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Bacteriological evaluation of minced meat with special reference to effect of thyme essential oil on it

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A B S T R A C T

Meat products are ideal medium for bacteria because of high moisture content, richness in nitrogenous compounds. Twenty five random samples of minced meat were collected from different supermarkets in, Menofia governorate. All collected samples were subjected to bacteriological examination for aerobic plate count, coliform Enterobacteriacea, Staphylococci count and detection of,*staph .aureus* organisms. Also twelve samples of minced beef were collected from different butcher shops in Menofia governorate were divided into untreated (control) and treated samples which homogenized with thyme oils in 0.5%, 1% and 1.5% concentrations. Each sample was analyzed bacteriological promptly during cold storage until spoilage for studying the antimicrobial efficiency of Thyme oil in minced beef.

Mean value of Aerobic Plate Count, coliforms Enterobacteriaceae and Staphylococci count of the examined minced beef were $6.52x\ 10^4 \pm 0.27x\ 10^4$, $7.8x10^2 \pm 0.15x10^2$, $5.82\times10^2 \pm 1.02\times10^2$ and $6.15\times10^2 \pm 1.36\times10^2$ respectively.

Seven strains of Coagulase + ve *S.aureus* , and *Staphylococcus epidermidis,* were isolated while 2 strains of *Staphylococcus saprophyticus* , *Staphylococcus intermedius Staphylococcus capitis* and *Micrococcispp* .were isolated from examinad samples.

In case of using thyme oil at the concentrations 0.5%, 1% and 1.5% the scores of sensory evaluation were 9,9 , 8, 6 ,5& 9 ,9, 8, 7,6 & 9,9,8,8 , 7after zero day, 1st day, 2nd day ,3rd day and 4th day of the cold storage period respectively, comparing to the scores of sensory evaluation in the control samples which were 9, 6,5,4 ,2 after zero day, 1st day, 2nd day ,3rd day and 4th day of the storage period respectively.

At zero day, for all samples the initial counts of APC was $5.٩x10^4+0.6x10^4$. For control sample and treated samples with 0.5%,1%,1.5%of thyme oil ,the mean counts were $8.2x10^4+0.7x10^4$، $2.1x10^5+1.6\ x\ 10^5$، $5.3x10^5+1.2x10^5$، $1.5x10^6+0.6x10^6$ $3.7x10^4+0.5x10^4$، $3.2x10^4+0.7x10^4$، $4.0x10^3+0.6x10^3$، $2.1x10^3+1.8x10^3$ & $2.5x10^4+0.4x10^4$، $1.9x10^3+1.1x10^3$ ، $3.6x10^3+1.4x10^3$ ، $1.6x10^3+0.8x10^3$ & $١.8x10^4+0.5x10^4$ ، $1.4x10^4+0.4x10^4$ ، $2.9x10^3+0.8x10^3$، $1.4.x10^3+1.2x10^3$ cfu/g at1st day,2nd day,3rdday and4th day respectively. Reduction % of APC were37.28% , 45.76%, 93.22%, 96.44%&57.63%, 96.78%, 93.90% ,97.29%&69.49%, 76.27%,95.08%, 97.63% at 1st day,2nd day,3rdday and4th day respectively.

At zero day, for all samples, the initial count of Enterobacteriaceae was $7.1x10^3+0.6x10^{3.}$. For control sample and treated samples with0.5%,1%,1.5%of thyme oil ,the mean counts were $7.5x10^4+0.7x10^4$, $8.1x10^4+1.1x10^4$, $9.4x10^4+1.5x10^4$,$3.1x10^5+1.2x10^5$&$5.7x10^3+0.5x10^3$,$5.3x4x10^3$, $4.1x10^3+0.4x10^3$,$3.8x10^3+1.8x10^3$&$4.8x10^3+0.4x10^3$,$4.3x10^3+1.1x10^3$, $3.5x10^3+1.4x10^3$,$2.9x10^3+0.8x10^3$&$4.0x10^3+0.5x10^3$,$3.2x10^3+0.4x10^3$, $2.3x10^3+0.8x10^3$, $1.4.x10^3+1.2x10^3$ cfu/g in the 1st day ,2nd day 3rdday and 4th day respectively .

Reduction% of Enterobacteriaceae count were 19.72% ,25.35% ,42.25% ,46.48% &32.39%,39.44%,50.80%,59.15%&43.66%,54.93%,67.61%,80.29 at 1st day,2nd day,3rdday and4th day respectively.

At zero day, for all samples the initialcountsofcoliformwas$6.6x10^3+0.6x10^3$ For control samples and treated samples with 0.5%,1%,1.5%of thyme oil , the mean counts were $4.2x10^4+0.7x10^4$, $8.9x10^4+1.6 x 10^4$, $1.3x10^5+1.2x10^5$,$2.5x10^5+0.6x10^5$ &$5.1x10^3+0.5x10^3$,$4.2x10^3+0.7x10^3$,$3.6x10^3+0.6x10^3$, $5.6x10^2+1.8x10^2$ &$3.5x10^3+0.4x10^3$, $2.8x10^3+1.1x10^3$, $2.3x10^3+1.4x10^3$, $6.8x10^2+0.8x10^2$ & $2.6x10^3+0.5x10^3$, $2.0x10^3+0.4x10^3$, $1.5x10^3+0.8x10^3$, $6.4.x10^2+1.2x10^2$ cfu/g. at 1st day,2nd day,3rdday and 4th day respectively.

Reduction%ofcoliformcountwere22.73%,36.36%,45.45%,91.52%&32.39%,46.97%,57.58%,89.70%&60.61%,69.70%,77.27%,90.30% at 1st day,2nd day,3rdday and 4th day respectively.

At zero day, for all samples the initial counts of Staphylococci was $5.1x10^3+0.6x10^3$.For control sample and treated samples with 0.5%,1%,1.5%of thyme oil , the mean counts were $8.2x10^4+0.7x10^4$, $2.1x10^5+1.6x10^5$,$5.٣x10^5+1.2x10^5$,$1.5x10^6+0.6x10^6$&$3.7x10^3+0.5x10^3$, $3.0x10^3+0.7x10^3$,$2.2x10^3+0.6x10^3$,$6.2x10^2+1.8x10^2$&$2.4x10^3+0.4x10^3$, $1.8x10^3+1.1x10^3$,$8.6x10^2+1.4x10^2$,$5.8x10^2+0.8x10^2$&$2.0x10^3+0.5x10^3$, $1.5x10^3+0.4x10^3$, $7.8x10^2+0.8x10^2$, $5.3.x10^2+1.2x10^2$cfu/g in 1st day, 2nd day 3rdday and 4th day respectively.

Reduction % of Staphylococci count were 27.45%,41.18%, 56.86% ,87.84% &52.94%, 64.71%, 83.14%, 88.61% & 60.78%, 70.59%, 84.71%, 89.61%, at 1st day, 2nd day , 3rdday and 4th day respectively .

There was a significant antimicrobial effect of different concentrations of thyme oil on bacteriological state of minced beef.

Keywords: minced beef, bacteriological profile thyme essential oil.

1. INTRODUCTION

Meat and meat products are an ideal medium for bacteria because of high moisture content, richness in nitrogenous compounds (essential amino acids, proteins), good source of minerals, vitamins and other growth factors. Furthermore, its pH is favorable for the growth of microorganisms (Alahakoon et al., 2015).

Ground beef is a popular food item that is used in a variety of dishes. Once the animal has been slaughtered, the meat is fabricated into wholesale or retail cuts. Trim and other cuts of meat are then further processed and ground. This increases the surface area of the meat which allows the increased adherence and growth of bacteria (Donsí et al., 2011).

There is growing consciousness among consumers for foods with high nutritional value, safe, healthy, microbiological safety and

free from synthetic chemical preservatives. The major problem with application of chemical preservatives is its carcinogenic nature; moreover, residual toxicity is increased due to these chemicals. Due to these reasons, consumers learn to be doubtful of chemical additives and so natural compounds derived from herbs or plants are recommended to be used either completely or partially substituting chemical preservatives Gammariello et al., 2008 and Tajkarimi and Ibrahim, 2011).

Natural products, such as essential oils which are produced by the secondary metabolism of herbs and/or spices and their constituents have been used in human consumption as functional food (nutraceuticals, biopolymers) and food additives (flavourings, antioxidant and antimicrobial) (Zengin and Baysal, 2014).

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, antifungal and antiviral activity, as well as antioxidant properties, they are used to prevent foodborne diseases, to extend shelf-life and to improve some meat characteristics (Akthar et al., 2014).

Essential oils (EOs) are aromatic and volatile oily liquids obtained from plant material (Hyldgaard et al., 2012).

Thyme essential oil is stated to posse's carminative, antispasmodic, antitussive, secretomotor, bactericidal, expectorant, astringent and anthelmintic properties. It is commonly used in foods mainly for its flavor and aroma (Küçükbay et al ., 2014).

As the level of contamination of minced beef with different food-borne pathogens constitutes serious problems for consumers, so, the present study was conducted to throw light over the bacterial status of meat minced beef at Menofia Governorate and study the effect of different concentrations of thyme essential oils on it.

2. MATERIALS AND METHODS

2.1. Samples:

A total of 25 fresh minced beef samples were collected from different markets. The samples were transferred in an ice box directly within an hour to the laboratory with a minimum delay to be bacteriologically examined.

2.2 Bacteriological examination:

2.2.1. Preparation of samples (APHA, 2001)

Twenty five grams of the samples under examination were taken under aseptic condition to sterile Stomacher bag then add 225 ml sterile 0.1% peptone water were added, the contents were homogenized at Stomacher (M A 106402, France, 450 to 640 strokes per minute) for 2 minutes, the mixture was allowed to stand for 5 minutes at room temperature The contents were transferred into sterile flask and thoroughly mixed by shaking . One ml of food homogenate was transferred into separate tube each containing 9 ml sterile 0.1% peptone water, from which tenth- fold serial dilutions were prepared. The prepared samples were subjected to the following bacteriological examinations:

2.2.2 Determination of Aerobic plate Count (ICMSF, 1996):

Streak one ml from each of chosen prepared dilution was inoculated separately onto duplicate sterile plates of plate count agar. Spread the inoculums using sterile bent glass,

the plates were incubated at 37°C for 24 hours. Plates containing 25-250 colonies were counted and Aerobic Plate Count (APC) per gram of the sample was calculated and recorded

2.2.3.Determination of Total Coliform count (ICMSF, 1996) :

The same technique of the previous surface plating method was applied using Violet Red Bile agar medium. The plates were incubated at 37°C for 24 hours. All pink colonies measuring 0.5 mm or more in diameter on uncrowded plates were then counted and the average number of colonies was determined. Numbers of colonies were calculated by multiplying by the dilution to obtain the number of Coliform organisms per gram of sample. .

2.2.4 Total Enterobacteriaceae count *(*(ICMSF, 1996)

The same technique of the previous pour plate method was carried out using Violet Red Bile Glucose agar medium (VRBG). The plates were incubated at 37°C for 24 hours. All purple colonies were then counted and the average number of colonies was determined. Hence, the Enterobacteriaceae count / g were calculated.

3.2.5 Determination of Total Staphylococci Count and Isolation of *S.aureus* (ICMSF, 1996):

Accurately, 0.1 ml from each of previously prepared serial dilutions was spread over duplicated plates of Mannitol agar using a sterile bent glass spreader. The inoculated and control plates were incubated at 37°C for 48 hours. The developed colonies (white, orange and yellow) were enumerated and the total Staphylococci count /g was calculated. Also, the colonies were picked up and purified on Semi-solid nutrient agar slopes for further identification. Moreover, yellow colonies surrounded by a halo zone (suspected *S.aureus*) were picked up and kept in Semi-solid agars lopes for morphological examination and biochemical identification by Quinn et al., 2002).

3.2.6 Studies the antimicrobial efficiency of thyme essential oils in minced beef : Jay (1992)

A grand total of 12 random samples of fresh minced beef were collected from different butcher shops in Menofia governorate. The samples were taken and transferred directly to the laboratory under complete aseptic conditions without undue delay. The samples were divided into untreated (control) and treated samples. The treated samples were homogenized with thyme oils in 0.5%, 1% and 1.5% concentrations. Each sample was packed in polyethylene bag, labeled and stored at 4 ºC. Each sample was analyzed promptly at 3 days intervals during storage and compared with control samples as follows:

3.2.6.1. Sensory examination:

It was carried out according to Pearson and Tauber (1984).

3.2.6.2. Determination of aerobic plate count (APC) which was performed according to (ICMSF, 1996)

3.2.6.3. Determination of total coliform count which was done according to (ICMSF, 1996)

3.3.6.4 Enumeration of *Enterobacteriaceae* which were carried out according to (ICMSF 1996).

3.3.6.5 Determination of total *Staphylococci* count which was performed according to (ICMSF, 1996).

3. RESULTS

In table (1) Aerobic Plate Count (cfu/g)(APC) of the examined minced meat varied from $6.1x10^4$to $8.4x10^4$with a mean value of $6.52x\ 10^4 \pm 0.27x\ 10^4$, the coliforms count varied from $3.4x10^2$to $7.1x10^2$with a mean value of $7.8x10^2 \pm 0.15x10^2$, the mean values of total Enterobacteriaceae count varied from $4.0\times10^2$to $6.4\times10^2$with a mean value of $5.82\times10^2 \pm 1.02\times10^{2,}$ minimum and maximum values of total staphylococci count were$4.8\times10^2$to $6.4\times10^2$with mean value of $6.15\times10^2 \pm 1.36\times10^{2.}$

In table (2) 22 strains Gram positive cocci were isolated (7strains of both Coagulase + ve *S.aureus* , and *Staphylococcus epidermidis,*while 2strains of each of *Staphylococcus saprophyticus* , *Staphylococcus intermedius* ,*Staphylococcus capitis* and *Micrococci spp.)*

Table (3) study the effect of different concentrations of thyme essential oil on sensory characters of minced beef during cold storage at 4ºC In case of using thyme oil at the concentrations 0.5%, 1% and 1.5% the scores of sensory evaluation were 9,٩ , 8, 6 ,5& 9 ,9, 8, 7,6& 9,9,8,8 , 7after zero day, 1st day, 2nd day ,3rd day and 4th day of the cold storage period respectively, comparing to the scores of sensory evaluation in the control samples which were 9, 6,5,4 ,2 after zero day, 1st day, 2nd day ,3rd day and 4th day of the storage period respectively.

In table (5) at zero days, for all samples the initial counts of APC was $5.٩x10^4+0.6x10^4$. For control sample and treated samples with 0.5%,1%,1.5%of thyme oil ,the mean counts were $8.2x10^4+0.7x10^4$، $2.1x10^5+1.6\ x\ 10^5$، $٥.٣x10^5+1.2x10^5$، $1.5x10^6+0.6x10^6$ $3.7x10^4+0.5x10^4$، $3.2x10^4+0.7x10^4$، $4.0x10^3+0.6x10^3$، $2.1x10^3+1.8x10^3$ & $2.5x10^4+0.4x10^4$، $1.9x10^3+1.1x10^3$ ، $3.6x10^3+1.4x10^3$ ، $1.6x10^3+0.8x10^3$ & $١.8x10^4+0.5x10^4$ ، $1.4x10^4+0.4x10^4$ ، $2.9x10^3+0.8x10^3$، $1.4.x10^3+1.2x10^3$ cfu/g at1st day,2nd day,3rdday and4th day respectively.In Table (6) reduction % of APC were37.28% , 45.76%, 93.22%, 96.44%&57.63%, 96.78%, 93.90% ,97.29%&69.49%, 76.27%,95.08%, 97.63% at 1st day,2nd day,3rdday and4th day respectively.

In table (7) At zero day, for all samples, the initial counts of Enterobacteriaceae was $7.1x10^3+0.6x10^{3.}$ For control sample and treated samples with0.5%,1%,1.5%ofthymeoil,themean countswere$7.5x10^4+0.7x10^4$،$8.1x10^4+1.1x10^4$،$9.4x10^4+1.5x10^4$،$3.1x10^5+1.2x10^5$&$5.7x10^3+0.5x10^3$،$5.3x4x10^3$، $4.1x10^3+0.4x10^3$،$3.8x10^3+1.8x10^3$&$4.8x10^3+0.4x10^3$،$4.3x10^3+1.1x10^3$، $3.5x10^3+1.4x10^{3,}$،$2.9x10^3+0.8x10^3$&$4.0x10^3+0.5x10^3$،$3.2x10^3+0.4x10^3$، $2.3x10^3+0.8x10^3$، $1.4.x10^3+1.2x10^3$ cfu/g in the 1st day ,2nd day 3rdday and 4th dayrespectively.Intable(8)Reduction%ofEntero bacteriaceaecountwere19.72%,25.35%,42.25%, 46.48%&32.39%,39.44%,50.80%,59.15%&43. 66%,54.93%,67.61%,80.29 at 1st day,2nd day,3rdday and4th day respectively.

In table (8), at zero day, for all samples the initial counts of coliform was $6.6x10^3+0.6x10^{3.}$ For control samples and treated samples with0.5%,1%,1.5%of thyme oil , the mean counts were $4.2x10^4+0.7x10^4$ ، $8.9x10^4+1.6x10^4$،$1.٣x10^5+1.2x10^5$،$2.5x10^5+0.6$

$x10^5$&$5.1x10^3+0.5x10^3$,$4.2x10^3+0.7x10^3$,$3.6x10^3+0.6x10^3$, $5.6x10^2+1.8x10^2$ &$3.5x10^3+0.4x10^3$, $2.8x10^3+1.1x10^3$, $2.3x10^3+1.4x10^3$, $6.8x10^2+0.8x10^2$ & $2.6x10^3+0.5x10^3$, $2.0x10^3+0.4x10^3$, $1.5x10^3+0.8x10^3$, $6.4.x10^2+1.2x10^2$ cfu/g at 1st day,2nd day,3rdday and 4th day respectively.

Intable(9),reduction%ofcoliformcountwere22.73%,36.36%,45.45%,91.52%&32.39%,46.97%, 57.58%,89.70%&60.61%,69.70%,77.27%,90.30% at 1st day,2nd day,3rdday and 4th day respectively.

In table (10), at zero day, for all samples the initial counts of staphylococci was $5.1x10^3+0.6x10^3$.For control sample and treated samples with 0.5%,1%,1.5%of thyme oil , the mean counts were $8.2x10^4+0.7x10^4$, $2.1x10^5+1.6$ x $10,^5$٥.٣$x10^5+1.2x10^5$ $1.5x10^6+0.6x10^6$&$3.7x10^3+0.5x10^3$, $3.0x10^3+0.7x10^3$, $2.2x10^3+0.6x10^3$, $6.2x10^2+1.8x10^2$&$2.4x10^3+0.4x10^3$, $1.8x10^3+1.1x10^3$, $8.6x10^2+1.4x10^2$, $5.8x10^2+0.8x10^2$&$2.0x10^3+0.5x10^3$, $1.5x10^3+0.4x10^3$, $7.8x10^2+0.8x10^2$, $5.3.x10^2+1.2x10^2$cfu/g in 1st day, 2nd day 3rdday and 4th day respectively. In table (11) reduction % of Staphylococci countwere27.45%,41.18%,56.86%,87.84%,&52.94%,64.71%,83.14%,88.61%,&60.78%,70.59%,84.71%, 89.61%, at 1st day, 2nd day , 3rdday and 4th day respectively .

Table (1): Bacteriological evaluation of minced beef samples (n=25).

Isolated organisms	Positive samples		Min.	Max.	Mean ± S.E.M.
	No	%			
Aerobic plate counts	25	100	$6.1x10^4$	$8.4x10^4$	$6.52x\ 10^4 \pm 0.27x\ 10^4$
coliform count	10	40	$3.4x10^2$	$7.1x10^2$	$7.8x10^2 \pm 0.15x10^2$
Enterobacteriaceae count	24	96	4.0×10^2	6.4×10^2	$5.82\times10^2 \pm 1.0^2\times10^2$
total Staphylococci count	23	92	4.8×10^2	6.4×10^2	$6.15\times10^2 \pm 1.36\times10^2$

S.E* = standard error of mean

All examined samples of minced beef were lower than 10^4 for aerobic plate counts and lower than 10^2 for coliform count, Enterobacteriaceae count and total Staphylococci count , so all samples were accepted following EEC,(2005) and EOS (2005b).

Table (2): Incidence of Gram positive cocci isolated from the examined minced beef samples (n=25).

Gram + ve cocci	No.	%
Staphylococcus aureus	7	28
Staphylococcus epidermidis	7	28
Staphylococcussaprophyticus	2	8
Staphylococcusintermedius	2	8
Staphylococcuscapitis	2	8
Micrococci spp.	2	8
Total	22	88

Table (3): Sensory evaluation of the untreated (control) and treated samples of minced beef during cold storage at 4ºC Samples

Groups	Zero day	1st day	2nd day	3rd day	4th day
Control	9	6	5	4	2
Thyme oil 0.5%	9	9	8	6	5
Thyme oil 1%	9	9	8	7	6
Thyme oil 1.5%	9	9	8	8	7

Table (4): Score System for Sensory Evaluation (Pearson and Tauber, 1984) Score System

Quality	Points
Excellent	9
Very very good	8
Very good	7
Good	6
Medium	5
Fair	4
Poor	3
Very poor	2
Very very poor	1

Table (5): Mean values of APC count of the examined untreated

(control) and treated samples of minced beef during cold storage at 4ºC

Groups	Zero day	1st day	2nd day	3rdday	4th day
Control	$5.٩x10^{4}+0.6x10^{4}$	$8.2x10^{4}+0.7x10^{4}$	$2.1x10^{5}+1.6 \times 10^{5}$	$٥.٣x10^{5}+1.2x10^{5}$	$1.5x10^{6}+0.6x10^{6}$
Thyme oil 0.5%	$5.٩x10^{4}+0.6x10^{4}$	$3.7x10^{4}+0.5x10^{4}$	$3.2x10^{4}+0.7x10^{4}$	$4.0x10^{3}+0.6x10^{3}$	$2.1x10^{3}+1.8x10^{3}$
Thyme oil 1%	$5.٩x10^{4}+0.6x10^{4}$	$2.5x10^{4}+0.4x10^{4}$	$1.9x10^{3}+1.1x10^{3}$	$3.6x10^{3}+1.4x10^{3}$	$1.6x10^{3}+0.8x10^{3}$
Thyme oil 1.5%	$5.٩x10^{4}+0.6x10^{4}$	$١.8x10^{4}+0.5x10^{4}$	$1.4x10^{4}+0.4x10^{4}$	$2.9x10^{3}+0.8x10^{3}$	$1.4.x10^{3}+1.2x10^{3}$

Table (6): Reduction % APC count of the examined untreated

(control) and treated samples of minced beef during cold storage at 4ºC

Groups	1st day	2nd day	3rdday	4th day
Thyme oil 0.5%	37.28	45.76	93.22	96.44
Thyme oil 1%	57.63	96.78	93.90	97.29
Thyme oil 1.5%	69.49	76.27	95.08	97.63

Table (7): Mean values of Enterobacteriaceae count of the examined untreated (control) and treated samples of minced beef during cold storage at 4ºC

Groups	Zero day	1st day	2nd day	3rdday	4th day
Control	$7.1x10^3+0.6x10^3$	$7.5x10^4+0.7x10^4$	$8.1x10^4+1.1 x 10^4$	$9.4x10^4+1.5x10^4$	$3.1x10^5+1.2x10^5$
Thyme oil 0.5%	$7.1x10^3+0.6x10^3$	$5.7x10^3+0.5x10^3$	$5.3x10^3+0.4x10^3$	$4.1x10^3+0.4x10^3$	$3.8x10^3+1.8x10^3$
Thyme oil 1%	$7.1x10^3+0.6x10^3$	$4.8x10^3+0.4x10^3$	$4.3x10^3+1.1x10^3$	$3.5x10^3+1.4x10^3$	$2.9x10^3+0.8x10^3$
Thyme oil 1.5%	$7.1x10^3+0.6x10^3$	$4.0x10^3+0.5x10^3$	$3.2x10^3+0.4x10^3$	$2.3x10^3+0.8x10^3$	$1.4.x10^3+1.2x10^3$

Table (8): Reduction % Enterobacteriaceae count of the examined untreated (control) and treated samples of minced beef during cold storage at 4ºC

Groups	1st day	2nd day	3rdday	4th day
Thyme oil 0.5%	19.72	25.35	42.25	46.48
Thyme oil 1%	32.39	39.44	50.80	59.15
Thyme oil 1.5%	43.66	54.93	67.61	80.29

Table (9): Mean values of coliform count of the examined untreated (control) and treated samples of minced beef during cold storage at 4ºC

Groups	Zero day	1st day	2nd day	3rdday	4th day
Control	$6.6x10^3+0.6x10^3$	$4.2x10^4+0.7x10^4$	$8.9x10^4+1.6 x 10^4$	$1.٣x10^5+1.2x10^5$	$2.5x10^5+0.6x10^5$
Thyme oil 0.5%	$6.6x10^3+0.6x10^3$	$5.1x10^3+0.5x10^3$	$4.2x10^3+0.7x10^3$	$3.6x10^3+0.6x10^3$	$5.6x10^2+1.8x10^2$
Thyme oil 1%	$6.6x10^3+0.6x10^3$	$3.5x10^3+0.4x10^3$	$2.8x10^3+1.1x10^3$	$2.3x10^3+1.4x10^3$	$6.8x10^2+0.8x10^2$
Thyme oil 1.5%	$6.6x10^3+0.6x10^3$	$2.6x10^3+0.5x10^3$	$2.0x10^3+0.4x10^3$	$1.5x10^3+0.8x10^3$	$6.4.x10^2+1.2x10^2$

Table (10): Reduction % coliform count of the examined untreated (control) and treated samples of minced beef during cold storage at 4ºC

Groups	1st day	2nd day	3rdday	4th day
Thyme oil 0.5%	22.73	36.36	45.45	91.52
Thyme oil 1%	32.39	46.97	57.58	89.70
Thyme oil 1.5%	60.61	69.70	77.27	90.30

Table(11):Mean values of Staphylococci count of the examined untreated(control) and treated samples of minced beef during cold storage at 4ºC

Groups	Zero day	1st day	2nd day	3rdday	4th day
Control	$5.1x10^3+0.6x10^3$	$8.2x10^4+0.7x10^4$	$2.1x10^5+1.6 x 10^5$	٥.٣$x10^5+1.2x10^5$	$1.5x10^6+0.6x10^6$
Thyme oil 0.5%	$5.1x10^3+0.6x10^3$	$3.7x10^3+0.5x10^3$	$3.0x10^3+0.7x10^3$	$2.2x10^3+0.6x10^3$	$6.2x10^2+1.8x10^2$
Thyme oil 1%	$5.1x10^3+0.6x10^3$	$2.4x10^3+0.4x10^3$	$1.8x10^3+1.1x10^3$	$8.6x10^2+1.4x10^2$	$5.8x10^2+0.8x10^2$
Thyme oil 1.5%	$5.1x10^3+0.6x10^3$	$2.0x10^3+0.5x10^3$	$1.5x10^3+0.4x10^3$	$7.8x10^2+0.8x10^2$	$5.3.x10^2+1.2x10^2$

Table (12): Reduction % Staphylococci count of the examined untreated (control) and treated samples of minced beef during cold storage at 4ºC

Groups	1st day	2nd day	3rdday	4th day
Thyme oil 0.5%	27.45	41.18	56.86	87.84
Thyme oil 1%	52.94	64.71	83.14	88.61
Thyme oil 1.5%	60.78	70.59	84.71	89.61

4. DISCUSSION

Minced beef are perishable foods and unless stored under proper conditions spoil quickly. In addition, if pathogens are present, minced beef become hazardous for consumers. Therefore, assurance of meat safety and bacteriological quality is the most important (Shimoni and Iabuza, 2000). It is well established fact that the main source of transmission for pathogenic bacteria is contaminated food; it is considered as the major cause of enteric diseases in developing countries and the major cause of mortality and morbidity *(*Gunasegaran et al., 2011).

Aerobic Plate Count is considered as the important parameter for the sanitation and hygienic importance of minced beef. This is the most widely used microbiological test of foods. It gives information about the general level of contamination of the product. *(*APHA, 2001).

Table (1) showed that Aerobic Plate Count (APC) of the examined minced meat varied from $6.1x10^4$to $8.4x10^4$with mean value of $6.52x\ 10^4 \pm 0.27x\ 10^4$ (cfu/g).

Nearly similar counts were recorded by Hassan and Soultan (2004) and Paulsen et al. (2006) Lamada-Hanan et al. (2012)

Although, APC of any food articles is not a sure indicative of their safety for consumption, yet it is of supreme importance in judging the hygienic condition under which food has been produced, handled and stored *(*Levine, 1987).

Coliforms counts reflect inadequate sanitation during production and handling of raw material, meat contact surfaces and employees. Meanwhile, the occurrence of large numbers of them on carcass surfaces are highly and undesirable and suggests mostly faecal contamination and points to potentially severe hazard *(*Eribo and Jay, 1985).

Results recorded in table (1) revealed that coliforms count / g of the examined minced

beef samples varied from $3.4x10^2$to $7.1x10^2$with mean value of $7.8x10^2 \pm 0.15x10^2$.

The results are relatively agree, to some extent, with those of Paulsen et al.,(2006), Badawi (2008) , Al-Mutairi (2011) Mousa et al.,(2014) .Lower results were recorded by Mansour & Basha (2009) and Saad et al., (2011). While the higher results were recorded by by Saleh and Salah El-Dien (2005) .

A significant higher coliforms count may be related to poor sanitary conditions prevailing at the abattoir. Therefore, the presence of coliforms bacteria in great numbers may be responsible for the inferior quality of meat resulting in economic losses and the possibility of the presence of enteric pathogens which constitute public health hazards (NA hS, 1985 and Trott and oburn, 1997).

In general ,Enterobacteriaceae count used to assess the general hygienic status of a food product and their presence in food indicates inadequate cooking or post-processing contamination(CFS, 2014). Members of this family have been considered a potent cause of food borne outbreaks (Centinkaya et al., 2008).

Results achieved in table (1) declared that the mean values of total Enterobacteriaceae counts in the examined minced meat samples varied from $4.0\times10^2$to $6.4\times10^2$with mean value of $3.53x10^2 \pm 0.24x10^2$These results agree with those of Hassan and Soultan (2004) , Paulsen et al.,(2006) ,Stagnitta et al.,(2006) and Gwida et al.,(2014) .

Higher count was recorded by Al-Mutairi (2011) and Gaafar-Rehab et al., (2012)

Generally, Enterobacteriaceae count may be used as abroad base in detecting the organisms which usually decontaminates the products after processing (ICMSF, 1978). Enterobacteriaceae have an epidemiological importance as some of their members are pathogenic and may cause serious infections and food poisoning outbreaks to human being. (Mosupye and Van Holy, 2000).

Total Staphylococci count is a good indication of inadequate sanitation and processing as well as the possibility for presence of enterotoxin producing strains of *S. aureus* (AS/NZS, 1999).

Results recorded in table(1) revealed that the minimum and maximum values of total staphylococci count / g. in examined minced beef $4.8\times10^2$to $6.4\times10^2$with mean value of $6.15\times10^2 \pm 1.36\times10^{2.}$

According to the results recorded for minced beef ,they are relatively agree, to some extent, with those obtained Youssef et al.(1999) , Zaki-Eman(2003).The lower results were recorded by Mansour and Basha (2009) and Amer (2013) .

Several staphylococci food poisoning outbreaks were attributed to the use of bar hands in preparation of food (Soliman, 1988).

Table (2) revealed that from 22 strains isolated from minced beef (7strains of both Coagulase + ve *S.aureus*,and *Staphylococcus epidermidis*,while 2strains of each of *Staphylococcus saprophyticus* , *Staphylococcus intermedius* ,*Staphylococcus capitis* and *Micrococci spp.*

Nearly similar results were recorded by Darwish et al. (1991), Youssef et al .(1999); Fathi and Thabet (2001); El- Leboudi et al. (2004)

All the results discussed for minced beef might harbor heavy load of

microorganisms. So bacteriological testing program are required to produce final.

Meat is subjected to contamination with several types of microorganisms, during the period that elapses from the time of slaughtering till consumption; such contamination may lead to production of inferior quality meat or even unfit for human consumption resulting in economic losses and may constitutes a public health hazard. (Nosseur, 2010).

There is an increase in using plant-origin food-preservative essential oils since the 1990s, with more utilization of spices and their essential oils as natural bio-preservatives, to increase shelf life and overall quality of food products (Simitzis et al., 2008).

Sensory evaluation is an easy, quick and efficient method for getting idea about the quality of the product and its overall acceptance; sensory methods were used to assess the degree of freshness based on organoleptic characteristics such as color, odor, texture and overall acceptability of the product (Haq et al., 2013).

The direct addition of essential oils to food may alter the sensory characteristics of food (Seydim and Sarikus, 2006).

Table (3) study the Effect of different concentrations of thyme oil on sensory characters of minced beef during cold storage at 4ºC , in case of using thyme oil at the concentrations 0.5%, 1% and 1.5% the scores of sensory evaluation were 9,٩ , 8, 6 and 5& 9 ,9, 8, 7 and 6& 9,9,8,8 and 7 at zero day, 1st day, 2nd day ,3rd day and 4th day of the cold storage period respectively, comparing to the scores of sensory evaluation in the control samples which were 9, 6,5,4 and 2 at zero day, 1st day, 2nd day ,3rd day and 4th day of the storage period respectively.

It is obvious from the results that the sensory properties of different treated minced beef samples during cold storage (4ºC) were enhanced by increasing the concentrations of oils compared to the untreated (control) samples at zero, 3rd and 6th day of the storage period. Nearly similar results were obtained by Rasooli and Mirmostafa (2003) and Salem-Amany et al., (2010). This may be due to thyme is traditionally used as flavoring agents in meat and meat products (Lawless, 1995). The antimicrobial properties of essential oils against various pathogenic microorganisms are contributed to the presence of a large number of alkaloids, phenols, trepans derivatives compounds and other antimicrobial compounds. These compounds possess hydrophobic characteristics, which enable them to partition the lipids of bacterial cell membrane and mitochondria and interact with different targets of microbial cell causing loss of cellular constituents, collapse of membrane structure, loss of membrane integrity, dissipation of proton motive force, sequential inhibition of respiration and ion transport processes, leading to cell death (Canillac and Mourey, 2004).

Table (5) illustrated that at zero day, for all samples, the initial counts of APC was $5.٩x10^4+0.6x10^4$. For control sample and treated samples with 0.5%,1%,1.5%of thyme oil ,the mean counts were $8.2x10^4+0.7x10^4$، $2.1x10^5+1.6$ x 10^5،٥ $.٣x10^5+1.2x10^5$and $1.5x10^6+0.6x10^6$&$3.7x10^4+0.5x10^4$, $3.2x10^4+0.7x10^4$, $4.0x10^3+0.6x10^3$and

$2.1x10^3+1.8x10^3$ & $2.5x10^4+0.4x10^4$, $1.9x10^3+1.1x10^3$, $3.6x10^3+1.4x10^3$ and $1.6x10^3+0.8x10^3$ & ١.$8x10^4+0.5x10^4$, $1.4x10^4+0.4x10^4$, $2.9x10^3+0.8x10^3$and $1.4.x10^3+1.2x10^3$ cfu/g at1st day,2nd day,3rdday and4th day respectively .In Table (6) reduction % of APC were37.28% , 45.76%, 93.22% and 96.44%&57.63%, 96.78%, 93.90% and 97.29% & 69.49%, 76.27%,95.08% and 97.63% at 1st day,2nd day,3rdday and4th day respectively.

The relatively high initial counts of control samples may be attributed to the grinding process, which compounds the problem by introducing the pathogens into the interior of the meat and contributes to the increase of total viable counts of meat (Mead and Griffin, 1998). APC counts were gradually increased during cold storage for all samples with different ratios depending on the concentration of oil. The incremental pattern in APCIn general, as the concentration of oil decreased, APC increased as discussed by Marino et al., (2001).

Table (7) declared that, at zero day, for all samples, the initial counts of Enterobacteriaceae was $7.1x10^3+0.6x10^3$. For control sample and treated sampleswith0.5%,1%,1.5% of thyme oil ,the mean counts were $7.5x10^4+0.7x10^4$,$8.1x10^4+1.1x10^4$,$9.4x10^4+1.5x10^4$and$3.1x10^5+1.2x10^5$&$5.7x10^3+0.5x10^3$,$5.3x10^3+0.4x10^3$,$4.1x10^3+0.4x10^3$and $3.8x10^3+1.8x10^3$ &$4.8x10^3+0.4x10^3$, $4.3x10^3+1.1x10^3$, $3.5x10^3+1.4x10^3$and $2.9x10^3+0.8x10^3$ &$4.0x10^3+0.5x10^3$,$3.2x10^3+0.4x10^3$, $2.3x10^3+0.8x103$ and $1.4.x10^3+1.2x10^3$ cfu/g in the 1st day ,2nd day 3rdday and4thdayrespectively.

In table (8) reduction% of Enterobacteriaceae countwere19.72%,25.35%,42.25%,46.48%&32.39%,39.44%,50.80%,59.15%&43.66%,54.93% ,67.61%,80.29 at 1st day,2nd day,3rdday and4th day respectively.

..Nearly similar results were obtained by Yassin-Nessrien and Abou-Taleb (2007)

Table (8) discussed that, at zero day, for all samples the initial count of coliform was $6.6x10^3+0.6x10^3$. For control samples and treated samples with0.5%,1%,1.5%of thyme oil , the mean counts were $4.2x10^4+0.7x10^4$, $8.9x10^4+1.6x10^4$,$1.٣x10^5+1.2x10^5$and$2.5x10^5+0.6x10^5$&$5.1x10^3+0.5x10^3$,$4.2x10^3+0.7x10^3$,$3.6x10^3+0.6x10^3$ and $5.6x10^2+1.8x10^2$ &$3.5x10^3+0.4x10^3$, $2.8x10^3+1.1x10^3$, $2.3x10^3+1.4x10^3$ and 6.8x102+0.8x102 & $2.6x10^3+0.5x10^3$, $2.0x10^3+0.4x10^3$, $1.5x10^3+0.8x10^3$ and $6.4.x10^2+1.2x10^2$ cfu/g at 1st day,2nd day,3rdday and 4th day respectively.

In table (9) reduction % of coliform count were 22.73%,36.36%,45.45%,91.52%&32.39%,46.97%,57.58%,89.70%&60.61%,69.70%,77.27%, 90.30% at1st day,2nd day,3rdday and 4th day respectively.

Nearly similar results were recorded by Gutierrez et al., (2008).

Table (10) revealed that , for all samples the initial counts of staphylococci was $5.1x10^3+0.6x10^3$.For control sample and treated samples with 0.5%,1%,1.5%of thyme oil at cold storage , the mean counts were $8.2x10^4+0.7x10^4$،$2.1x10^5+1.6x10^5$،٥.٣$x10^5+1.2x10^5$and$1.5x10^6+0.6x10^6$&$3.7x10^3+0.5x10^3$,$3.0x10^3+0.7x10^3$,$2.2x10^3+0.6x10^3$and$6.2x10^2+1.8x10^2$&$2.4x10^3+0.4x10^3$,$1.8x10^3+1.1x10^3$,$8.6x10^2+1.4x10^2$and$5.8x10^2+0.8x10^2$&$2.0x10^3+0.5x$

10^3, $1.5x10^3+0.4x10^3$, $7.8x10^2+0.8x10^2$ and $5.3.x10^2+1.2x10^2$cfu/g in 1st day, 2nd day 3rdday and 4th day respectively.

In table (11) reduction %of Staphylococci count were 27.45% ,41.18% ,56.86% and 87.84% & 52.94% ,64.71% ,83.14% and 88.61% & 60.78%, 70.59% ,84.71% and 89.61%, at 1st day, 2nd day , 3rdday and 4th day respectively .

The essential oils will result in immediate reduction of bacterial population (Seydim and Sarikus, 2006) and might be more effective against food borne pathogens and spoilage bacteria when applied directly on foods ready to be used, containing a high protein level at acidic pH, as well as, lower levels of fat or carbohydrates (Gutierrez et al., 2008).
The antimicrobial activity of thyme oil has been thoroughly investigated (Ozcan et al., 2006) and found to be active against food borne and spoilage flora (Solomakos et al., 2008). This significant rate of antibacterial activities is mostly attributable to the phenolic compounds (cavracrol) and to the hydrocarbons wh
be bactericidal or bacteriostatic depen
their effective concentration (Yassin – M
and Abou-Taleb, 2007).
As an overall conclusion thyme oils
general enhancement in sensory and microbial
attributes

5. CONCLUSION

We can concluded that in minced beef there are high load of aerobic plate count , Enterobacteriacra count, coliforms and Staphylococci count ,this may be due to mishandling and the negligence of hygienic aspects either at production levels or selling of meat with expired dates. The sensory properties of treated minced beef during cold storage (4ºC) were improved by using thyme compared to the control samples after 3rdand 6th day of the cold storage period. The sensory properties of the samples were enhanced by increasing the concentrations of thyme during the storage period as, samples containing 1.5% oil, demonstrated the highest enhancement of sensory attributes, while the samples treated with 0.5% oils demonstrated the lowest enhancement. The inhibition of bacterial load is related to the concentration of the studied essential oils, since they declined, when increasing the concentration of the studied essential oils.

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