

Antibacterial efficiency of both natural and chemical compounds in minced meat

Hemmat El gendy¹, Faten Hasanine¹, Amani Mohamed Salem¹, Nahla Ahmed Abo Elroos²

¹Department of Food Hygiene, Faculty of Veterinary Medicine, Benha University ²Animal Health Research Institute, Shibin El Kom Branch, Egypt

ABSTRACT

A total of 4200g fresh minced beef samples were divided into both large 2 groups, the first group (A) for E.coli and the second (B) for S.aureus then each group was divided into 7 equal subgroups (100g of each). Escherichia coli was inoculated into the first 7 subgroups with 4×106 cfu/g and S.aureus was inoculated into the second 7 subgroups with 3×106 cfu/g. The first (A) group was inoculated with cinnamon, garlic and rosemary at concentration of (1%) separately and the second (B) group was inoculated samples were stored at $4\pm1^{\circ}$ C in refrigerator till be used. The inoculated subgroups were examined at zero time, 3rd, 6th and 9thdays for bacterial count. The highest effect with 100% reduction of E.coli was in group (A) for rosemary and garlic oils at 9th days of storage. Also, the highest effect of chemical compounds on E.coli was achieved by nisin (100ppm) at 6th & 9th days of storage with reduction percentage was in group (B) artificially inoculated with S.aureus was for rosemary oil at 6th and 9th day of storage. Also, the highest effect of (100% reduction) of nisin on S.aureus was indicated at9th days of storage. Generally, essential oils proved to be efficient similar to chemical preservatives in addition to their safety for human consumption.

Keywords: Minced meat, E.coli, S. aureus, essential oils, chemical preservatives.

Received: 11 June 2019, Accepted: 28 August 2019 (<u>http://www.bvmj.bu.edu.eg</u>) (BVMJ-36(2): 138-149, 2019)

1. INTRODUCTION

Meat is a nutritious protein –rich food which is highly perishable and has a short-life unless preservation methods are used (Olaoye and Onilude, 2010). However, it gets easily contaminated by pathogenic microorganisms present in animal prior to slaughter. It is therefore important to make meat safe for consumers in terms of stability, transportation and storage.

Shelf life and maintenance of meat quality are influenced by a number of inter-related factors including holding temperature, which can result in determinable changes in the quality attributes of meat. Spoilage by microbial growth is the most important factor in relation to keeping quality of meat (Lambert et al., 1991).

As the food industry is facing great challenges to produce safe and at the same time food without synthetic chemical preservatives. So the essential oils make their way into the scientific focus, due to their antibacterial and antioxidant properties, they are used to prevent food borne diseases and extend shelf-life and to improve some meat characteristics (Akthar *et al.*, 2014).

Cinnamon can be used to obtain sweet flavoring and spicy characteristics, easily absorbed and do not have any adverse effects. important It also plays an role in pharmacological effects such as: carminative, stomachic, astringent, stimulant. antiantimicrobial, inflammatory. antioxidant. antispasmodic, antiulcer and treating the common cold (Mishra and Behak, 2010).

Garlic is one of the most commonly used ingredients as a flavor enhancement. Garlic has a wide spectrum of actions, not only antibacterial, antifungal and antiprotozoal, but also it has beneficial effects on the cardiovascular and immune systems (Harris *et al.*, 2001). During the last decade, the antimicrobial activity of garlic and garlic derived organosulfur compounds was widely investigated against both food spoilage bacteria and food borne pathogens (Leuschner and Ielsch, 2003).

Rosemary, the antioxidant activity of rosemary extracts has been primarily related to two phenolic diterpenes: carnosic acid and carnosol (Frankel et al., 1996). Several compounds found in this oil have been reported to be inhibitory to several microorganisms (Mangena and Muyima, 1999).

In Concern to meat products, good quality and improvements in equipment of production and hygiene were recommended to reduce contamination of meat products (Depoureq and Poucke, 1991). In addition to chemical preservatives which have been used as additional barriers to limit the number of microorganisms capable to grow in foods through inactivating or inhibiting the growth of undesirable microorganisms. Many methods of preservation are available to food processors including thermal processing, refrigeration, addition of chemical preservatives or a combination of these methods, but customer preferences have led researches to develop the use of natural inhibitors from plant, animal, and microbial sources (Shahidi *et al.*, 1991).

Nisin is the only one internationally accepted as a safe and natural food bio preservative in certain foods for several decades (Papagianni and Anastasiadou, 2009). Nisin is a broad spectrum bacteriocin with bactericidal activity, even in very low concentrations, towards a wide range of Gram –positive bacteria, including *S.aureus* (Parada *et al.*, 2007).

Sodium nitrite (50ppm) has a role in meat products by stabilizing the pink coloration of meat and enhances the meat product flavor (Eleiwa- Nesreen, 2003). Nitrite is considered as an essential curing ingredient responsible for fixing the characteristic color associated with cured meats, creating a unique flavor profile that is distinguishable from products not containing nitrite, providing control of the oxidation of lipids, and serving as an effective antimicrobial by itself or synergistically with other ingredients (Pegg, 2004).

Potassium sorbate is used to improve microbial quality of the meat and increase its shelf –life. It did not cause and adverse effect on the quality characteristics of the meat (Hussein et al., 2012).

It is known that the effectiveness of preservatives depend not only on their concentration and type, but also on the species of microorganism on which they act. Experiment results of preservatives combinations exhibited synergistic effects (Stanojevic *et al.*, 2009).

Control of food poisoning bacteria remain an important objective for sectors of food production industry, Therefore, the main goal of the present work was carried out to evaluate the efficacy of some essential oils and chemical preservatives against two serious pathogens (*E. coli* and *S.aureus*) in minced beef stored at $4\pm1^{\circ}$ C.

2. Materials and methods

2.1. Bacterial strains:

Escherichia coli and S.aureus strain were obtained from Media Unite, Food Hygiene Department, Animal Health Research Institute, Dokki, Giza, Egypt, with recommended dose 104cfu/ml for E.coli and 106cfu/ml for S.aureus.

2.2. Natural oil :

Cinnamon oil (1%), garlic oil (1%) and rosemary oil (1%), were purchased from El Captain Company (CAP PHARM), Al-Obour City- Cairo-Egypt.

2.3. Chemical preservatives :

- Nisine 10ppm
- Sodium Nitrite 100ppm
- Potassium Sorbate 0.2%
- 2.4. Experimental application:

Accurately, 4200 g of the fresh minced beef used in this study was purchased from butcher shops in El Menofia Governorate. The minced beef samples were immediately prepared and divided into two main groups (2100 g of each) and then sterilized in Gamma irradiation units, The Egyptian Atomic Energy Authority (EAEA), Naser city, Cairo. Egypt as recorded by (Huq et al., 2013).Each group divided into equal7 subgroups (3×100g of each). The first 7subgroups were inoculated with E.coli (4×106 cfu/g) and the other 7 subgroup were inoculated with S.aureus (3×106cfu/g).

1st Group:

Control +ve: inoculated with *E.coli* strain only. A: Treated with garlic oil1%+*E.coli* strain.

B: Treated with cinnamon oil 1%+*E.coli* strain.

C: Treated with rosemary oil1%+*E.coli* strain.

D: Treated with nisin 100ppm+*E.coli* strain.

E: Treated with sodium nitrite 100ppm+*E.coli* strain.

F: Treated with potassium sorbate 0.2%+*E.coli* strain.

2 nd Group:

Control +ve: inoculated with *S.aureus strain* only.

A: Treated with garlic oil1%+*S.aureus strain*.

B: Treated with cinnamon oil 1%+ *S.aureus strain*.

C: Treated with rosemary oil1%+ S.aureus strain.

D: Treated with nisin 100ppm+ S.aureus strain.

E: Treated with sodium nitrite 100ppm+ *S.aureus strain.*

F: Treated with potassium sorbate 0.2%+ *S.aureus strain.*

The inoculated samples were stored at $(4\pm1^{\circ}C)$ till be used .The inoculated subgroups were examined at 3^{rd} , 6th and 9^{th} days of inoculation for:

Sensory examination: The color, odor and overall acceptability were determined for each sample of meat according to (Fik and Fik, 2007).

E.coli and *S.aureus* counts according to (FDA, 2002).

The experiment was performed in triplicate.

2.5. *Reduction percentage*:

 $Reduction = \frac{Initial \ load \ - \ new \ count}{Initial \ load}$

2.4. Statistical Analysis:

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Rosner (2002).

3. RESULTS

Table (1& 2) revealed that sensory analysis of control (untreated sub groups) remain accepted until 2nd days while treated subgroups with Cinnamon 1% & potassium sorbate (0.2%) remain accepted until 6th days of storage. Garlic oil 1% &sodium nitrite (100ppm) remain accepted until 6th and9th days of storage. Moreover, rosemary oil (1%) and nisin (100ppm) showed good grade at 6thday and acceptable at 9th days.

From the obtained results there was decline of sensory attributes begin after the first day of storage, with marked reduction of odor in case of oils mainly in garlic oil (pungent odor)

Table (3) illustrated the antimicrobial effects and reduction percentage of cinnamon, garlic and rosemary oils at concentration (1%) on counts of E.coli artificially inoculated into minced beef. Cinnamon oil (1%) decreased count of *E.coli* from 4×10^6 (initial load)to $1.68 x 10^6 \pm 0.4 x 10^6 \text{ cfu/g}, \ 6.29 x 10^5 \pm \ 1.1 x 10^5$ cfu/g and $3.51 \times 10^5 \pm 0.7 \times 10^5$ cfu/g with reduction percentage 58%,84. 27% & 91.23% on 3rd,6th and 9th days of storage, respectively. Garlic oil (1%) decreased counts of E.coli to $7.12 \times 10^5 \pm 1.5 \times 10^5$, $4.53 \times 10^4 \pm 0.8 \times 10^4$ cfu/g at 3rd and 6th days of storage with reduction % 85.21% and 98.87% and complete reduction (100%) of E.coli count was attained at 9th days. Rosemary oil (1%) decreased count of E.coli at 3^{rd} and 6^{th} days of storage to $9.84 \times 10^3 \pm 2.0 \times 10^4$ cfu/g and $1.6x10^3 \pm 0.2x10^2$ with reduction percentage 99.75% & 99.9% and not detected count of E.coli at 9th days of storage with reduction percentage 100%.

Table (4) declared the effects and reduction percentage of potassium sorbate (0.2%), sodium nitrite (100ppm) and nisin (100ppm) on counts of E.coli artificially inoculated into minced beef. Potassium sorbate (0.2%) decreased count of *E.coli* from 4×10^6 (initial load) to 2.15×10^{6} $\pm 0.3 \times 10^{6}$ $cfu/g, 1.07x10^{6} \pm 0.1x10^{6} cfu/g$ and $5.93x10^{5} \pm$ 0.9×10^5 cfu/g with reduction percentage 46.25%, 73.25% and 85.17% on 3rd,6th and 9th days of storage, respectively. Sodium nitrite (100ppm) decreased counts of E.coli to $1.30 \times 10^6 \pm 0.2 \times 10^6$, $3.65 \times 10^5 \pm 0.5 \times 10^5$ and $9.47 \times 10^3 \pm 2.1 \times 10^5$ cfu/g at 3rd, 6th and 9th days of storage with reduction % 67.50%, 99.76%, respectively. Nisin 90.88% and (100ppm) decreased count of E.coli to $5.19 \times 10^5 \pm 1.0 \times 10^5$. $8.33 \times 10^3 \pm 1.6 \times 10^3$ &

 $9.45 \times 10^2 \pm 1.9 \times 10^2$ cfu/g with reduction percentage 87.03%, 99.79% & 99.995 at 3rd,6th and 9th days of storage, respectively.

Table (5) illustrated the antimicrobial effects and reduction percentage of cinnamon, garlic and rosemary oils at concentration (1%) on counts of S.aureus artificially inoculated into minced beef. Cinnamon oil (1%) decreased count of S.aureus from 3×10⁶(initial load) to $1.02 \times 10^6 \pm 0.1 \times 10^6 \text{cfu/g}, 3.41 \times 10^5 \pm$ 0.6×10^{5} cfu/g and $9.67 \times 10^{4} \pm 1.8 \times 10^{5}$ cfu/g with reduction percentage 66%, 88.63% & 96.78% on 3rd,6th and 9th days of storage, respectively. Garlic oil (1%) decreased counts of S.aureus to $4.29 \times 10^5 \pm 0.8 \times 10^5$ and $1.73 \times 10^4 \pm 0.4 \times 10^4$ cfu/g at 3rd and 6th days of storage with reduction % 85.21% and 99.425 and not detected S.aureus count at 9th days with R% 100%. Rosemary oil (1%) decreased count of S.aureus at 3^{rd} day of storage to $2.50 \times 10^4 \pm$ 0.3×10^4 cfu/g with reduction percentage 99.17% and not detected count of S.aureus at 6th and 9th days of storage with reduction percentage 100%.

Table (6) pointed out the antimicrobial effects and reduction percentage of potassium sorbate (0.2%), sodium nitrite (100ppm) and nisin (100ppm) on counts of S.aureus artificially inoculated into minced beef. Potassium sorbate (0.2%) decreased count of S.aureus from 3×10^6 (initial load) to $1.46 \times 10^{6} \pm 0.3 \times 10^{6}$ cfu/g, $8.36 \times 10^{5} \pm 1.4 \times 10^{5}$ cfu/g and $1.58 \times 10^5 \pm 0.3 \times 10^5$ cfu/g with reduction percentage 51.33%, 72.13% and 94.73% on 3rd, 6th and 9th days of storage, respectively. Sodium nitrite(100ppm) decreased counts of S.aureus to $7.70 \times 10^5 \pm$ 2.1×10^5 , $1.61 \times 10^5 \pm 0.3 \times 10^5$ and $5.24 \times 10^3 \pm$ 1.4×10^5 cfu/g at 3rd, 6th and 9th days of storage with reduction % 74.33%,94.63% and 99.82%, respectively. Nisin (100ppm) decreased count aureus to $1.04 \times 10^5 \pm 0.1 \times 10^5$, of S. $1.10 \times 10^4 \pm 0.2 \times 10^3$ reduction cfu/g with

percentage 96.53%, 99.63% &100% at 3^{rd} , 6^{th} and 9^{th} days of storage ,respectively.

Table1: Changes in sensory traits of control and essential oil (1%) treated minced meat stored at	
4±1 °C (n=5).	

Trait Storage time	Color (5)	odor (5)	Appearance (5)	Consistenc y (5)	Overall (5)	Grade
1. <u>Control:</u>						
Zero time	5	5	5	5	5	Very good
3 days	2.2	2.0	2.6	2.4	2.3	Unacceptable
6 days	1.0	1.2	1.0	1.0	1.1	Bad
9 days	S	S	S	S	S	Spoiled
2. Cinnamon oil:						
Zero time	5	5	5	5	5	Very good
3 days	4.0	3.6	4.2	3.8	3.9	Good
6 days	3.4	2.6	3.2	3.0	3.1	Acceptable
9 days	2.6	2.2	2.0	2.4	2.3	Unacceptable
3. Garlic oil:						
Zero time	5	5	5	5	5	Very good
3 days	4.2	3.8	4.0	4.2	4.1	Good
6 days	3.6	3.0	3.4	3.6	3.4	Acceptable
9 days	3.2	2.6	3.0	3.2	3.0	Acceptable
4. Rosmary oil:						
Zero time	5	5	5	5	5	Very good
3 days	4.4	4.2	4.0	4.4	4.3	Good
6 days	4.0	3.6	3.8	4.2	4.0	Good
9 days	3.2	3.0	3.4	3.0	3.2	Acceptable
Very good	4:	Good		3: Acceptab	le	
Unacceptable	1:	Bad		S: Spoiled		

Trait Storage time	Color (5)	odor (5)	Appearanc e (5)	Consistenc y (5)	Overal l (5)	Grade
1. <u>Control:</u>						
Zero time	5	5	5	5	5	Very good
3 days	2.2	2.0	2.6	2.4	2.3	Unacceptabl
6 days	1.0	1.2	1.0	1.0	1.1	Bad
9 days	S	S	S	S	S	Spoiled
2. Pot. Sorbate (0.2%):						
Zero time	5	5	5	5	5	Very good
3 days	4.2	3.6	4.4	3.8	4.0	Good
6 days	3.4	2.8	3.6	3.4	3.3	Acceptable
9 days	2.8	2.0	2.2	2.6	2.4	Unacceptabl
3. <u>Sod. Nitrite (100</u>						
<u>ppm):</u> Zero time	5	5	5	5	5	Very good
3 days	4.4	4.0	4.2	4.4	4.3	Good
6 days	3.6	3.2	3.4	3.8	3.5	Acceptable
9 days	3.2	2.8	3.0	3.4	3.1	Acceptable
4. <u>Nisin (100 ppm):</u>						
Zero time	5	5	5	5	5	Very good
3 days	4.4	4.2	4.2	4.6	4.4	Good
6 days	4.2	4.0	4.0	4.2	4.1	Good
9 days	3.2	3.4	3.2	3.4	3.3	Acceptable
Very good	4: Goo	od	3	: Acceptable		
. 1 1	1 D 1			- L C 1 - 1		

Table 2: Changes in sensory traits of control and chemical preservatives treated minced meat stored
at 4±1°C (n=5).

2: Unacceptable

1: Bad

S: Spoiled

Hemmat et al. (2019). BVMJ-36(2): 138-149

Table 3: The effects and Reduction % of different essential oils on *E.coli* counts (cfu/g) inoculated into minced meat samples (n=5).

Treatment	Control		Cinnamon 1%		Garlic oil 1%		Rosemary oil 1%	
Storage time	Count	R%	Count	R%	Count	R %	Count	R%
Zero time 4	$1.0 \times 10^{6} \pm 0.1 \times 10^{6}$	06 -	$4.0x10^{6} \pm 0.1x1$	06 -	$4.0x10^{6} \pm 0.1x10^{6}$) ⁶ -	$4.0 \times 10^6 \pm 0.1$	1x10 ⁶ ·
3 rd days 3.8	7x10 ⁶ ±0.2x10 ⁶	5 3.25	1.68x10 ⁶ ±0.4x1	10 ⁶ 58	$7.12 \times 10^5 \pm 1.5 \times 10^5$	10 ⁵ 82.21	$9.84 \times 10^3 \pm 2.0 \times 10^3$	99.75 ⁴
6 th day 3.69	$9x10^{6} \pm 0.3x10^{6}$	7.75	$6.29 \times 10^5 \pm 1.1 \times 10^5$	10 ⁵ 84	.27 4.53x10 ⁴ ±0	.8x10 ⁴ 98.87	$1.6 x 10^3 \pm 0.2 x$	10 ² 99.9
9 th days 3.5	$2x10^{6} \pm 0.1x10^{6}$	5 12	$3.51 \times 10^5 \pm 0.7 \times 10^5$	×10 ⁵ 91	1.23 ND	100	ND	100
*=Reduction	- %				ND=Not Dete	cted		

Table 4: The effects and Reduction % of some chemical preservatives on *E.coli* counts (cfu/g) inoculated into minced meat samples (n=5).

Treatment	Control	0.29	6 Potassium	sorbate	100ppm So	dium nitrite	-	100 ppm nisin	
Storage time	Count R%)	Count	R%	Co	ount	R%	Count	R%
Zero time 4.0	$0x10^{6} \pm 0.1x10^{6}$	⁶ -	$4.0 \text{ x} 10^6 \pm 0.$	1x10 ⁶ -	4.0x10 ⁶	$\pm 0.1 x 10^{6}$	- 4	$4.0 \times 10^6 \pm 0.1 \times 10^6$	-
3 rd days 3.87	$x10^{6} \pm 0.2 x10^{6}$	3.25	$2.15 \times 10^{6} \pm 0$.3x10 ⁶ 46	5.25 1.30x10	$0^{6} \pm 0.2 \times 10^{6}$	67.50	$5.19 x 10^5 \pm 1.0 x 10^5$	87.03
6 th days 369	$9x10^{6} \pm 0.3x10^{6}$	⁶ 7.75	1.07x10 ⁶ ±0	0.1x10 ⁶ 73	3.25 3.65x1	$0^{5} \pm 0.5 \times 10^{5}$	90.88	$8.33 x 10^3 \pm 1.6 x 10^3$	99.79
9 th days 3.52	x10 ⁶ ±0.1x10 ⁶	12	5.93x10 ⁵ ± ().9x10 ⁵ 8	5.17 9.47x1	0 ³ ±2.1x10 ⁵	99.76	$9.45 \times 10^2 \pm 1.9 \times 10^2$	99.99

Table 5: The effects and Reduction % of different essential oils on *S. aureus* counts (cfu/g) inoculated into minced meat samples (n=5).

Treatment	Control	Cinna	mon oil 1%	Garlic o	il 1%	Rosemar	y oil 1%	
Storage time C	count	R% Count	R%	Count	R%	Count	R%	
Zero time 3.0x	$10^{6} \pm 0.1 \times 10^{6}$	- 3.0x10 ⁶ ±	0.1x10 ⁶ - 3	3.0x10 ⁶ ±0.1x1	06 -	$3.0x10^6 \pm 0.1x$	- 106	
3 rd days 2.75x1	$0^{6} \pm 0.3 x 10^{6}$ 6	$.25 \ 1.02 \mathrm{x10^{6} \pm 0^{6}}$	0.1x10 ⁶ 66 4.29	9x10 ⁵ ± 0.8x10	5 85.21	$2.50 \times 10^4 \pm 0.3 \times 10^4$	10 ⁴ 99.17	
6 th days 2.49x1	$0^6 \pm 0.2 \times 10^6$ 12	$2.75 \ 3.41 \mathrm{x10^5} \pm$	0.6x10 ⁵ 88.63	1.73x10 ⁴ ±0.4	x10 ⁴ 99.4	2 ND	100	
9 th days 2.38x10	$0^6 \pm 0.2 \times 10^6$ 1.	5.50 9.67x10 ⁴	±1.8x10 ⁵ 96.78	ND	100	ND	100	

Table 6: The effects and Reduction % of chemical preservatives on *S. aureus* counts inoculated into minced meat samples (n=5).

Treatment	Control	0.2	% Potassium	sorbate	100ppm Sodiur	n nitrite	100ppm Nisi	n
Storage time	Count H	R %	Count	R%	Count	R%	Count	R%
Zero time	$30x10^{6} \pm 0.1x$	×10 ⁶ -	$3.0 \times 10^{6} \pm 0$).1x10 ⁶ -	3.0x10 ⁶ ±	0.1x10 ⁶	- 3.0x10	$6 \pm 0.1 \times 10^{6}$ -
3 days 2.75x	10 ⁶ ±0.3x10 ⁶	6.25	$1.46 \times 10^6 \pm 0.3$	3x10 ⁶ 51.33	$7.70 \times 10^5 \pm 2.1$	x10 ⁵ 74.33	$1.04 x 10^5 \pm 0$.1x10 ⁵ 96.53
6 days 2.49x	$10^{6} \pm 0.2 \times 10^{6}$	12.75	$8.36 \times 10^5 \pm 1.4$	x10 ⁵ 72.13	$1.61 \times 10^5 \pm 0.$	3x10 ⁵ 94.63	$1.10 \times 10^4 \pm 0$	2x10 ³ 99.63
9 days 2.38x1	10 ⁶ ±0.2x10 ⁶ 1	5.50 1.5	58x10 ⁵ ± 0.2x1	0 ⁵ 94.73 5	$5.24 \text{x} 10^3 \pm 1.4 \text{x}^3$	0 ⁵ 99.82	ND 1	.00

4. DISCUSSION

Minced meat is a highly perishable product due to its biological composition and the shelf life of refrigerated minced meat is limited by the growth and biochemical activities of aerobic bacteria. Minced beef can be easily contaminated with microorganisms and ,if not properly handled and preserved, support growth of pathogenic bacteria, leading to loss of quality and potential public health problems (Vernozy-Rozand et al. 2002).

4.1. Antibacterial activity of cinnamon, garlic and rosemary essential oils at concentration 1% against E.coli and S.aureus artificially inoculated in minced beef by intensity of $4x10^{6}$ /g during cold storage at $(4\pm1^{\circ}C)$

The presence of *E.coli* in food is considered as indicator of faults during preparation, handling or storage. It is also, considered as indicator of fecal contamination, beside. It may induce severe diarrhea in infants and young children, as well as food poisoning and gastroenteritis among the adults (Hassan, 2007).

Staphylococcus aureus is a leading cause of food poisoning resulting from the consumption of contaminated food with staphylococcal enterotoxins. Different foods act as a good medium for *S. aureus* such meat products (Guven et al. 2010). The Presence of *S.aureus* in meat products may be attributed to direct contact with workers with hand or arm lesions caused by *S.aureus*, or by coughing and sneezing, which is common during respiration infections. Food handlers are frequently the source of food contamination on staphylococcal outbreaks (Jennifer Hait, 2012).

Essential oils can act against resistance mechanisms in bacteria and tumor cells via plasmid curing and inhibition of efflux act indirectly processes and may by stimulating the defense mechanisms and the immune response (Trajano et al. 2010). Certain components of essential oils can act as uncouplers, which interfere with proton translocation over a membrane vesicle and subsequently interrupt ADP phosphorylation (Parasa et al. 2012). They can also interfere with membrane- integrated or associated enzyme-proteins, stopping their production or activity. Essential oils are also able to inhibit the synthesis of DNA, RNA, proteins and polysaccharides bacterial in cells (Chorianopoulos et al. 2007 and Akthar et al. 2014).

Table (3 &5) indicated that the studied essential oils possess a significant antibacterial activity against *E. coli* and *S. aureus*, in the following order: Rosemary> garlic> cinnamon. These essential oils may be selected for use as potential antibacterial efficient and extend shelf life of minced meat. These findings were similar with those of Pranto et al., (2005), Barbosa et al. (2009), Babu et al. (2011) and Jolly and Menon (2015). The better effectiveness of essential oils against Gram- positive bacteria (*S. aureus*) than Gram- negative bacteria (*E. coli*) may be due to volatile action of essential oils and due to absence of lipo-polysaccharide layer in Gram-positive bacteria that might function as an effective barrier against any incoming biomolecule (Delaquis et al, 2002).

In general, α -Pinene is as major component of rosemary EO, followed by 1, 8cineole, β -myrcene, camphor and borneole. It was determined that Rosemary essential oil exhibits antimicrobial activity by passing through the cell wall and cytoplasm membranes and disrupting their structures as a typical lipophilic substance. Since damaged cell membrane increases its permeability, small molecular weight molecules such as phosphates and potassium ions first leach out from the cell, followed by larger molecules, such as DNA and RNA. 1.8-cineole exhibits a strong toxic effect on eukaryotic cells (Stojanovic-Radic et al. 2010).

These results may be attributed to the antibacterial effect of garlic apparently which results from interaction of sulfur compounds, like allicin, with sulfur (thiol)groups of microbial enzymes (such as trypsin and other proteases), leading to an inhibition of microbial growth (Jolly and Menon, 2015).

Cinnamon EO has gained greater acceptance among food technologists due to better sensory evaluation their and antimicrobial properties (Fischer and Phillips, 2006). The major component of cinnamon, cinnamaldehyde, possesses antimicrobial effects on microorganisms, as it inhibited cell wall biosynthesis, membrane function, and specific enzyme activities. More specific cellular targets of cinnamaldehyde are still required to be studied in detail (Sheraz et al. 2016).

4.2. Antibacterial activity of potassium sorbate (0.2%), Sodium nitrite(100ppm) and Nisin (100ppm) chemical preservatives against E.

coli and S. aureus artificially inoculated into minced beef by intensity of $4x10^6/g$ for E. coli and $3x10^6$ for S. aureus during cold storage at $(4\pm1^\circ C)$

Food preservation is designed to enhance or protect food safety while maintaining or improving product quality by inactivating or inhibiting the growth of undesirable microorganisms (Ray, 1992).

Table (4&6) revealed that nisin (100ppm) and sodium nitrite (100 ppm) had a higher antibacterial effect than that obtained by (0.2% potassium sorbate).

Also, *S. aureus* more sensitive to preservatives than *E.coli*, this results of the present study agreed with those obtained by Abdel-Shakour et al. (2014) who found that *S.aureus* as G +ve bacteria showed high sensitivity to sodium nitrite higher than other G -ve strains and found also when nisin was added as food preservative no growth observed for Gm + ve bacteria as *S. aureus*. This also agreed with Abou Hussein- Reham, (2012) who found that the growth of *S. aureus* in minced meat samples was completely inhibited after being treated with nisin.

Potassium sorbate inhibits certain amino acids uptake by *E.coli* interfering with its growth .However, the action of potassium sorbate is greatly dependant on PH of the food article where the low PH (1-4) had an apparent synergistic inhibition action caused by sorbate on the amino acid uptake by *E.coli* (Eklude,2003).

The resistance of *E. coli* as Gram-negative bacteria against nisin depends on the type of lipopolysaccharide of the cell membrane. Consequently, addition of an agent to change the nature of the outer membrane of *E.coli* is very necessary to render the organism sensitive to nisin (Ganzle et al. 1999).

From these results illustrated in tables (4 & 6), one can concluded that the use of chemical preservatives in certain concentrations have a benefit results in inhibiting such foodborne microorganisms that affect human health and causing public health hazards . It was concluded that the reduction percentage in *S. aureus* and *E. coli* counts artificially inoculated into minced beef samples increased by increasing the period of preservation.

Therefore, the effectiveness of preservatives depends not only on their concentration and type, but also on the species of microorganism on which they act (Stanojevic et al., 2009).

Finally, cinnamon, garlic, rosemary oils (1%), potassium sorbate (0.2%), sodium nitrite (100ppm) and nisin (100ppm) can be used meat preservatives with antibacterial activities against food borne pathogens specially rosemary oil (1%), garlic oil (1%), nisin (100ppm) and sodium nitrite (100ppm) and therefore may be useful in maintaining the meat quality, extending shelf- life of meat products, preventing economic loss.

5. Conclusion

Rosemary oil (1%) showed higher inhibitory effect on *E.coli* and *S.aureus* than that obtained by cinnamon and garlic oil (1%). Nisin (100ppm) showed higher inhibitory effect on *E.coli* and *S.aureus* than that obtained by potassium sorbate (0.2%) and sodium nitrite (100ppm). *Staphylococcus aureus* were more sensitive for essential oils and chemical preservatives than *E.coli*.

6. REFERENCES

- Abdel-Shakour, E.H., Elouboudy, S.S.,
 Abdelaziz, Z.K., Hassan, M.A. and
 Emara, M. B. (2014): Application of
 Bacteriocin as Bio-preservative in
 Foods" Adv. J. N Y Sci., 7(6):87-93.
- Abo Hussien Reham, A. (2012): Detection of food mediated pathogens in some meat and chicken products by using recent techniques. ph. D. Thesis, Fac. of Vet. Med. Benha University.

- Akthar, M.S, Degaga, B., 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.
- Babu, A., Sundari, A., Indumathi, J., Srujan,
 R.V.and Sravanthi, M. (20110): Study on the Antimicrobial activity and Minimum Inhibitory Concentration of Essential Oils of Spices. Veterinary World, 4 (7):311-316.
- Barbosa, L.N., Rall, V.L., Fernades, A., Ushimaru, P., Da Silva, I. and Fernades, A. (2009): Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne pathogens and Disease, 6:725-728.
- Chorianopoulos, N.G., Evergetis, E.T., Aligiannis, N., Mitakou, S., Nychas, G. Haroutounian. and S.A. (2007): Correlation between chemical composition of Greek essential oils and their antibacterial activity against foodborne pathogens. Natural Product Communications, 2:419-426.
- Delaquis, P.J., Stanich, K., Girard, B.and Mazza, G. (2002): Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int. J. Food Microbiol., 74:101-109.
- Depoureq, G. and Poucke, L.V. (1991): "Evaluation of the microbial quality of minced meat ". P. 200 Food Policy Trends in Europe. Nutrition Technology, Analysis and Safety. 7476:75-79.
- Eklude, T. (2003): The antimicrobial effect of dissociated and undissociated sorbic acid at different PH levels.J.Appl.Bacteriol; 72 (5): 249-255.

- Eleiwa- Nesreen, Z.H. (2003): "Effect of chemical preservatives on food poisoning bacteria in some locally manufactured meat products". Ph.D. Thesis (Meat Hygiene), Fac. Vet. Med., Zagazig Univ. (Benha branch), Egypt.
- Fik, M. and Fik, A. (2007): Microbiological and sensory changes in minced beef treated with potassium lactate and sodium diacetate during refrigerated storage .Inter.J.Food Properities, 10: 589-598.
- Fisher, K. and Phillips, C.A.(2006):The effect of lemon,orange and bergamot essential oils and their components on the survival of *Campylobacter jejuni*, *Escherichia coliO157;H7*, *Listeria Monocytogenes*, *Bacillus cereus* and *Staphyococcus aureus* in vitro and in foodsystems.J.Appl.Microbiol.101(6):1 232-1240.
- Food and Drug Adminstration "FDA (2002):" Food borne illness, what consumer need to know. Food Safety and Inspection Service.
- Frankel, E., Huang, S.and Aeschbach, R. (1996): Prior E. Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol and rosmarinic acid, in bulk oil and oil-in-water emulsion. J. Agric Food Chem 44:131-135.
- Ganzle, M.G., Herd, C. and Hammes, W.P. (1999): "Resistance of *Escherichia coli* and *Salmonella* against nisin and curvacin A". Int.J.Food Microbiol., 48(1):37-50.
- 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.

- Huq,M., Dutta,P.L., Suatan, N. and Rahman,A. (2013): Production and quality assessmentof fish burger from the grass crap, ctenopharyngodon idella (Cuvier and Valenciennes,1844),Journal of Fisheries,1(1):42-47.
- Harris, J.C., Cottrell, S.L., Plummer,S. L. and LIoyd, D.(2001): Antimicrobial properties of garlic. J. Appl. Microbiol and Biotechnol.57:282-286.
- 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.
- Hussein, A.M., El-Ghareeb, W.R., Lotfy,O.O. (2012): Shelf life improvement of camel meat treated with potassium sorbate0.3%. Journel of American Science, 8(4):507-511.
- Jolly, D. and Menon, V. (2015): Antibacterial effect of garlic and ginger extracts on *Escherichia coli and Listeria monocytogenes*. International Journal of Applied and Pure Science and Agriculture (IJAPSA), 1(2): 111-118.
- Lambert, A.D., Smith, J.P., Dodds, K.L. (1991): Shelf life extension and microbiological safety of fresh meat. Food Microbiol.9:267-297.
- Leuschner, R. G. K. and Ieisch, V. (2003): Antimicrobial effects of garlic, clore and red hot chilli on *Listeria monocytogenes* on broth model systems and soft cheese. Int. J. of Food Sci. and Nut., 54:127-133.
- Mangena, T. and, Muyima, N.Y. (1999): Comparative evaluation of the

antimicrobial activities of essential oils of Artemisia afra, Pteronia incana and Rosmarinus. Officinalis on selected bacteria and yeast strains. Lett Appl. Microbiol. 28:291-296.

- Mishra,N. and Behak, K.K.(2010): Antimicrobial activity of some spices against selected microbes. Int. J. Pharmacy and Pharmaceutical Sci., 2(3):187-196.
- Olaoye, O.A. and Ntuen, I.G. (2011): Spoilage and preservation of meat a general appraisal and potential of lactic acid bacteria as biological preservatives. Review Paper. Int. Res. J. Biotechnol., 2:33-46.
- Papagianni, M. and Anastasiadou, S. (2009): Pediocins: The bacteriocins of pediococci. Sources, production, properties and applications. Review. Microbial Cell Fractories, 8:1-16.
- Parada, J.L., Caron, C.R., Medeiros, A.B.P and Soccol, C.R. (2007): Bacteriocins from lactic acid bacteria purification, properties and use as bio preservatives. Int. J. Brazilian Archives of Biology and Technol., 50:521-542.
- Parasa, L.S., Tumati, S.R., Prasad, C. and Kumar, L.C.A. (2012) In vitro antibacterial activity of culinary spices aniseed, star anise and cinnamon against bacterial pathogens of fish. Int. J. Pharmacy and Pharmaceutical Sciences, 4(3):667-670
- Pegg, R.B. (2004): "Curing". In Encyclopedia of Meat Sciences. W. K. Jensen, C. Devine, and M. Dikeman, ed. Elsevier Ltd., Oxford, UK.
- Pranoto,Y., Salokhe, V.M. and Rakshit, S.K.(2005): Physical and antibacterial properties of aliginate based edible film

incorporated with garlic oil. Food Research International, 38(3):267-272.

- Ray, B. (1992): "Nisin of Lactococcus lactis sub sp. Lactis as a food biopreservative".In: Food Biopreservatives of microbial origin. CRC Press, Boca Raton, Fla.PP:207-264.
- Rosner, B. (2002): Fundamental of Biostatistics. 4th Ed. (ed. P. W. Kent), Publishing Co. California, USA.
- Shahidi, F. J., Arachchi, K.V. and Jeon, Y. (1991-1999): "Food applications of chitin and chitosans". Trends Food Sci. Technol., 10:37-51.
- Stanojevic, D., Comic, L., Stefanovic, O. and Solujic-Sukdolak, S. I. (2009):
 "Antimicrobial effects of sodium benzoate, sodium nitrite and potassium sorbate and their synergestic action in vitro". Bulgarian Journal of Agricultural Science, 15(4):307-311.
- Stojanovic-Radic, Z., Nesic, M., Comic, L. and Niko Radulovic, N. (2010): Antimicrobial activity and cytotoxicity of commercial rosemary essential oil (*Rosmarinus officinalis L.*) Biological Nyssana, 1(1-2):83-88.
- Trajano, V., Lima, E., Travassos, A. and, Souza, E.(2010): Inhibitory effect of the essential oil from Cinnamomum zeylanicum Blume leaves on some food- related bacteria. Cienc. Tecnol. Aliment., 30 (3):771-775.
- Vernozy-Rozand, C., Ray-Gueniot, S., Ragot, C., Bavai, C., Mazuy, C., Montet, M., Bouvet, J. and Richard, Y. (2002): "Prevalence of *Escherichia coli 0157;H7* in industrial minced beef".Lett.Appl.Microbiol.,35:7-11.