Acceptable |
| 9 th day | 2.8±0.1 | 2.8±0.1 | 3.0±0.1 | 2.6±0.2 | 2.6±0.2 | Un acceptable |
| 12 th day | S | S | S | S | S | Spoiled |
| 15 th day | S | S | S | S | S | Spoiled |
| 18 th day | S | S | S | S | S | Spoiled |

4.5-5 :Very good 4-4.5 : Good 3-4: Acceptable 2-3: Un acceptable 1-2:Bad S:Spoiled

Table (3) sensory characteristic scores of minced meat treated with 1.5% cinnamon oil during chilling at 4 °c (n=3)

| Trait Storage time | Color | Odor | Appearance | Consistency | Over all acceptability | Grade |
|-----------------------|---------|---------|------------|-------------|------------------------|---------------|
| Zero time | 4.8±0.3 | 4.8±0.1 | 4.8±0.1 | 4.8±0.2 | 4.8±0.1 | Very good |
| 3 rd day | 4.6±0.1 | 4.4±0.1 | 4.6±0.2 | 4.4±0.1 | 4.5±0.1 | Good |
| 6 th day | 4.2±0.2 | 4.2±0.2 | 4.0±0.2 | 4.2±0.1 | 4.2±0.1 | Good |
| 9 th day | 3.2±0.2 | 3.0±0.1 | 3.0±0.2 | 2.8±0.2 | 3.0±0.1 | Acceptable |
| 12 th day | 2.6±0.1 | 2.2±0.2 | 2.8±0.1 | 2.4±0.1 | 2.5±0.2 | Un acceptable |
| 15 th day | S | S | S | S | S | Spoiled |
| 18 th day | S | S | S | S | S | Spoiled |

4.5-5 :Very good 4-4.5 : Good 3-4: Acceptable 2-3: Un acceptable 1-2:Bad S:Spoiled

Table (4) sensory characteristic scores of minced meat treated with 1.5% Moringa olifera extract during chilling at 4 °c (n=3)

| Trait | Color | Odor | Appearance | Consistancy | Over all acceptability | Grade |
|----------------------|---------|---------|------------|-------------|------------------------|------------|
| Storage time | | | | | | |
| Zero time | 4.8±0.3 | 4.8±0.1 | 4.8±0.1 | 4.8±0.2 | 4.8±0.1 | Very good |
| 3 rd day | 4.6±0.2 | 4.6±0.1 | 4.6±0.1 | 4.4±0.1 | 4.6±0.1 | Very good |
| 6 th day | 4.4±0.1 | 4.2±0.1 | 4.2±0.2 | 4.4±0.2 | 4.3±0.2 | Good |
| 9 th day | 4.2±0.1 | 4.0±0.2 | 3.8±0.2 | 4.2±0.1 | 4.0±0.2 | Good |
| 12 th day | 3.6±0.2 | 3.2±0.1 | 3.2±0.1 | 3.0±0.1 | 3.3±0.1 | Acceptable |
| 15 th day | 3.4±0.1 | 3.0±0.1 | 3.0±0.1 | 2.8±0.2 | 3.1±0.2 | Acceptable |
| 18 th day | S | S | S | S | S | Spoiled |

4.5-5 :Very good 4-4.5 : Good 3-4: Acceptable 2-3: Un acceptable 1-2:Bad S:Spoiled

Table (5) Effect of natural essential oils on *Staph. aureus* experimentally inoculated (5.0×10^6 /g) to minced meat stored at 4°C (n=3)

| Additives | | 1.5% Cumin oil | | 1.5% Cinnamon oil | | M.olifera extract | |
|----------------------|---------------------------------------|---------------------------------------|------|---------------------------------------|------|---------------------------------------|------|
| Storage time | Control (+ve) | Mean±S.E | R% | Mean±S.E | R% | Mean±S.E | R% |
| Zero time | $5 \times 10^6 \pm 0.3 \times 10^6$ | $5 \times 10^6 \pm 0.3 \times 10^6$ | | $5 \times 10^6 \pm 0.3 \times 10^6$ | | $5 \times 10^6 \pm 0.3 \times 10^6$ | |
| 3 rd day | $7.4 \times 10^6 \pm 0.6 \times 10^6$ | $5.9 \times 10^6 \pm 0.5 \times 10^6$ | 20.3 | $5.5 \times 10^6 \pm 0.4 \times 10^6$ | 25.7 | $5.2 \times 10^6 \pm 0.4 \times 10^6$ | 29.7 |
| 6 th day | $9.5 \times 10^6 \pm 0.8 \times 10^6$ | $7.1 \times 10^6 \pm 0.6 \times 10^6$ | 25.2 | $6.4 \times 10^6 \pm 0.6 \times 10^6$ | 32.6 | $5.8 \times 10^6 \pm 0.5 \times 10^6$ | 38.9 |
| 9 th day | S | $8.9 \times 10^6 \pm 0.8 \times 10^6$ | | $7.3 \times 10^6 \pm 0.6 \times 10^6$ | | $6.5 \times 10^6 \pm 0.5 \times 10^6$ | |
| 12 th day | S | S | | $9.2 \times 10^6 \pm 0.7 \times 10^6$ | | $8.0 \times 10^6 \pm 0.6 \times 10^6$ | |
| 15 th day | S | S | | S | | $9.1 \times 10^6 \pm 0.8 \times 10^6$ | |
| 18 th day | S | S | | S | | S | |

**Mean values with different superscript litters in the same rows are significantly different (P<0.05).

S.E = Standard error

R % = Reduction %

S= Spoiled

Table (6) Effect of natural essential oils on Enteropathogenic *E.coli* experimentally inoculated (2.0×10^6 /g) to minced meat stored at 4°C (n=3)

| Additives | | 1.5% Cumin oil | | 1.5% Cinnamon oil | | M.olifera extract | |
|----------------------|---------------------------------------|---------------------------------------|------|---------------------------------------|------|---------------------------------------|------|
| Storage time | Control (+ve) | Mean±S.E | R% | Mean±S.E | R% | Mean±S.E | R% |
| Zero day | $2.0 \times 10^6 \pm 0.1 \times 10^6$ | $2.0 \times 10^6 \pm 0.1 \times 10^6$ | | $2.0 \times 10^6 \pm 0.1 \times 10^6$ | | $2.0 \times 10^6 \pm 0.1 \times 10^6$ | |
| 3 rd day | $3.9 \times 10^6 \pm 0.2 \times 10^6$ | $2.6 \times 10^6 \pm 0.2 \times 10^6$ | 33.3 | $2.4 \times 10^6 \pm 0.2 \times 10^6$ | 38.5 | $2.3 \times 10^6 \pm 0.1 \times 10^6$ | 41 |
| 6 th day | $7.9 \times 10^6 \pm 0.6 \times 10^6$ | $4.8 \times 10^6 \pm 0.4 \times 10^6$ | 39.2 | $4.1 \times 10^6 \pm 0.3 \times 10^6$ | 48.1 | $3.7 \times 10^6 \pm 0.3 \times 10^6$ | 53.2 |
| 9 th day | S | $7.0 \times 10^6 \pm 0.6 \times 10^6$ | | $5.9 \times 10^6 \pm 0.5 \times 10^6$ | | $5.2 \times 10^6 \pm 0.4 \times 10^6$ | |
| 12 th day | S | S | | $7.3 \times 10^6 \pm 0.6 \times 10^6$ | | $6.5 \times 10^6 \pm 0.6 \times 10^6$ | |
| 15 th day | S | S | | S | | $7.7 \times 10^6 \pm 0.6 \times 10^6$ | |
| 18 th day | S | S | | S | | S | |

**Mean values with different superscript litters in the same rows are significantly different (P<0.05).

S.E = Standard error

R % = Reduction %

S= Spoiled

Table (1) illustrated the sensory characteristics of control (untreated) minced meat samples refrigerated at 4 °c .The results revealed that control samples were completely spoiled at 9th day of cold storage. Results tabulated in tables (2 , 3 , 4) revealed the sensory characteristic scores of minced meat samples treated with different natural preservatives . Addition of 1.5% M.olifera extract maintained the overall acceptability of the sensory parameters until 15th day , 1.5% Cinnamon oil maintained the overall acceptability until 9th day , while Cumin oil 1.5% conserved the overall acceptability until 6th day of cold storage .

Regarding to the influence of natural essential oils on artificially inoculated *Staph.aureus* in minced meat samples, table (5) revealed that the mean value of examined strain count at zero time was $5 \times 10^6 \pm 0.3 \times 10^6$ (CFU/g) in control and treated groups . After the day 3 , there was progressive increasing in *Staph.aureus* count in control group which recorded $7.4 \times 10^6 \pm 0.6 \times 10^6$ (CFU/g) , while the mean values of *Staph.aureus* counts were $5.9 \times 10^6 \pm 0.5 \times 10^6$, $5.5 \times 10^6 \pm 0.4 \times 10^6$ and $5.2 \times 10^6 \pm 0.4 \times 10^6$ (CFU/g) with reduction rate of 20.3% , 25.7% and 29.7% for Cumin oil, Cinnamon oil and M.olifera extract , respectively .

On the 6th day of storage , *Staph.aureus* count increased significantly in control group with mean value of $9.5 \times 10^6 \pm 0.8 \times 10^6$ (CFU/g) , conversely all treated samples exhibited a significant effect in controlling *Staph.aureus* growth with mean values of $7.1 \times 10^6 \pm 0.6 \times 10^6$, $6.4 \times 10^6 \pm 0.6 \times 10^6$ and $5.8 \times 10^6 \pm 0.5 \times 10^6$ (CFU/g) and reduction rate of 25.2 % , 32.6% and 38.9% for Cumin oil, Cinnamon oil and M.olifera extract , respectively .Furthermore , On the 9th day of storage the control sample got spoiled but treated samples were still accepted with mean values of $8.9 \times 10^6 \pm 0.8 \times 10^6$, $7.3 \times 10^6 \pm 0.6 \times 10^6$ and $6.5 \times 10^6 \pm 0.5 \times 10^6$ (CFU/g) for Cumin oil, Cinnamon oil and M.olifera extract , respectively . Cumin oil treated samples spoiled at the 12th day of the storage period , Cinnamon oil treated samples recorded $9.2 \times 10^6 \pm 0.7 \times 10^6$ (CFU/g) at the 12th day of storage then got spoiled at the 15th day of the storage period , while M.olifera extract treated samples did not show signs of spoilage till the end of the experiment and recorded $8.0 \times 10^6 \pm 0.6 \times 10^6$ and $9.1 \times 10^6 \pm 0.8 \times 10^6$ (CFU/g) for *Staph.aureus* count at the 12th and 15th day of the storage period, respectively, and spoiled at 18th day.

The inhibitory effect of tested natural preservatives on artificially inoculated *E. coli* strain into minced meat samples was estimated . Data presented in table (6) showed that the mean value of *E.coli* at zero time was $2.0 \times 10^6 \pm 0.1 \times 10^6$ (CFU/g) in control and treated groups . *E.coli* count at the 3rd day of storage demonstrated higher rate in control group with mean value of $3.9 \times 10^6 \pm 0.2 \times 10^6$ (CFU/g) , whereas in treated groups , *E.coli* counts showed more lowering as compared with the control group with mean values of $2.6 \times 10^6 \pm 0.2 \times 10^6$, $2.4 \times 10^6 \pm 0.2 \times 10^6$ and $2.3 \times 10^6 \pm 0.1 \times 10^6$ (CFU/g) and reduction rate of 33.3 % , 38.5% and 41% for Cumin oil, Cinnamon oil and M.olifera extract , respectively . On the day 6 , the control group showed dramatic increase in *E.coli* count with mean value of $7.9 \times 10^6 \pm 0.6 \times 10^6$ (CFU/g) . On the other hand , treated groups displayed a significant effect in controlling *E.coli* growth with mean values of $4.8 \times 10^6 \pm 0.4 \times 10^6$, $4.1 \times 10^6 \pm 0.3 \times 10^6$ and $3.7 \times 10^6 \pm 0.3 \times 10^6$ (CFU/g) and reduction rate of 39.2% , 48.1% and 53.2% for Cumin oil, Cinnamon oil and M.olifera extract , respectively. At the 9th day of storage control group revealed signs of decomposition , nevertheless all treated samples continued their positive antibacterial effect with mean values of $7.0 \times 10^6 \pm 0.6 \times 10^6$, $5.9 \times 10^6 \pm 0.5 \times 10^6$ and $5.2 \times 10^6 \pm 0.4 \times 10^6$ (CFU/g) for Cumin oil, Cinnamon oil and M.olifera extract

, respectively . By the time there was a gradual elevation in *E.coli* counts in all treated samples , Cumin oil treated samples got spoiled by the 12th day of the experiment . *E.coli* count in Cinnamon oil treated group was $7.3 \times 10^6 \pm 0.6 \times 10^6$ (CFU/g) at the 12th day, after that spoilage was noticed by the 15th day of the experiment , while the group treated with M.olifera extract remained acceptable until the 15th day of the experiment with mean values of $6.5 \times 10^6 \pm 0.6 \times 10^6$ and $7.7 \times 10^6 \pm 0.6 \times 10^6$ (CFU/g) at the 12th and 15th day of the storage period , respectively ,then spoiled at the 18th day.

4. Discussion

Natural preservatives have a potent antimicrobial properties as they can act against resistance mechanisms of bacteria , they can hinder membrane- integrated or associated enzyme-proteins, stopping their activity or production. They also could inhibit the synthesis of DNA, RNA, proteins and polysaccharides in bacterial cells (23, 24).

The sensory evaluation is generally the main guide of the product quality from consumer point of view. It is a quick , easy , and efficient technique for receiving an idea about the quality of the product and its overall acceptability (25)

It is evident from results that the sensory properties were improved by using natural preservatives compared to control samples and that were in agreement with (26) who said that natural antioxidants improved color and flavor stability in meat.

This study showed that the sensory characteristics of M. olifera treated samples were significantly improved and the overall acceptability continued till 15th day of the storage period , these results agree with (27) who found that the treated meat balls with M.olifera extract 1% , 2% showed overall acceptability until 15th , 18th day of the storage period, respectively.

Furthermore , (28) revealed that samples treated with 1% M.olifera extract maintained the overall acceptability until 14th day of cold storage , compared to control one which got spoiled after 6th day of cold storage , As well as it could extend the shelf life of chicken meat without any alteration in sensory traits . The leaves of M. olifera are rich in polyphenolics , flavonoids, carotenoids, and other bioactive compounds which gave the food special favorable taste and aroma (5) . In addition (29) said that untreated samples of Poultry meat stored at chilling were completely spoiled at 6th day and the addition of 1%

M.olifera extract maintained the acceptability until 14th day .

On the other hand (30) said that the incorporation of Moringa extract decreased some of the sensory characteristics of cooked patties such as color, taste and overall acceptability . Also, (11) indicated that increasing *M. olifera* concentration more than 1% may have changed the characteristic sensory attributes of the chicken sausages.

The results also revealed the better effect of Cinnamon oil on the sensory attributes and that were in agreement with (31) who recorded that 1.5% cinnamon oil demonstrated highest enhancement of sensory characteristics and their treated sample did not show spoilage until the 12th day of cold storage.

The samples treated with cumin oil 1.5% demonstrated the lowest improvement in sensory characters ,these results were nearly similar to (32) who found that the addition of cumin oil (0.3% ,0.5% ,1.0%) to minced meat samples showed a slight improvement in sensory characters compared to other oils under study which revealed a better improvement .

Food poisoning caused by *Staph. aureus* is mainly due to the consumption of contaminated food with staphylococcal enterotoxins (33). The existence of *Staph. aureus species* in meat may be due to direct contact with workers, with hand or arm lesions, or by coughing and sneezing . Food handlers are often the major source of food contamination on staphylococcal outbreaks (34). *E.coli* is another important food borne pathogen and its presence in food considered as indicator of fecal contamination, the symptoms may be severe diarrhea in infants and young children, as well as food poisoning and gastroenteritis among the adults (35).

The results in table (5,6) indicated that the control samples had the highest count of *Staph .aureus* and *E.coli* compared to other treatments , *M.olifera* extract showed the maximum antibacterial activity . These findings are nearly similar to (27) who recorded that the incorporation of *M.olifera* extract 1% , 2% into minced meat revealed a significant decrease in *Staph. aureus* and *E.coli* counts and extended the shelf life till the end of the experiment (18 day) . Also, (36) studied the antibacterial effect of *M.olifera* extract against *Staph. aureus* and *E.coli* in chicken fillet , they found that *M.olifera* extract had a significant antibacterial effect against tested strains when compared to control sample. Furthermore , (37) recorded that the bacterial counts of *Staph. aureus* and *E.coli* decreased by

using three different levels (1%,2%,3%) of *M.olifera* extract in smoke dried catfish.

Moriga oleifera leaves was reported to contain a chemical constituent called pterygospermin which readily splits into two molecules of benzyl isothiocyanate, that is known to have antimicrobial characters (11).

A previous study conducted by (38) showed that *M.olifera* extract at level 100g/kg caused complete inhibition in *Staph .aureus* and *E.coli* growth during 12th day of refrigeration in treated chicken patties as compared to the control group which recorded higher bacterial counts ,however *Staph .aureus* was detected in treated chicken patties on the day 12 of refrigeration which may be due to external contamination. The same authors reported that this antimicrobial activity may be owing to abundance of phytochemical substances such as flavonoids , polyphenols as well as some proteins and peptides . Accordingly , (5) found that in vitro effect of *M.olifera* extracts showed a remarkable antimicrobial activity against Gram-negative bacteria, but the maximum inhibitory effect of the extract was found toward *E. coli* .

The current study revealed a good antimicrobial effect of Cinnamon oil 1.5 % against *Staph. aureus* and *E. coli* as it extended the shelf life until the 15th day of the experiment ,thus it has a potential applicability as a natural preservative to improve food safety. These results match with those obtained by (39) who reported that adding cinnamon oil 1% to inoculated chicken minced meat samples with *Staph .aureus* revealed lower counts with reduction percent 23%,37% at 4th ,6th day of cold storage , respectively , however the same authors found that cinnamon oil exerted a potent antibacterial effect against *E. coli* with reduction percent 100% at the 5th day of the storage period . Additionally , (40) found that Cinnamon oil (1.5%) had various degrees of inhibition against the two bacterial strains as it reduced *E. coli* count with reduction percent reached 75% after 5 days while *Staph .aureus* growth was completely inhibited with reduction percent 100% after 2 days of cold storage.

Our results was partially different from those obtained by (41) who recorded reduction percent 96.78% and 91.23% for *Staph .aureus* and *E. coli*, respectively, on the 9th day of the experiment when using cinnamon oil 1%.

Concerning to the results of Cumin oil usage , it exhibits lower antibacterial effect , which were agree with (32) who declared that using of cumin oil 1 % showed inhibition of *Staph .aureus* growth with a reduction percent 14 .59 % ,10.69% after

72 hrs, 96 hrs , respectively, they said that Cumin oil showed the lowest antibacterial effect compared to other tested essential oils . These findings disagreed with those obtained by (42) who reported a significant inhibitory effect of cumin extract against *Staph aureus* and *E.coli* with reduction percent 99,97 % for both after 7days of the experiment.

5. Conclusion

The current study proved that *Moringa olifera* extract , Cinnamon and Cumin oils can be used as natural preservatives with remarkable antibacterial effect as well as accepted organoleptic properties . *Moringa olifera* extract showed the highest inhibitory effect on *Staph .aureus* and *E. coli* followed by cinnamon oil and finally cumin oil . Therefore, they may be useful to maintain the meat quality , extending its shelf life at cold storage , preventing economic loss and providing the consumer with healthy products

References:

- 1- Gong,H.; Yang Z. and Liu, M. et al.(2017): “Time-dependent categorization of volatile aroma compound formation in stewed Chinese spicy beef using electron nose profile coupled with thermal desorption GC-MS detection” *Food Science and Human Wellness* 6, (3): 137–146.
- 2- Al-Mutairi, M.(2011): The incidence of Enterobacteriaceae causing food poisoning in some meat products. *Advance J of Food Science and Technology* (3) :116-121.
- 3- Shahidi, F. and Zhong, Y. (2010) : Novel antioxidants in food quality preservation and health promotion.*Eur. J. Lipid Sci. Technol.* (112): 930–940.
- 4- Lucera, A.; Costa, C.; Conte, A. and Del Nobile, M.A. (2012): Food applications of natural antimicrobial compounds. *Front. Microbiol.*, (3), 287.
- 5- Falowo, A.B.; Muchenje, V.; Hugo, C.J. and Charimba, G.(2016): In vitro antimicrobial activities of *Bidens pilosa* and *Moringa oleifera* leaf extracts and their effects on ground beef quality during cold storage. *CyTA J. Food* (14): 541–546.
- 6- Olatunde, O.O. and Benjakul, S.(2018): Natural preservatives for extending the shelf-life of seafood: A revisit. *Compr. Rev. Food Sci. Food Saf.* (17): 1595–1612.
- 7- Spandana, U. and Srikanth, P. A (2016): Review on Meracle tree: *Moringa oleifera*. *J. Pharmacogn. Phytochem.*, 5, 189. Available online:
<https://www.phytojournal.com/archives/2016/vol5issue6/PartC/5-5-35-246.pdf> (accessed on 2 December 2022)
- 8- Gopalakrishnan, L.; Doriya ,K. and Kumar ,D.S. (2016): *Moringa oleifera*: a review on nutritive importance and its medicinal application. *Food Sci Hum Wellness.* (5):49–56.
- 9-Siddhuraju,P. and Becker, K.(2003): Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam). *J Agric Food Chem.*, (15):2144–55.
- 10– Bakar, A.; Uba, A. and Oyeyi ,T.I.(2010): Antimicrobial profile of *Moringa oleifera* Lam. extracts against some foodborne microorganisms. *Bayero J of Pure Appli Sci.* ,(3):43–8.
- 11– Jayawardana, B.C.; Liyanage, R.; Lalantha, N.; Iddamalgoda, S. and Weththasinghe, P.(2015): Antioxidant and antimicrobial activity of drumstick (*Moringa oleifera*) leaves in herbal chicken sausages *LWT – Food Sci Technol.*,(64):1204–8
- 12- Jham, G.N.; Dhingra, O.D.; Jardin, C.M. and Valente, M.M. (2005): Identification of the major fungi toxic component of cinnamon bark oil. *Fitopatol Bras.*, (30): 404-408.
- 13– Akhtar, S .; Khan ,M.I. and Faiz ,F .(2013) “ effect of thawing on frozen meat quality :A comprehensive review “ , *Pakistan Journal of food sciences* , vol .23 (4): 198 – 211 .
- 14- Bahraminejad, A.; Mohammed-Nejad, G. and Abdulkhadir, M.(2010): Genetic diversity evaluation of Cumin (*Cuminum Cyminum* L.) based on phenotypic characteristics. *Australian Journal of Corp science*, 5(3): 304-310.
- 15- Iacobelis, N.S., cantrop, L.O.; Capasso, F. and Senatore, F. (2005): Anyibacterial Activity of *Cuminum Cyminu,l* and *Carum Carvi* essential Oil S. *J. Agricul. and Food Chemi.*, (53): 57-61
- 16- Shah, M.A.; Bosco, S.J.D. and Mir, S.A.(2015): Effect of *Moringa oleifera* leaf extract on the physicochemical properties of modified atmosphere packaged raw beef. *Food Packag. Shelf Life* (3): 31–38. <https://doi.org/10.1016/j.fpsl.2014.10.001>
<https://doi.org/10.1016/j.fpsl.2014.10.001>
- 17- Barbosa, L.; Rall, V.; Fernades, A.; Ushimaru, P.; De Silva, I. and Fernandes, T. (2009): Essential oils against food borne pathogens

- and spoilage bacteria in minced meat. Foodborne Pathogens and Disease (6): 725-728.
- 18- Dorsa, W.J.(1997): New and established carcass decontamination procedures commonly used in the beef processing industry. J. Food Protec., (60): 1146- 1151.
 - 19-Fik, M. and Fik, A. (2007): Microbiological and sensory changes in minced beef treated with potassium lactate and sodium diacetate during refrigerated storage. J. Inter. Food properties (10): 589-598.
 - 20-Food and Drug Administration "FDA" (2001): *Staphylococcus aureus*. Bacteriological analytical manual .8th Ed. Chapter12. Academic Press, Gaithersburg, UK.
 - 21- Food and Drug Administration "FDA (2002):" Food borne illness, what consumer need to know. Food Safety and Inspection Service.
 - 22 – Greenhouse , S.W. and Geisser ,S (1959) . “ on methods in the analysis of profile data “. Psychometrika (24): 95 – 112.
 - 23-Chorlanopoulos ,N.G.; Evergetis ,E.T.; Aliogiannis ,N .; Mitakoa , S.; Nychas,G. and Haroutounian ,S .A. (2007): correlation between chemical composition of greek essential oils and their antibacterial activity against food borne pathogens . Natural product communications (2) :419 – 426 .
 - 24-Akthar , M.S; Degaga ,B. and Azam ,T.(2014) : Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms :A review . Biological Sciences and Pharmaceutical Research , 2 (1) :1-7 .
 - 25- Haq, M.; Dutta, P.L.; Sultana, N. and Rahman, A. (2013): Production and quality assessment of fish burger from the grass carp, *Ctenopharyngodonidella* (Cuvier and Valenciennes, 1844). Journal of Fisheries, (1): 42-47.
 - 26-. Sasse, A.; Colindres, P. and Brewer, M.S. (2009): Effect of natural and synthetic antioxidants on oxidative stability of cooked, frozen pork patties. J Food Sci (74): S30-S35
 - 27- Abdallah, R.; Mostafa, N.Y.; Kirrella, G.A.K.; Gaballah, I.; Imre, K.; Morar, A.; Herman, V.; Sallam, K.I. and Elshebawy, H.A.(2023): Antimicrobial Effect of Moringa oleifera Leaves Extract on Foodborne Pathogens in Ground Beef. Foods(12), 766. <https://doi.org/10.3390/foods12040766>
 - 28- Youssef ,E. A; Saad ,S.M; Hassan ,M. A and Ibrahim, H. M.(2021): “Assessment of the effects of selected plant extracts on quality indices and shelf life of raw chilled chicken meat” Benha Veterinary Medical Journal (40) :146-151
 - 29- Diao Eldin ,I.M.; Abou Sayed-Ahmed, E. T.; Hassan,H. M. ; Shaltout,F. A. and El-shorbagy ,G. A. (2023) : “ Influence of Plant Extracts on Acceptability of Chilled Poultry Meat “EC NUTRITION Research Article .
 - 30-Khomola, G. T; Ramatsetse, K. E; Ramashia ,S. E and Mashau ,M. E. (2021): The incorporation of Moringa oleifera leaves powder in mutton patties: Influence on nutritional value, technological quality, and sensory acceptability Open Agriculture (6): 738–748 <https://doi.org/10.1515/opag-2021-0043>
 - 31– Shaltout,F.A; Thabet,M.G and Koura,H.A (2017) : Impact of some essential oils on the quality aspect and shelf life of meat Benha Veterinary Medical Journal Vol 33,No (2):351-364.
 - 32– Mousa ,M. M.; Mohamed-Shawky Yousef , Ahmed,A.A and Dewedar ,R. S.(2021): “Essential Oils as Antimicrobial Agents in Minced Meat” AJVS. Vol.70 (1): 67-
 - 33- Guven,K.; Mutla, B.M.; Gulbandila, A. and Cakir, P.(2010):Occurrence and characterization of Staph.aureus isolated from meat and dairy products consumed in Turkey. J .Food Safety (30):196-212.
 - 34 – Hait ,J; Tallent ,S; Melka ,D; Keys ,C and Bennett ,R. (2012):“Staphylococcus aureus outbreak investigation of an Illinois bakery “ journal of food safety 32(4) :435-444.
 - 35-Hassan, Z.H.(2007): studies on food poisoning microorganisms in some meat products .M.V.Sc.Thesis (Meat hygiene), fac.vet.med. Menofia univ .,Sadat branch
 - 36- Bahi Eldin, R. M ; Talaat, D ; Elbaba ,A.H and Ibrahim ,M .S(2020): Antibacterial Activity of some plant extracts on different bacteria in chicken fillet. ejpmr, 7(1): 84-95.
 - 37–Adeyemi,K.D.; Ahmed El-Imam AM, Dosunmu,O.O. and Lawal, O. K.(2013): Effect of M.Oleifera marinade on microbial stability of smoke-dried african catfish (*clariasgariepinus*). Ethiopian Journal of Environmental Studies and Management, 6: 1. doi: <http://dx.doi.org/10.4314/ejesm.v6i1.12>
 - 38 -Elhadi, D.A.; Elgasim, E.A. and Mohamed Ahmed, I.A. (2017): Microbial and oxidation characteristics of refrigerated chicken patty incorporated with moringa (*Moringa oleifera*) leaf powder. CyTA J. Food (15): 234–240.

<https://doi.org/10.1080/19476337.2016.1242157>.

- 39–Hassanien,F.S; Shaltout,F.A; Hamouda, S.N and Arakeeb,S.M.(2019): Natural preservatives in row chicken meat .Benha Veterinary Medical Journal (37) :41 _45 .
- 40-Reham, A. A.(2013): Screening of antibacterial activity of cinnamonn, clove and rosemary essential oils against common food borne pathogens in minced beef ,Benha Veterinary Medical Journal, VOL. 25, NO.(2):151-164.
- 41– El gendy,H. ; Hasanine, F.; Salem ,A .M and Abo Elroos ,N. A.(2019) : “Antibacterial efficiency of both natural and chemical compounds in minced meat” .Benha Veterinary Medical Journal, VOL. 36, NO. (2) :138-149.
- 42– Saad,S.M; Ibrahim,H.M; Hassan,M.A and Shehab Eldin,S.N.(2020) :Antibacterial effect of Pepper and Cumin extracts on some pathogens contaminating chicken meat . Benha Veterinary Medical Journal(39) 130-134 .