Studies on Antimicrobial and Antioxidant Efficiency of Some Essential Oils in Minced Beef

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Abstract: In this study, the antioxidant and antibacterial effect of garlic (G), thyme (T) and lemon grass (L) oils were investigated in refrigerated minced beef. It is noticed that, all essential oils used had considerable effectiveness in decreasing aerobic plate count (APC), *Enterobacteriaceae* count, Coliform count and *Staphylococci count*, as well as chemical indices as pH, total volatile nitrogen (TVN) and thiobarbituric acid (TBA). Sensory analysis indicated significant advantages in using lemon grass and thyme oils in refrigerated minced beef. In addition, a highly significant differences ($P<0.05$) between the different oils were noticed. Also, results indicated that the bacterial counts, pH, TVN and TBA values decrease as the concentration of the oil increases since the concentration (1.5%) gives the best effectiveness. The antioxidant and antibacterial activities of the added essential oils followed the order lemon grass oil > thyme oil > garlic oil. The treated minced beef samples extend the shelf life of the treated samples more than the control samples by 6 days. In conclusion, lemon grass, thyme and garlic oils can play an important role as antioxidant and antibacterial agents in refrigerated minced beef, but lemon grass oil is the best one.

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1. Introduction:

Meat and its products have experienced increasing popularity and become widely spread all over the world. The appearance of food is one of the major determinants of its appeal to consumers and consequently, sales of the product. However, during storage, quality attributes of the products deteriorate due to lipid oxidation and bacterial growth which are the main factors that determine food quality loss and shelf life reduction. Lipid oxidation leads to the degradation of lipids and proteins which, in turn, contribute to the reduction in nutritional quality as well as deterioration in flavor, color and texture of displayed meat products (Aguirrezábal *et al.*, 2000), while bacterial contamination can precipitate major public health hazard and economic loss in terms of food poisoning and meat spoilage (Fernández –López *et al.*, 2005).

Although several synthetic food additives have been widely used in the meat industry to extend food shelf life, inhibit lipid oxidation and delay or inhibit the growth of pathogenic microorganisms, the trend is to decrease their use because of the growing concern among consumers about such chemical additives. Consequently, search for natural additives, especially of plant origin, has notably increased in recent years indicating that the application of natural food additives possessing both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf life and preventing economic loss (Yin and Cheng, 2003 and Mielnik *et al.*, 2008).

Essential oils are regarded as natural alternatives of chemical preservatives and their use in food meets the demands of consumers for mildly processed or natural products, since in modern food industries, mild processes are applied in order to obtain safe products which have a natural or "green" image (Burt, 2004). However, the practical application of essential oils is limited because of flavor considerations, as well as, their effectiveness is moderate due to their interaction with food ingredients and structure (Skandamis *et al.*, 2001). Essential oils of herbs and their components, products from the secondary metabolism of plants, have many applications in ethno-medicine, food flavoring and preservation as well as the fragrance and pharmaceutical industries (Fabian *et al.*, 2006).

Garlic is one of the most commonly used ingredients as a flavor enhancement for sausage. In addition, garlic is appreciated for its medical properties. 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).

Thyme is commonly used in foods mainly for its flavor and aroma. Also, thymol, which is found in thyme, has been commercially available as part of mouthwash for more than hundred years. Besides, it is active against *E. coli* and *St. aureus* and spoilage flora in meat products (Solomakos *et al.,* 2008), completely stops the growth of fungi at low concentrations (Soliman and Badeaa, 2002) and inhibits the aflatoxin production (Chiasson *et al.,* 2004), so it has a role as pharmaceutical and preservative (Hammer *et al.,* 1999).

Lemon grass is a rich source of citral, which is used in perfumery and pharmaceutical industries, and bioactive compounds (flavonides and vitamin C). The natural flavonides are also attracting more and more attention not only due to their antioxidant properties, but as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects (Martin *et al.,* 2002).

Moreover, some researchers reported that there is a relationship between the chemical structures of the most abundant compounds in plants and their above mentioned functional properties (Deans and Svoboda, 1990).

The objective of the present study was to investigate the antioxidant as well as the antimicrobial effectiveness of three essential oils (garlic, thyme & lemon grass oils) at various concentrations on the quality of fresh minced beef during refrigerated storage (4ºC).

2. Materials and methods

A grand total of thirty random samples of fresh minced beef were collected from different butcher shops in Kaluobyia 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 garlic, thyme and lemon grass oils in 0.5%, 1% and 1.5% concentrations for each oil. Each sample was packed in polyethylene bag, labeled and stored at 4 ºC. Each sample was analysed promptly at 3 days intervals during storage as follows:

1. Sensory examination:

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

2. Chemical examination includes:

2.1. PH values were carried out according the technique recommended by ISO (1979).

2.2. Total volatile nitrogen (TVN) was done according the technique recommended by FAO (1980).

2.3. Thiobarbituric acid (TBA) was carried out according the technique recommended by Vyncke (1970).

3. Microbiological examination:

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

3.2. Determination of total coliforms count which was done according to APHA (1985).

3.3 Enumeration and identification of *Enterobacteriaceae* which were carried out according to ICMSF (1996).

3.4. Determination of total *Staphylococci* count which was performed according to Oxoid (1986).

4. Statistical Analysis:

ANOVA was carried out on data of the sensory, chemical and microbiological evaluations. Data are expressed as mean + SE (Gomez and Gomez, 1984).

3. Results and Discussion:

It is obvious from results obtained in table (1) 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. Generally, samples containing 1.5% lemon grass oil, thyme and garlic oils, respectively demonstrated the highest enhancement of sensory attributes, while the samples treated with 0.5% garlic oil demonstrated the lowest enhancement. The direct addition of essential oils to food may alter the sensory characteristics of food (Seydim and Sarikus, 2006). Nearly similar results were obtained by El-Desouky *et al.,* (2006) and Mielnik *et al.,* (2008).

Lipid oxidation and other degradation reactions lead to the formation of low molecular compounds, which contribute to the sensory profile. Hydroperoxides and secondary oxidation products can react with protein and amino acids during processing and storage period affecting the flavor, odour and texture of meat products (Frankel, 1998).

The differences in pH mean values between different treated and untreated samples were significant during storage at 4ºC (table 3). The results showed a significant ($P<0.05$) increase in pH mean values in different treatments during storage by different rates. The highest incremental rates (pH values) were found in the untreated (control) samples. The samples treated with 1.5% and 1% lemon grass oil, showed the highest significant ($P<0.05$) effect on

pH lowering its values than those of untreated samples, followed by samples treated with 1.5%, 1% and 0.5% thyme oil, respectively, and finally the samples treated with 1.5%, 1% and 0.5% garlic oil, respectively, till the end of the storage period. There was significant ($P<0.05$) increase in pH mean values of all untreated and treated samples with garlic, thyme and lemon grass oils at all concentrations at the 6th day of the storage period. This may be due to the activation effect of microbial load which may cause protein hydrolysis with the appearances of alkyl groups (Yassin - Nessrien, 2003).

The mean values of TVN are summarized in table (3) estimating the degree of meat deterioration during the storage period (zero, 3rd and 6th day). As the storage period at 4^{o}C increased, the TVN values increased as shown in table (3) for all minced meat samples with different rates depending on the nature of treatments. This may be attributed to the breakdown of proteins as a result of activity of microbial strains and proteolytic enzymes (Yassin - Nessrien, 2003). EOS (2005) stated that 20 mg TVN/ 100 gm raw samples indicates the spoilage of minced meat. The highest rate of increase of TVN values was recorded in control samples. The treatments with 1.5% lemon grass, thyme and garlic oils, respectively, were more effective in delaying the rate of TVN increase during the subsequent cold storage. This may be attributed to the role of such oils on microbial population and bacterial growth as antimicrobial agents (Sacchetti *et al.,* 2005).

The evaluation of TBA mean values of control and treated samples during storage at 4^{o}C are shown in table (3). The highest incremental rate was recorded in the untreated (control) samples, while the lowest significant incremental rate was recorded in samples treated with 1.5% lemon grass oil, followed by samples treated with 1% of the same oil. The incremental pattern in TBA values for all the stored samples with advancing the chilling storage time may be due to the auto-oxidation of meat lipids, bacteriological and/or oxidative rancidity. TBA value is routinely used as an index of lipid oxidation in meat products in store (Raharjo and Sofos, 1993) and the rancid flavor is initially detected in meat products between TBA values of 0.5 and 2.0 (Gray and Pearson, 1987).

Thus, it has been reported that thyme oil may act as a high scavenger of radicals involved in lipid peroxidation protecting lipids from oxidation during cold storage as discussed by Kulisic *et al.* (2005) and Bozin *et al.* (2006), while garlic oil posses effective antioxidant activity (Jackson *et al.,* 2002) which is mainly attributed to a variety of sulphur-containing compounds and their precursors (Song *et al.,* 2004). In the same field, Fernández – López *et al.* (2005) found that about 50% of the rancidity of meat products can be controlled by the citrus preparations (e.g. lemon grass oil) with significant advantages in acceptability and aroma in rancidity-susceptible meat products. This antioxidant activity has been mainly attributed to flavonoids and ascorbic acid in citrus fruits (hesperidin, neohesperidin and eriocitrin) (Schwarz *et al.,* 2001). All of these polyphenolic compounds have the ability to act as antioxidants by a free radical scavenging mechanism and also through their known ability to chelate transition metals (inactivation of iron ions) (Martin *et al.,* 2002).

In the past few years, a variety of plant materials containing phenolic compounds have been to be effective antioxidants in model systems. Since ancient times, herbs and spices have been added to food to improve sensory properties and prolong shelf life. Among the main objections against the use of spices as antioxidants, is the characteristic flavor which they give to the meat products. However, this could be turned towards a positive new exciting sensory sensation. The acceptability of the taste of highly spiced food is transmitted both culturally and genetically, and the countries with hotter climate use spices more frequently and at much higher levels than countries with cooler climates. Essential oils rich in polyphenols exhibit antioxidative activities as they scavenge free radicals, similar to synthetic phenolic antioxidants (Cuvelier *et al.,* 1996 and Billing and Sherman, 1998).

The mean values of total aerobic counts (APC) of different untreated and treated minced beef samples during cold storage were shown in table (4). The control samples showed the highest APC counts comparing to others containing lemon grass, thyme or garlic oils with different concentrations (table 4). 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 (Nychas *et al.,* 1991 and 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 APC

can be arranged in a descending order as follows: samples treated with garlic, thyme and finally lemon grass oil at 0.5%, 1% & 1.5% concentration levels, respectively. In general, as the concentration of oil decreased, APC increased as discussed by Marino *et al.* (2001).

As shown in table (4), it could be observed that the control samples had the highest counts of *Enterobacteriaceae* and coliform at any time of cold storage compared to other treatments. It is clear that lemon grass, thyme and garlic oils at concentration 1.5% have strong effects against the growth of *Enterobacteriaceae* and coliform, and as the concentration of these essential oils increases, the counts of *Enterobacteriacea*e and coliform reduce especially at the 3rd and 6th days of cold storage.

Data presented in table (5) showed staphylococci counts of different treated and untreated minced beef samples during cold storage. As demonstrated by the different treatments, the treated samples with lemon grass, thyme and garlic oils at concentration 1.5% respectively showed the lowest counts in this parameter at zero, 3rd and 6th days of cold storage.

Table (6) summarized the initial microflora of chilled minced beef samples related to *Enterobacteriaceae*. The contribution of this group to the final flora depends on the type and the concentration of the used essential oil. It needs to be stressed that the rate of growth, lag phase and the final incidence of enteric bacteria were affected by the addition of essential oils (table 6). During the cold storage of minced beef, *Enterobacteriaceae* count reached the highest level with the 6th day of the storage period. *Proteus vulgaris* being the most dominant (66.67%) followed by *Citrobacter freundii* (55.56%) and then *Enterobacter aerogenes* (44.45%) in control samples. The addition of essential oils reduces the growth of *C. diversus, C. freundii, E. aerogenes, K. pneumoniae, P. mirabilis & P. vulagaris* and suppressed the growth of *E. cloacae* & *Serratia liquefaciens* completely. The addition of lemon grass oil showed pronounced inhibition of *C.diversus, E. cloacae, K. pneumoniae, Proteus mirabilis* & *Serratia liquefaciens* and reduced the incidence of *C. freundii* (11.11%), *E. aerogenes* (11.11%) & *P. vulgaris* (22.2%) when compared to the control and the other treated samples with thyme and garlic oils.

Nearly similar results were obtained by Yassin-Nessrien and Abou-Taleb (2007) and Gutierrez *et al.* (2008).

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 Mielnik *et al.*, 2008) 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 which can be bactericidal or bacteriostatic depending on their effective concentration (Bozin *et al.*, 2006 and Yassin – Nessrien and Abou – Taleb, 2007).

Also garlic oil provides antimicrobial benefits (Sallam *et al.*, 2004), where garlic oil is rich in organosulfur compounds and their precursors (allicin, dially sulfide & diallyl trisulfides) (Ankri and Mirelman, 1999) inhibiting the growth of a lot of pathogens as APC, *E. coli* & *S. aureus* by reacting with their cystine, inactivating the thio-containing enzymes or affecting the metabolism of lipids (Song *et al.*, 2004) and subsequently, extending the shelf life of the product, so the garlic extracts are potentially useful in preserving meat products (Pranoto *et al.*, 2005).

Moreover, lemon grass oil was observed to possess high antimicrobial activity, so it can be used as a way of combating the growth of common causes of food poisoning (Fisher and Phillips, 2006). In the same field, Adegoke and Odesola (1996) added that some bacteria associates with food spoilage / intoxication were inhibited by the essential oil of lemon grass. Lemon grass oil was found to be effective at all concentrations (Chahal *et al.*, 2007), where it is composed of three main components, the alpha and beta-citral components which elicit antibacterial action on Gram-positive and Gram-negative organisms, while the third component is mycrene which provided enhanced activities when mixed with either of the two main previously identified components (Onawunmi *et al.*, 1984)

As an overall conclusion, garlic, thyme and lemon grass oils showed general enhancement in sensory, chemical and microbial attributes due to the

action of these oils in retarding oxidation as well as microbial population in the fresh minced meat during cold storage at 4ºC and the action of these oils is concentration dependent. Also we found that lemon grass oil is the most effective at all concentrations especially at 1.5% than thyme and garlic oils, respectively. The low effectiveness of garlic oil in comparison with thyme & lemon grass oils could be attributed to the losses of volatile sulfur compounds, which have high biological activity, during distillation, and also due to the nature of garlic oil itself, which is volatile and hydrophobic (Sallam *et al.*, 2004).

Therefore, it is suggested that garlic, thyme and lemon grass oils can be used as natural meat preservatives with both antioxidants and antimicrobial activities against food borne pathogens and spoilage organisms, and therefore may be useful in maintaining the meat quality, extending shelf- life of meat products, preventing economic loss and providing the consumer with food containing natural additives, which might be seen more healthful than those of synthetic origin. Further research is necessary to explore the efficiency and palatability of suitable concentrations of natural oils in meat industry.

Table (1): Sensory evaluation of the untreated (control) and treated samples of minced beef during cold storage at 4ºC

Samples	Zero day	3rd day	6th day
Control	Excellent	Very poor	Very very poor
Garlic oil			
0.5%	Excellent	Fair with the presence of garlic odour	Very very poor
1%	Excellent	Fair with the presence of garlic odour	Very poor
1.5 %	Excellent	Fair with the presence of garlic odour	Very poor
Thyme oil			
0.5%	Excellent	Fair with the presence of thyme odour	Very poor
1%	Excellent	Fair with the presence of thyme odour	Very poor
1.5 %	Excellent	Medium with the presence of thyme odour	Fair with the presence of thyme odour
Lemon grass oil			
0.5%	Excellent	Excellent with the presence of lemon odour	Very good with the presence of lemon odour
1%	Excellent	Excellent with the presence of lemon odour	Very good with the presence of lemon odour
1.5 %	Excellent	Excellent with the presence of lemon odour	Very good with the presence of lemon odour

Table (2): Score System for Sensory Evaluation (Pearson and Tauber, 1984)

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

(3): Mean values of chemical induces of the examined untreated (control) and treated samples of minced beef during cold storage at 4ºC

Samples	pH			TVN			TBA		
	Zero day	3rd day	6th day	Zero day	3rd day	6th day	Zero day	3rd day	6th day
Control*	5.71± 0.03	6.76 ± 0.02	7.05 ± 0.04	6.33 ± 0.21	44.29 ± 0.41	63.68 ± 1. 17	0.04 ± 0.01	0.42 ± 0.02	0.58 ± 0.01
Garlic oil 0.5 %	5.71± 0.03	6.34 ± 0.03	6.86 ± 0.03	6.33 ± 0.21	26.06 ± 0.32	41.58 ± 0.87	0.04 ± 0.01	0.26 ± 0.01	0.44 ± 0.02
1 %	5.71± 0.03	6.23 ± 0.01	6.73 ± 0.05	6.33 ± 0.21	22.81 ± 0.22	36.25 ± 0.45	0.04 ± 0.01	0.21 ± 0.01	0.38 ± 0.02
1.5 %	5.71± 0.03	6.19 ± 0.02	6.67 ± 0.02	6.33 ± 0.21	20.34 ± 0.75	29.89 ± 0.33	0.04 ± 0.01	0.20 ± 0.01	0.36 ± 0.03
Thyme oil 0.5 %	5.71± 0.03	6.17 ± 0.02	6.78 ± 0.04	6.33 ± 0.21	20.72 ± 0.31	35.56 ± 0.61	0.04 ± 0.01	0.22 ± 0.03	0.38 ± 0.03
1%	5.71± 0.03	6.09 ± 0.01	6.61 ± 0.02	6.33 ± 0.21	18.64 ± 0.22	31.25 ± 0.43	0.04 ± 0.01	0.19 ± 0.01	0.36 ± 0.02
1.5 %	5.71± 0.03	6.02 ± 0.03	6.37 ± 0.03	6.33 ± 0.21	16.81 ± 0.17	28.51 ± 0.37	0.04 ± 0.01	0.18 ± 0.02	0.30 ± 0.02
Lemon grass oil 0.5%	5.71± 0.03	6.09 ± 0.02	6.57 ± 0.03	6.33 ± 0.21	18.41 ± 0.30	32.90 ± 0.51	0.04 ± 0.01	0.19 ± 0.02	0.34 ± 0.02
1%	5.71± 0.03	6.00 ± 0.01	6.33 ± 0.02	6.33 ± 0.21	16.77 ± 0.21	27.36 ± 0.38	0.04 ± 0.01	0.16 ± 0.01	0.29 ± 0.01

+ ($P < 0.05$)

TVN 20 mg / 100 gm raw minced beef (EOS, 2005)

TBA 0.9 mg Melanoaldehyde / kg raw minced beef (EOS, 2005)

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

Samples	*APC*			*Enterobacteriaceae* count		
	Zero day	3rd day	6th day	Zero day	3rd day	6th day
Control*	$5.61x10^5 \pm 0.92x10^5$	$6.29x10^7 \pm 1.22x10^7$	$3.08x10^9 \pm 0.62x10^5$	$8.79x10^3 \pm 2.25x10^3$	$6.81x10^5 \pm 1.06x10^5$	$9.25x10^6 \pm 2.89x10^6$
Garlic oil						
0.5 %	$5.61x10^5 \pm 0.92x10^5$	$1.15x10^7 \pm 0.31x10^7$	$9.94x10^8 \pm 2.75x10^8$	$8.79x10^3 \pm 2.25x10^3$	$3.66x10^5 \pm 0.57x10^5$	$5.81x10^6 \pm 0.92x10^6$
1 %	$5.61x10^5 \pm 0.92x10^5$	$8.52x10^6 \pm 2.16x10^6$	$7.27x10^8 \pm 1.53x10^8$	$8.79x10^3 \pm 2.25x10^3$	$1.03x10^5 \pm 0.16x10^5$	$3.78x10^6 \pm 0.64x10^6$
1.5 %	$5.61x10^5 \pm 0.92x10^5$	$7.36x10^6 \pm 1.46x10^6$	$7.01x10^8 \pm 1.29x10^8$	$8.79x10^3 \pm 2.25x10^3$	$7.56x10^4 \pm 1.83x10^4$	$8.49x10^5 \pm 2.38x10^5$
Thyme oil						
0.5 %	$5.61x10^5 \pm 0.92x10^5$	$3.58x10^7 \pm 0.62x10^7$	$7.92x10^8 \pm 1.80x10^8$	$8.79x10^3 \pm 2.25x10^3$	$2.07x10^5 \pm 0.35x10^5$	$3.58x10^6 \pm 0.76x10^6$
1%	$5.61x10^5 \pm 0.92x10^5$	$8.13x10^6 \pm 2.40x10^6$	$3.67x10^8 \pm 0.75x10^8$	$8.79x10^3 \pm 2.25x10^3$	$6.95x10^4 \pm 1.10x10^4$	$1.14x10^6 \pm 0.30x10^6$
1.5 %	$5.61x10^5 \pm 0.92x10^5$	$4.21x10^6 \pm 0.72x10^6$	$8.39x10^7 \pm 2.14x10^7$	$8.79x10^3 \pm 2.25x10^3$	$5.12x10^4 \pm 0.78x10^4$	$5.63x10^5 \pm 0.87x10^5$
Lemon grass oil						
0.5%	$5.61x10^5 \pm 0.92x10^5$	$7.93x10^6 \pm 2.05x10^6$	$4.73x10^8 \pm 0.82x10^8$	$8.79x10^3 \pm 2.25x10^3$	$8.14x10^4 \pm 2.36x10^4$	$8.73x10^5 \pm 2.21x10^5$
1%	$5.61x10^5 \pm 0.92x10^5$	$2.45x10^6 \pm 0.37x10^6$	$1.96x10^7 \pm 0..38x10^7$	$8.79x10^3 \pm 2.25x10^3$	$2.75x10^4 \pm 0.49x10^4$	$4.09x10^5 \pm 0.75x10^5$
1.5 %	$5.61x10^5 \pm 0.92x10^5$	$1.70x10^6 \pm 0.24x10^6$	$9.01x10^4 \pm 3.12x10^6$	$8.79x10^3 \pm 2.25x10^3$	$1.18x10^4 \pm 0.22x10^4$	$8.15x10^4 \pm 2.51x10^4$

*** Significant differences as a result of oil treatments (P< 0.05)**

Table (5): Mean values of coliform and Staphylococci counts of the examined untreated (control) and treated samples of minced beef during cold storage at 4ºC

Samples	Coliform count			*Staphylococci* count		
	Zero day	3rd day	6th day	Zero day	3rd day	6th day
Control*	$5.12x10^3 \pm 0.95x10^3$	$4.27x10^5 \pm 0.81x10^5$	$6.49x10^6 \pm 1.24x10^6$	$2.37x\ 10^4 \pm 0.55x10^4$	$9.96x10^5 \pm 3.01x10^5$	$8.73x10^6 \pm 2.58x10^6$
Garlic oil 0.5 %	$5.12x10^3 \pm 0.95x10^3$	$1.53x10^5 \pm 0.46x10^5$	$3.05x10^6 \pm 0.68x10^6$	$2.37x\ 10^4 \pm 0.55x10^4$	$7.31x10^5 \pm 1.69x10^5$	$5.43x10^6 \pm 1.17x10^6$
1 %	$5.12x10^3 \pm 0.95x10^3$	$8.19x10^4 \pm 1.98x10^4$	$1.18x10^6 \pm 0.27x10^6$	$2.37x\ 10^4 \pm 0.55x10^4$	$2.48x10^5 \pm 0.85x10^5$	$2.18x10^6 \pm 0.49x10^6$
1.5 %	$5.12x10^3 \pm 0.95x10^3$	$5.67x10^4 \pm 1.01x10^4$	$5.39x10^5 \pm 0.97x10^5$	$2.37x\ 10^4 \pm 0.55x10^4$	$1.15x10^5 \pm 0.42x10^5$	$6.92x10^5 \pm 1.56x10^5$
Thyme oil 0.5 %	$5.12x10^3 \pm 0.95x10^3$	$9.78x10^4 \pm 3.04x10^4$	$1.27x10^6 \pm 0.33x10^6$	$2.37x\ 10^4 \pm 0.55x10^4$	$4.87x10^5 \pm 0.96x10^5$	$2.51x10^6 \pm 0.64x10^6$
1%	$5.12x10^3 \pm 0.95x10^3$	$4.93x10^4 \pm 0.82x10^4$	$8.41x10^5 \pm 1.99x10^5$	$2.37x\ 10^4 \pm 0.55x10^4$	$1.09x10^5 \pm 0.34x10^5$	$6.03x10^5 \pm 1.41x10^5$
1.5 %	$5.12x10^3 \pm 0.95x10^3$	$2.46x10^4 \pm 0.53x10^4$	$3.89x10^5 \pm 0.77x10^5$	$2.37x\ 10^4 \pm 0.55x10^4$	$7.65x10^4 \pm 2.12x10^4$	$1.24x10^5 \pm 0.38x10^5$
Lemon grass oil 0.5%	$5.12x10^3 \pm 0.95x10^3$	$6.48x10^4 \pm 1.31x10^4$	$6.92x10^5 \pm 1.49x10^5$	$2.37x\ 10^4 \pm 0.55x10^4$	$1.19x10^5 \pm 0.27x10^5$	$7.82x10^5 \pm 1.53x10^5$
1%	$5.12x10^3 \pm 0.95x10^3$	$9.22x10^3 \pm 2.71x10^3$	$9.85x10^4 \pm 2.63x10^4$	$2.37x\ 10^4 \pm 0.55x10^4$	$6.58x10^4 \pm 1.75x10^4$	$3.45x10^5 \pm 0.71x10^5$
1.5 %	$5.12x10^3 \pm 0.95x10^3$	$8.52x10^3 \pm 2.39x10^3$	$6.01x10^4 \pm 1.26x10^4$	$2.37x\ 10^4 \pm 0.55x10^4$	$2.91x10^4 \pm 0.63x10^4$	$8.16x10^4 \pm 2.33x10^4$

* Significant differences as a result of oil treatments ($P < 0.05$)

Table (6): Incidence of enteric bacteria isolated from untreated (control) and treated samples of minced beef during cold storage at 4ºC

Group / Identified *Enterobacteriaceae*	Control		Garlic oil treated group		Thyme oil treated group		Lemon grass oil treated group	
	No.	%	No.	%	No.	%	No.	%
Citrobacter diversus	3	33.33	1	11.11	1	11.11	–	–
Citrobacter freundii	5	55.56	2	22.22	1	11.11	1	11.11
Enterobacter aerogenes	4	44.45	2	22.22	2	22.22	1	11.11
Enterobacter cloacae	1	11.11	–	–	–	–	–	–
Klebsiella pneumoniae	3	33.33	1	11.11	1	11.11	–	–
Proteus mirabilis	2	22.22	1	11.11	–	–	–	–
Proteus vulgaris	6	66.67	3	33.33	2	22.22	2	22.22
Serratia liquefaciens	2	22.22	–	–	–	–	–	–

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