Annals Of Agric. Sc., Moshtohor,
Vol. 44(4): 1675-1695, (2006).

EFFECT OF SOME ESSENTIAL OILS AND PRESERVATIVES ON THE GROWTH OF *E. COLI* O157:H7 AND QUALITY OF REFRIGERATED MINCED MEAT.

BY

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ABSTRACT

In this study, seven essential oils, cumin (C), marjoram (M), thyme (T), rosemary (R), lemongrass (L), clove (CL) and citronella (CI) and three preservatives, tri-sodium phosphate (TSP), sodium lactate (SL) and sodium acetate (SA) were studied for their antimicrobial effect on the growth of *E. coli* O157:H7. Oils of T, M and R and SL gave considerable effectiveness against *E. coli* O157:H7. The effect of 21 mixtures from 7 concentrations of T, M or R oils with SL on *E. coli* O157:H7 growth was studied. All mixtures had bacteriostitic and some of them had bactericidal effect. Sensory evaluation for meat patties containing the mention different mixtures were done to select the highly acceptable formula. ANOVA indicated that there are high significant differences ($p<0.01$) between the different mixtures. Patties contained mixtures of T, M or R (0.1 and 0.25%) and SL (0.25 and 0.5%) had high score. These mixtures were selected to complete the study. Results lead to advice that low concentration from essential oils are accepted for use in minced meat. Minced meat samples were inoculated by *E. coli* O157:H7 then treated by selected mixtures and stored at $4\pm0.5^{o}C$ to study quality of refrigerated minced meat. Control minced meat sample spoiled at 6 days. The treated minced meat samples had shelf life more than control between 9 and 15 days. Moreover, addition of essential oils minimize the growth of *E. coli* O157:H7 in treated minced meat corresponding with control sample. Finally, thyme, marjoram and rosemary oils can play an important role as antimicrobial and antioxidant agents in refrigerated minced meat.

INTRODUCTION

Several food additives are used to extend food shelf life and delay or inhibit the growth of pathogenic microorganisms (Wagner and Busta, 1985). Essential oils are natural products classified as generally recognized as safe (GRAS) and known to possess antimicrobial activity (Nychas, 1995 and Burt, 2004). In Egypt many essential oils are produced and some are imported. The essential oils have been shown to inhibit the growth of bacteria and yeast and moulds (Conner, 1993; Cox *et al.*, 2000; Dorman and Deans, 2000; Nielsen and Rios, 2000; Delaquis *et al.*, 2002; Mejholm and Dalgaard, 2002 and Burt and Reinders, 2003). DelCampo *et al.* (2000) reported that rosemary oil has antimicrobial effect. Thyme and oregano (wild type from marjoram) have

commonly been used in foods mainly for their flavor and aroma (Aureli *et al.*, 1992). Soliman and Badeaa (2002) found that essential oils could be safely used as preservative materials on some kinds of foods, such as thyme oil, which completely stopped the growth of fungi at low concentrations. Coriander, clove, oregano and thyme oils were found to be effective in inhibiting *Listeria monocytogenes*, *Aeromonas hydrophila* and spoilage flora in meat products (Aureli *et al.*, 1992; Stecchini *et al.*, 1993; Hao *et al.*, 1998 a, b; Tsigarida *et al.*, 2000 and Skandamis *et al.*, 2001). Besides antibacterial properties (Mourey and Canillac, 2002) essential oils or their components have been shown to exhibit antiviral (Bishop, 1995), antimycotic (Mari *et al.*, 2003), antitoxigenic (Juglal *et al.*, 2002), antiparasitic (Pessoa *et al.*, 2002) and insecticidal (Karpouhtsis *et al.*, 1998) properties.

Essential oil fractions of oregano and pimento are efficient against various foodborne bacteria such as *Salmonella sp.* (Helander, *et al.*, 1998) and *Escherichia coli* O157:H7 (Burt and Reinders, 2003). Direct addition of essential oils to food will result in immediate reduction of bacterial population and may alter the sensory characteristics of added food (Seydim and Sarikus, 2006). Lactate are naturally present in meat, it had been permitted, as a natural preservative, by the USDA-Food Safety and Inspection Service (USDA-FSIS) at a level of up to 4.8% in meat products (Shelef, 1994; USDA/FSIS, 2000 and Bedie *et al.*, 2001). Fresh meat products are commonly marketed at refrigerated temperatures (2-5 ºC). However, many undesirable changes of the products can occur during refrigeration due to microbial growth and lipid oxidation, which give rise to quality reduction, meat spoilage, and economic loss. The growth of microorganisms in meat and meat products may cause spoilage of foodborne diseases (Fernandez-lopez *et al.*, 2005). The shelf life of meats from freshly slaughtered sheep and goat carcasses at 5-7 ºC was extended after spraying by preservatives included sodium lactate (Ahmed *et al.*, 2003).

Escherichia coli has been identified as a foodborne pathogen since 1982 (Doyle, 1991). The relatively recent emergence of *E. coli* O157:H7 as a foodborne pathogen has a significant impact on the food industry (McClure, 2000). Meat probably becomes contaminated at the time of slaughter and grinding may compound the problem by introducing the pathogen into the interior of the meat, where it is more likely to survive cooking (Mead and Griffin, 1998). *Escherichia coli* O157:H7 generally, survives well in foods at refrigeration temperatures. Minimum water activity for growth is 0.95 (ICMSF, 1996). Ground beef is the most susceptible form of meat to microbial contamination during processing and handling and it is also the most susceptible to discoloration (Nam and Ahn, 2003). Most of the previous researches worked with essential oils or preservatives only. On the other hand, Seydim *et al.* (2006) worked with mixture from sodium lactate and rosemary oil and found that mixing of sodium lactate and rosemary oil gave considerable antimicrobial effect and was better than using each of them alone.

Therefore, the purpose of this study was to evaluate the antimicrobial activity of some essential oils and preservatives alone on the growth and survival of *E. coli* O157:H7 on solid and liquid media. Also, to study the effect of some essential oils and sodium lactate mixtures on the growth and survival of *E. coli* O157:H7 on liquid media. Moreover, to study the sensory evaluation for meat patties containing the mention different mixtures to select the most acceptable treatments. Finally, to evaluate the behavior of *E. coli* O157:H7 and quality of refrigerated minced meat with upon adding some mixtures of essential oils and sodium lactate to extend the shelf life of minced meat.

MATERIALS AND METHODS

Materials:

Source of essential oils:

Cumin (*Cuminum cyminum* L.), marjoram (*Origanum marjorana* L.), thyme (*Thymus vulgaris* L.), rosemary (*Rosmarinus officinalis L*), lemongrass (*Cymbopogon citrates L*), clove (*Dianthus coryophyllus L*) and Citronilla (*Cymbopogon nardus L.*) pure oils were obtained from sugar industrial integrated company (SIIC) Cairo, Egypt. Fragrance and extraction factory.

Preservatives:

Tri-sodium phosphate (TSP), sodium lactate (SL) and sodium acetate (SA), were obtained from El-Nasr Co., Cairo, Egypt.

Culture:

Escherichia coli O157:H7 ATCC 69373 strain was obtained from Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt and checked for purity.

Fresh minced beef meat:

Fresh minced beef meat was obtained from butcher shop in Kaha City, Qaliuobia Governorate and immediately transported to our laboratory.

Methods:

Cultures propagation:

E. coli O157:H7 strain was grown on Tryptic Soy Broth (TSB, Biolife) at 37°C for 16 hrs. (until the logarithmic phase) to make a stock and inoculation culture.

Determination of sensitivity:

The sensitivity of *E. coli* O157:H7 against the essential oils and preservatives was determined by the paper disc and spot diffusion methods (Sagdic *et al.*, 2002 and Schillinger and Lucke, 1989, respectively). Sterile paper discs (Whatman No 1, 6.0 mm in diameter) were soaked with essential oils, which serially diluted. The used dilutes were 1:1, 1:2, 1:4, 1:8 and 1:16 v/v of each oil extract in absolute ethanol (as a solvent agent). The dilutes were then filtered by a sterilized filter 45µm and collected in a sterilized vials, and absolute ethanol was also used as control. Preservatives were diluted in sterilized distilled

water (w/v) and used in the same manner as previously mentioned oils. After that the discs were placed on the surface of the *E. coli* O157:H7 inoculated plate count agar medium (PCA supplemented with tween 80, Biolife). On the other side, for spot diffusion method, 5µl of diluted essential oils or preservatives were spotted on the surface of the *E. coli* O157:H7 inoculated plate count agar medium. The plates were incubated at 37 ºC for 24-48 hr and the zones of inhibition were measured in mm.

Effect of essential oils or preservatives on growth of *E. coli* O157:H7 at 37 ºC:

Essential oils or preservatives were added to TSB (supplemented with tween 80 for essential oils only) bottles after autoclaving to make concentration 1, 2 and 3 % for each substances (except one bottle as a control) under aseptic condition. Each bottle was inoculated by *E. coli* O157:H7 to have a final number approximately 10^6 to 10^7. The inoculated bottles were incubated at 37 ºC for 7 days. Every day, 1 ml from each bottle was taken and *E. coli* O157:H7 was enumerated by using MacConkey sorbitol agar media (MSA, Biolife) then the inoculated plates were incubated at 37 ºC for 24-48 hr. Inhibition percentage was calculated according to Sagdic *et al.* (2002) as follows:

$$\text{Inhibition percentage} = \frac{\text{Control count} - \text{treatment count}}{\text{Control count}} \times 100$$

Effect of combined selected essential oils and sodium lactate on growth of *E. coli* O157:H7 at 37ºC:

Mixtures (21) were prepared from selected three essential oils (which gave high inhibition percentage against *E. coli* O157:H7) and sodium lactate to make a combination between each oil which dissolved in absolut ethanol and sodium lactate as indicated in Table (1).

Table (1): Mixtures formula from essential oils and sodium lactate (SL).

Oil concentration # %	Sodium lactate concentration*	Thyme oil (T) +SL	Marjoram oil (M) +SL	Rosemary oil (R) +SL
0.1	0.25	T1	M1	R1
0.25	0.50	T2	M2	R2
0.50	1.00	T3	M3	R3
0.75	1.50	T4	M4	R4
1.00	2.00	T5	M5	R5
1.25	2.50	T6	M6	R6
1.50	3.00	T7	M7	R7

calculated as (volume from oil/ volume from medium). *calculated as (weight from preservative/volume from medium).

Preparation of meat patties and sensory evaluation:

The meat was divided into 22 batches, 21 of them were formulated to contain all mentioned formulas in Table (1) without inoculation with *E. coli* O157:H7 (essential oil and sodium lactate were adding to minced meat as v/w and w/w, respectively) and the last one as control. Meat patties were made according

to Molins *et al.,* (1987). The meat was mixed by electric mincer meat "moulinex" Type 643- cod 211, France. This mixture was shaped using a commercial burger maker (9 cm diameter) to obtain patties of approximately 70g and 1cm thickness.

Sensory evaluation was done according to Liu *et al.* (1991). Meat patties were cooked by grilling for 3.5 min (2 min. on the first side and 1.5 min. on the other) to simulate a normal fast food restaurants. Sensory evaluation of the treatments included color, juiciness, flavor, texture and overall acceptability, with scores of 25, 25, 25, 25 and 100, respectively.

Minced meat preparation:

Fresh minced beef meat was inoculated by *E. coli* O157:H7 and divided into 7 batches, which formulated to contain mixtures T1, T2, M1, M2, R1, R2 or no additives (control), respectively. After adding the additives, the samples were mixed by electric meat mincer. Each sample was packaged in polyethylene bags, labeled and stored at 4 ºC$\pm$0.5. Minced meat was sampled at 3 days intervals during storage for analysis.

Physicochemical properties of refrigerated minced meat:

The following examination were done for fresh and refrigerated minced meat: Total volatile nitrogen (TVN) was measured, the results were calculated as mg TVN/100g. and thiobarbituric acid value (TBA.) was colorimeterically determined in minced meat as described by Harold *et al.* (1987). The pH values were measured using a pH meter model Consort P107 according to Defreitas *et al.,* (1997). Color index was determined according to Krzywicki (1982).

Microbiological examination:

The following determinations were done for fresh and refrigerated minced meat: Total viable bacterial count (TVBC), psychrophilic bacterial count (Ps.B) and coliform group (CG) were enumerated according to the methodology of the American Puplic Health Association (1992). *E. coli* O157:H7 was enumerated according to (Ogden *et al.,* 2001) using MacConkey sorbitol agar media (MSA, Biolife).

Statistical analysis:

ANOVA was carried out on data of the sensory evaluation applying the function of two factors with replicates, "Excel" Software of Microsoft office 2000. L.S.D. analysis was adapted according to Gomez and Gomaz (1984). Data are expressed as mean $\pm$ SE.

RESULTS AND DISCUSSION

Growth inhibition zones of *E. coli* O157:H7 by essential oils and preservatives.

Antimicrobial inhibition zone for some essential oils and preservatives against *E. coli* are shown in Table (2). *E. coli* showed high susceptibility with high concentration of essential oils and preservatives for spot and disc diffusion methods.

Table (2): Inhibition zones* (diameter, mm) of *E. coli* O157:H7 growth by essential oils and preservatives at five dilutions (diameter of paper disc, 6mm included).

Essential oils & preservatives	Essential oils and preservatives diluents										R^2 value
	1:1		1:2		1:4		1:8		1:16		
	D	S	D	S	D	S	D	S	D	S	
Control (ethanol)	6	6	6	6	6	6	6	6	6	6	---
Cumin oil	27	29	25	26	21	21	16	18	7	12	0.964
Marjoram oil	29	30	26	27	22	24	19	19	14	15	0.983
Thyme oil	27	45	13	29	11	26	10	19	9	13	0.879
Rosemary oil	17	35	12	22	11	17	11	14	9	10	0.967
Lemongrass oil	29	34	27	28	25	22	17	18	13	18	0.778
Clove oil	18	32	14	24	13	22	9	18	8	17	0.962
Citronella oil	17	26	16	23	10	18	8	17	8	14	0.930
Tri-sodium phosphate	14	20	13	17	11	11	10	9	8	8	0.894
Sodium lactate	20	22	16	14	11	11	8	10	7	9	0.907
Sodium acetate	10	12	8	10	6	6	6	6	6	6	0.908

D: Disc method **S**: Spot method

* The values were calculate as average for three replicates.

Nearly the inhibition zone of spot was bigger than disc diffusion methods. This variation of zone diameter was related to the different volume of essential oils and preservatives on the two methods, meanwhile, the response of scatter plots exhibited a high degree of relationship between the paper disc and spot diffusion methods (R^2 value) for detect sensitivity of *E. coli* O157:H7 to the essential oils and preservatives except lemongrass oil. Our results are in agreement with many workers such as Oussalah *et al*., (2006), Dusan *et al*., (2006) Moreira *et al*., (2005), and Sagdic *et al*., (2002) and can be attributed the effect to factors such as growth stage, ecological condition, and other plant factor and the essential oils are a heterogeneous group of complete mixtures organic substances (Ozcan and Erkmen, 2001).

Effect of essential oils and preservatives on growth of *E. coli* O157:H7 during seven days on TSB at 37ºC.

Nearly in this study, the disc and spot methods were done just to know the degree of inhibition for essential oils and preservatives on *E. coli* O157:H7. So, the effect of essential oils and preservatives against growth of *E. coli* on broth media was investigated. Fig. (1) showed the effect of essential oils and preservatives at different concentrations on the survival of *E. coli* O157:H7 at 37 ºC during seven days. In addition, inhibition percentage of essential oils and preservatives was given in Table (3). The influence of the additives appeared to depend on the degree of concentration of the additives. About, the essential oils, generally all of them have a bacteriostatic effect on the growth of *E. coli* O157:H7. Also, a bactericidal effect was shown at high concentrations of essential oils during the incubation period. Thyme oil at 2 and 3 % showed the bactericidal effect for fifth and second days, respectively. In addition, other concentrations of thyme oil exhibited a bacteriostatic effect on the strain. Some workers such as Cosentino *et al.,* (1999), Dorman and Deans (2000) and Rauha *et al.*

(2000) reported that thyme oil showed an inhibitory effect on *E. coli* O157:H7. Marjoram oil at 2 and 3% showed the bactericidal effect on the third and sixth days, respectively. These results are coinciding with Sagdic *et al.* (2002) and Burt and Reinders (2003). Rosemary oil at 2 and 3% showed bactericidal effect for seventh and third days, respectively. These results are in agreement with Hammer *et al.*, 1999 and Pintore *et al.* (2002). Cumin oil has bactericidal effect at 2 and 3% for sixth and seventh days, respectively. These results are coincide with those obtained by Smith-Palmer *et al.* (1998), Hammer *et al.* (1999) and Dzcan and Erkmen (2001).

Table (3): Inhibition percentage (IP) of essential oils and preservatives on *E. coli* O157:H7 during seven days in TSB at 37ºC.

Additives		First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day
Cumin oil	1%	13.45	40.08	41.52	38.61	51.93	47.01	70.40
	2%	30.56	50.28	60.36	60.90	61.13	62.96	100.00
	3%	33.95	52.01	55.54	67.43	68.19	100.00	100.00
Marjoram oil	1%	32.86	52.40	52.35	50.44	37.95	30.03	44.76
	2%	42.66	49.67	61.81	63.18	63.42	100.00	100.00
	3%	56.03	73.14	100.00	100.00	100.00	100.00	100.00
Thyme oil	1%	27.76	43.64	50.48	48.26	50.76	50.12	61.68
	2%	36.84	47.62	63.62	67.86	100.00	100.00	100.00
	3%	48.09	100.00	100.00	100.00	100.00	100.00	100.00
Rosemary oil	1%	30.09	44.45	47.75	53.95	65.35	64.38	63.57
	2%	38.80	44.39	57.25	64.82	68.28	77.43	100.00
	3%	42.09	59.62	74.12	100.00	100.00	100.00	100.00
Lemongrass oil	1%	27.71	45.13	43.14	36.30	43.15	31.03	44.94
	2%	36.84	47.23	54.84	39.93	61.85	60.40	63.85
	3%	36.95	48.10	72.39	68.41	73.03	64.05	100.00
Clove oil	1%	17.60	35.50	52.43	48.93	52.92	53.15	56.83
	2%	27.56	43.31	54.61	61.66	58.66	58.63	59.06
	3%	29.13	50.74	55.20	58.47	62.49	72.27	73.06
Citronella oil	1%	14.09	22.53	27.25	15.92	15.96	7.56	8.97
	2%	16.14	34.21	28.57	22.39	17.94	15.44	18.86
	3%	29.26	46.67	36.46	33.08	23.54	17.09	17.81
Tri-Sodium Phosphate	1%	24.27	38.85	39.20	46.31	44.18	38.12	54.73
	2%	15.04	38.91	51.47	43.14	58.25	59.51	100.00
	3%	45.89	63.62	65.17	69.27	100.00	100.00	100.00
Sodium Lactate	1%	14.37	33.28	31.75	46.28	45.41	41.81	50.88
	2%	45.25	50.30	64.02	65.16	68.73	100.00	100.00
	3%	59.18	69.50	100.00	100.00	100.00	100.00	100.00
Sodium Acetate	1%	19.19	35.75	37.58	39.09	43.53	55.16	59.02
	2%	15.39	39.30	41.72	54.89	66.82	68.41	100.00
	3%	35.90	56.01	72.01	100.00	100.00	100.00	100.00

Three percent of lemongrass oil was bactericidal at seventh day. While, clove oil has a bacteriostatic effect with all concentrations. These results are in

harmony with Smith-Palmer *et al.* (1999) and Hammer *et al.* (1999). Citronella oil was effective on the growth of *E. coli* O157:H7 but less than clove oil. The effect (inhibition percentage) for all oils was shown to be high with increasing the incubation period. From the obtained data, the effectiveness of the essential oils nearly followed the sequence: thyme > marjoram > rosemary > cumin > lemongrass > clove > citronella. With regard to the preservatives, the data in Table (3) indicated that 3% of sodium lactate, sodium acetate and tri-sodium phosphate had bactericidal for third, forth and fifth days, respectively. While 2 % of the same preservatives was bactericidal for sixth, seventh and seventh days, respectively. So, sodium lactate was the best effectiveness among others. The trend of our results was in agreement with Jordan *et al.* (1999), McWilliam Leithal and Stewat (2002) and Whiting and Golden (2003).

Effect of combined selected essential oils and sodium lactate against *E. coli* O157:H7 during seven days on TSB at 37ºC:

From the previous experiment we found that thyme, marjoram and rosemary oils had the best effect against *E. coli* O157:H7 and 1% of selected oils had bacteriostitic properly. So, the effect of low concentration of oils against *E. coli* O157:H7 beside the accumulating effect between oil and sodium lactate were studied. Effect of twenty one mixtures as formulated in Table (1) against *E. coli* O157:H7 during seven days on TSB at 37ºC was investigated to select the highly effective mixtures with low concentration.

Table (4) showed the combined effect of essential oils and sodium lactate at different concentration of the survival of *E. coli* O157:H7 during seven days at 37ºC. The mixture No. 7 form all combinations had a highly bactericidal effect after the first day. Also, the mixture No. 6 for thyme and marjoram oils had a highly bactericidal effect after the first day. The mixture No.1 form all others had bacteriostatic effect only. On the other hand, the mixture No. 2 for thyme oil only showed bactericidal effect after the fifth day. Generally for other mixtures the Fig. (2) illustrated that the bactericidal effect of (thyme oil + lactate) occurred early before (marjoram oil + lactate) and (rosemary oil + lactate), respectively. Oussalah *et al.*, (2006), Dusan *et al.*, (2006) Moreira *et al.*, (2005), and Sagdic *et al.*, (2002) working with essential oils or preservatives only except Seydim *et al.*, (2006) found that mixing of sodium lactate and rosemary oil observed highly antimicrobial effect better than using each of them alone.

Sensory evaluation of untreated and treated meat patties with the mixture of essential oils and sodium lactate:

As in all foods, the organoleptic tests are generally the final guide of the quality from the consumers point of view. Thus, it is beneficial to make a comparison between sensory evaluation for untreated and treated meat patties with the mixture of essential oils and sodium lactate. The obtained data are tabulated in Table (5). To study the acceptability of untreated and treated meat patties with the mixture of essential oils and sodium lactate, colour, juiciness, flavor, texture and overall acceptability were evaluated.

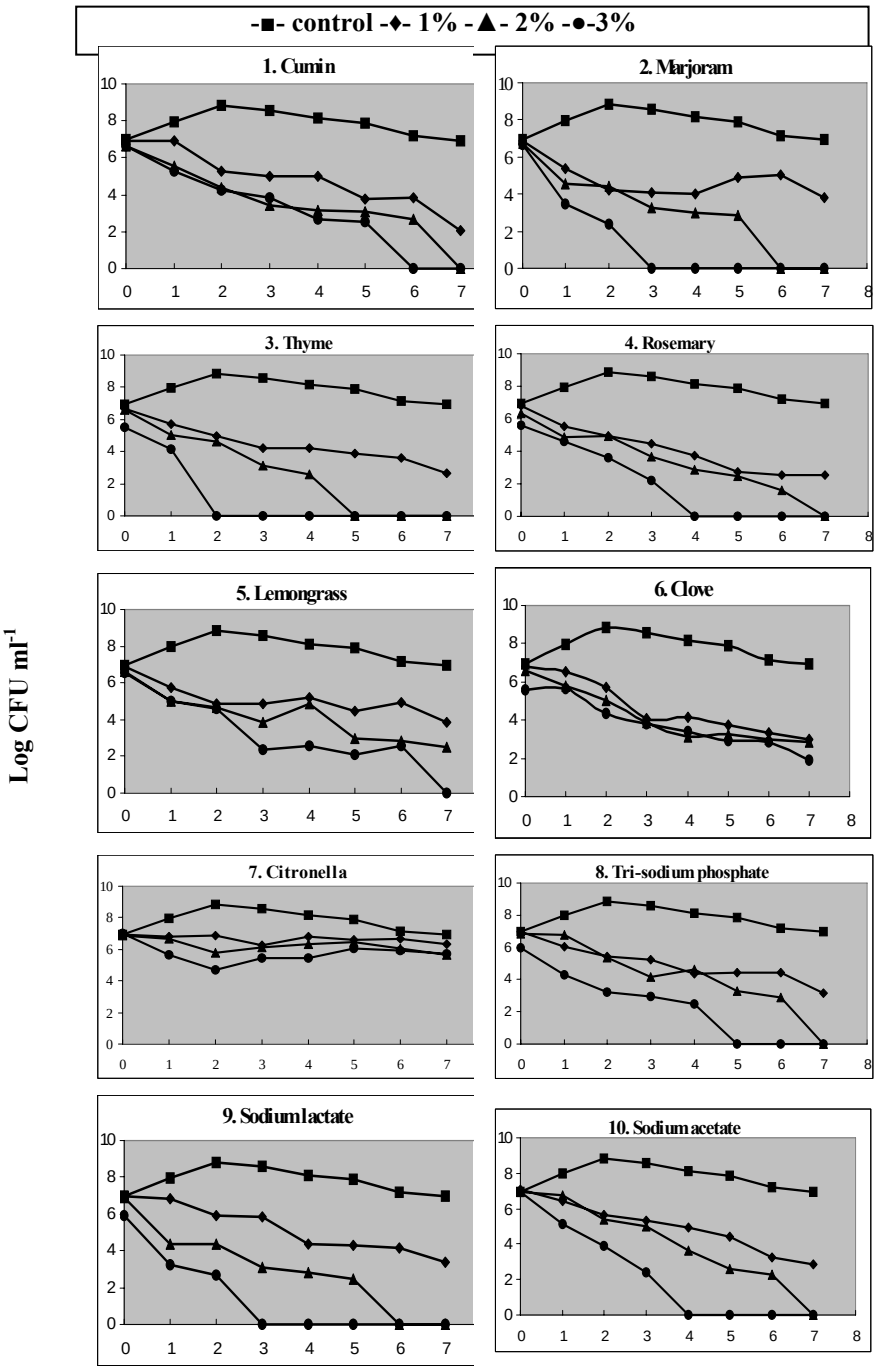

Fig. (1): Effect of essential oils and preservatives on survival of *E. coli* O157:H7 during seven days on TSB at 37 ºC.

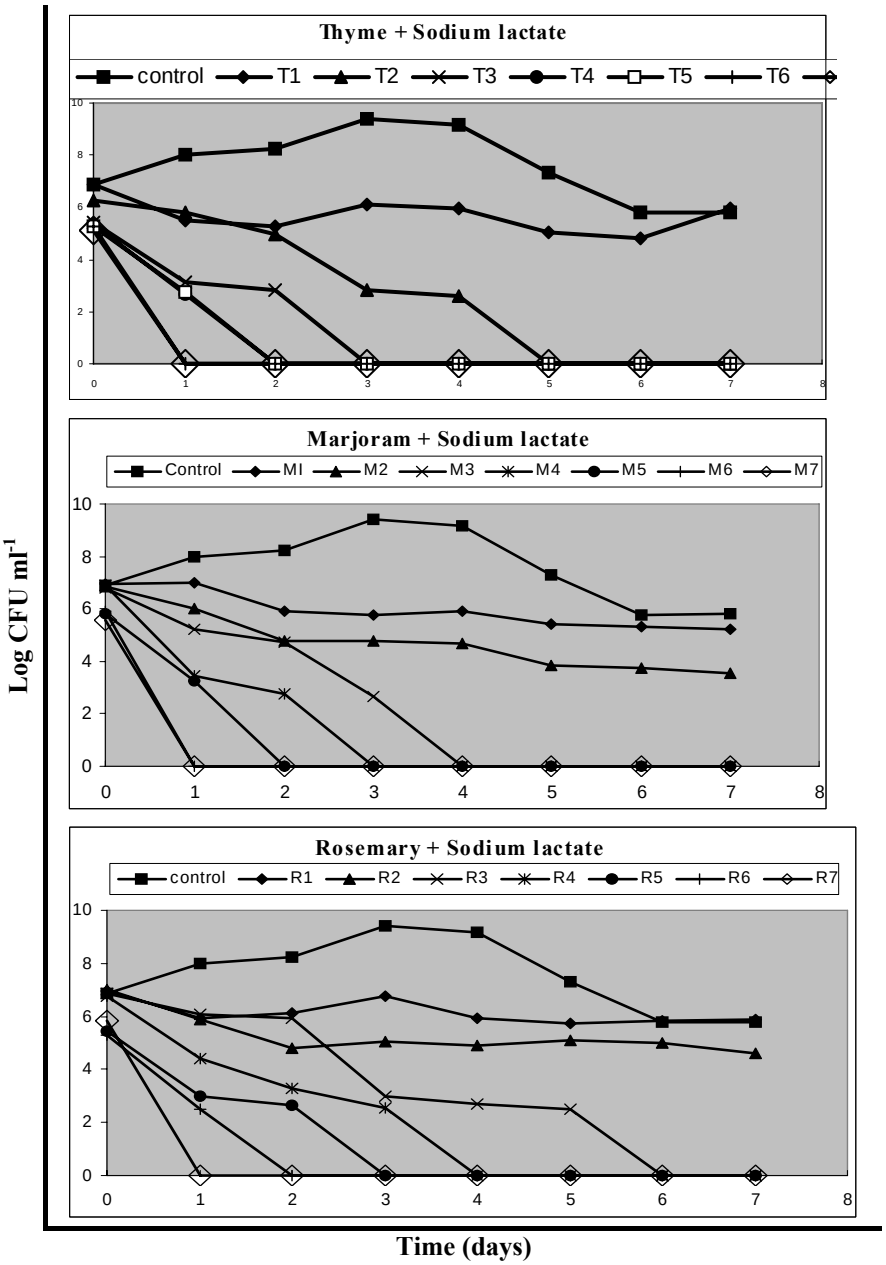

Fig. (2): Effect of combination between selected essential oils (thyme, marjoram and rosemary) and sodium lactate on survival of *E. coli* O157:H7 during seven days in TSB at 37ºC.

Table (4): Effect of combined selected essential oils and sodium lactate against *E. coli* O157:H7 during seven days in TSB at 37ºC.

Additives		IP*						
		First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day
Thyme oil + sodium lactate (T)	T1	31.57	35.62	35.00	34.83	30.74	17.10	-2.67
	T2	27.30	39.36	69.99	72.07	100.00	100.00	100.00
	T3	60.99	65.82	100.00	100.00	100.00	100.00	100.00
	T4	66.95	100.00	100.00	100.00	100.00	100.00	100.00
	T5	65.15	100.00	100.00	100.00	100.00	100.00	100.00
	T6	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	T7	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Marjoram oil + sodium lactate (M)	M1	12.53	28.31	38.43	35.42	25.74	8.30	9.88
	M2	24.78	41.65	49.11	48.97	47.04	35.56	38.97
	M3	34.47	42.75	71.56	100.00	100.00	100.00	100.00
	M4	56.70	66.25	100.00	100.00	100.00	100.00	100.00
	M5	59.07	100.00	100.00	100.00	100.00	100.00	100.00
	M6	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	M7	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Rosemary oil + sodium lactate (R)	R1	25.44	25.52	28.02	35.50	21.60	-1.14	-1.72
	R2	26.40	41.44	46.18	46.58	29.96	13.35	20.55
	R3	23.98	27.96	68.43	70.48	65.97	100.00	100.00
	R4	44.71	59.89	73.06	100.00	100.00	100.00	100.00
	R5	62.36	67.71	100.00	100.00	100.00	100.00	100.00
	R6	68.88	100.00	100.00	100.00	100.00	100.00	100.00
	R7	100.00	100.00	100.00	100.00	100.00	100.00	100.00

* Inhibition percentage

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As shown in Table (5) ANOVA indicated that there are high significant differences ($P < 0.01$) between different concentrations and between different oils. On the other hand, the scores of the meat patties showed high significant differences in colour, juiciness, flavor, texture and overall acceptability. These results may depend on the effect of essential oils and sodium lactate. The meat patties which contain mixture concentration 1 and 2 had the higher score, while the other mixtures concentration had the smaller scores in all sensory attribute.

These results for (flavor and overall acceptably) may be related to the effect of the type of essential oils more than sodium lactate. Regardless that sodium lactate was acceptable in meat products from 0.5- 4.8% with no deleterious effect on sensory properties (Shelef, 1994; Sallam and Samejima, 2004 and USDA/FSIS, 2000). From all the scores of attributes especially overall acceptability we can show that the mixtures 1&2 had a high score. In the previous researches, the authors focused on the effect of essential oils on pathogenic microbes on liquid or solid media without application in food system to know the acceptability of essential oils except (Fernandes-Lopes *et al.,* 2005 and Seydim, *et al.,* 2006).

Table (5): Sensory evaluation of untreated and treated meat patties with the mixture of essential oils and sodium lactate (mean ± stander error).

A. Color					
Mixture concentration (oils+sodium lactate)	**Mixtures**				**Average**
	Control	**Thyme oil + sodium lactate**	**Marjoram oil + sodium lactate**	**Rosemary oil + sodium lactate**	
0.1 + 0.25	22.0± 0.41	23.08±0.34	22.25±0.33	22.58±0.29	22.48±0.18a
0.25 + 0.5		21.92±0.34	21.92±0.34	21.17±0.84	21.75±0.26a
0.5 + 1.0		20.41±0.43	20.67±0.41	19.75±0.39	20.71±0.23b
0.75 + 1.5		18.58±0.71	19.83±0.55	19.42±0.63	19.69±0.34c
1.0 + 2.0		18.67±0.69	18.58±0.87	17.91±0.78	19.29±0.41c
1.25 + 2.5		16.83±1.20	18.25±0.84	18.5±0.88	18.90±0.51cd
1.5 + 3.0		15.33±0.33	15.17±0.42	13.41±0.81	16.47±0.54e
Average		19.26±0.73a	19.52±0.33a	18.46±0.39ab	
LSD (concentration)		**0.74**			
LSD (mixture)		**0.82**			
LSD (mixture within concentration)		**1.64**			
B. Juiciness					
0.1 + 0.25	23.25± 0.49	22.67±0.26	21.92±0.34	22.17±0.34	22.50±0.19a
0.25 + 0.5		20.58±0.48	21.00±0.37	22.00±0.30	21.71±0.25b
0.5 + 1.0		20.08±0.66	19.67±0.61	19.25±0.68	20.56±0.38c
0.75 + 1.5		17.50±0.57	18.08±0.61	18.00±0.95	19.21±0.47d
1.0 + 2.0		15.33±0.99	17.08±0.92	16.42±0.87	18.02±0.61e
1.25 + 2.5		14.92±0.51	15.75±1.09	16.92±0.10	17.71±0.63e
1.5 + 3.0		10.92±0.36	11.00±0.63	9.92±0.34	13.77±0.83f
Average		17.43±0.46a	17.79±0.45a	17.81±0.50a	
LSD (concentration)		**0.78**			
LSD (mixture)		**0.88**			
LSD (mixture within concentration)		**1.76**			
C. Flavor					
0.1 + 0.25	23.17± 0.44	22.67±0.58	22.92±0.19	22.75±0.28	22.88±0.20a
0.25 + 0.5		20.83±0.72	20.75±0.30	20.75±0.97	21.38±0.35b
0.5 + 1.0		17.92±0.70	18.50±0.60	17.25±1.30	19.21±0.49c
0.75 + 1.5		12.92±0.82	16.08±0.80	17.17±0.83	17.33±0.65d
1.0 + 2.0		10.25±0.85	13.75±0.82	14.25±1.16	15.35±0.81e
1.25 + 2.5		8.25±0.39	11.25±1.18	15.75±1.07	14.60±0.92f
1.5 + 3.0		7.42±0.36	7.92±0.31	8.83±0.47	11.83±0.98g
Average		14.32±0.67b	15.88±0.60a	16.68±0.60a	
LSD (concentration)		**0.85**			
LSD (mixture)		**0.95**			
LSD (mixture within concentration)		**1.90**			

Table (5): Cont.

D. Texture					
Mixture concentration (oils+sodium lactate)	**Mixtures**				**Average**
	Control	**Thyme oil + sodium lactate**	**Marjoram oil + sodium lactate**	**Rosemary oil + sodium lactate**	
0.1 + 0.25	22.50±0.47	22.25±0.39	22.17±0.39	22.17±0.39	22.27±0.20a
0.25 + 0.5		21.17±0.42	20.92±0.38	20.33±0.91	21.23±0.31b
0.5 + 1.0		19.25±0.59	19.42±0.47	18.00±0.81	19.79±0.38c
0.75 + 1.5		17.58±0.61	18.83±0.55	18.50±0.98	19.35±0.43c
1.0 + 2.0		13.92±1.31	16.75±0.99	16.67±1.05	17.46±0.67d
1.25 + 2.5		14.00±1.07	14.92±1.05	18.61±1.25	17.19±0.68d
1.5 + 3.0		9.25±0.51	9.17±0.67	8.67±0.54	12.40±0.89e
Average		16.77±0.55a	17.45±0.51a	17.38±0.54a	
LSD (concentration)		**0.89**			
LSD (mixture)		**1.00**			
LSD (mixture within concentration)		**2.00**			
E. Overall acceptability					
0.1 + 0.25	92.58±1.83	93.92±1.38	91.67±1.31	91.92±0.85	92.52±0.68a
0.25 + 0.5		88.83±1.08	84.00±1.42	83.08±4.04	87.13±1.28b
0.5 + 1.0		77.17±2.11	76.42±1.78	78.25±3.70	81.10±1.55c
0.75 + 1.5		62.17±1.66	63.75±1.96	70.33±3.59	72.21±2.11d
1.0 + 2.0		47.75±1.86	51.85±1.79	57.67±4.84	62.40±2.95e
1.25 + 2.5		43.67±1.73	47.25±3.80	56.08±5.40	59.90±3.27e
1.5 + 3.0		32.83±2.22	41.75±1.27	45.92±3.08	53.27±3.55f
Average		63.76±2.48b	65.20±2.10b	69.04±2.18a	
LSD (concentration)		**3.12**			
LSD (mixture)		**3.48**			
LSD (mixture within concentration)		**6.97**			

Values represent of 12 panelists (Mean+S.E)

A, b three is no significant difference ($P \geq 0.05$) between any two averages of different meat patties treatment, or different additives concentration have the same superscripts, within the same acceptability attribute.

From data on sensory evaluation we can recommend the use of low concentration from essential oils in minced meat. Therefore, minced meat were treated by those mixtures and inoculated by *E. coli* O157:H7 and stored at 4ºC to study the behavior of *E. coli* O157:H7 and consequently the quality of refrigerated minced meat.

Physicochemical properties of refrigerated minced meat:

The mean values of total volatile nitrogen (TVN) are given in Table (6). With the progression of cold storage TVN rapidly increased for all investigated treatments occurred with different rates depending on the initial treatments. The

control samples showed the highest incremental rate compared to other treatments and had TVN about 20.74 mg/100g at 6 days. However, the TVN values of about 20 mg TVN/100g raw samples indicates spoilage of minced meat (Egyptian Standards, 2005). Along shelf life storage (15 days) were found for sample treated with thyme oil (0.25)+sodium lactate (0.5) which had 17.97 mg TVN/100g. The same result was found for sample treated with same concentration of marjoram oil and sodium lactate. First treated sample spoiled was found at 12 days for sample treated with rosemary oil (0.10)+sodium lactate (0.25), followed by other treated sample with the same concentration at 15 days. The samples treated with thyme oil and sodium lactate showed the lowest incremental rate compared to other treatments. This is may be due to the effectiveness of this essential oil and sodium lactate on microorganisms. The progressive increase in TVN during cold storage might be attributed to the breakdown of nitrogenous substances as a result of microbial activity and any autolytic enzymes found naturally in meat tissues.

Concerning the thiobarbituric acid (TBA) values as lipid oxidation indicator, it could be noticed that the TBA values were increased for all the stored samples with advancing the chilled storage time may be due to the auto-oxidation of meat lipids, bacteriological and/or oxidative rancidity. The incremental rate of TBA was rapidly increased for control sample which nearly higher than the treated sample in any samples period. After 6 days the control sample had TBA value about 0.959 mg malonaldehyde/kg that higher than 0.9 mg malonaldehyde/kg recommended by the Egyptian standard (2005). The samples treated with thyme oil+sodium lactate still acceptable until 15 days as indicated by TBA values. Samples treated with high concentration of marjoram oil+sodium lactate showed the same trend while samples treated with low concentration showed opposite trend which was refused in the same time. While the sample treated by rosemary oil was refused at 12 days (Table, 6).

In the same Table, it could be noticed that at zero time the pH value was higher for control sample than that of treated samples. This may be due to addition of sodium lactate and pH values were decreased with increasing percentage addition of sodium lactate. The pH values gradually increased during the storage period for control and all treated samples. The sample treated with rosemary oil had pH values higher than other treated samples during the storage period. Changing in pH values may be due to the activation effect of microbial load which may cause protein hydrolysis with the appearance of alkaline groups.

Data in Table (6) indicated the color index of control and different treated minced meat samples. The color index was ranged from 0.975 to 0.820 (as O.D. at 540 nm) at zero time of cold storage. During subsequent cold storage the color index was generally decreased with increasing the time. During cold storage color deterioration of control and different treated minced meat was occurred. This may be due to many reasons, i.e. the oxidation of myoglobin and oxymyoglobin to metamyoglobin, a scape of some pigments (as water soluble protein) with and the decomposition of pigments as a result of bacterial action. The control sample had a decremental trend higher than treated sample and had pigment concentration of 0.319 O.D. at 540 nm at 6 days (sample was rejected). Addition of essential oils and sodium lactate showed an important effect on pigments concentration. Minced meat samples

treated by high concentration of thyme oil+sodium lactate had highly stability followed by treated samples with marjoram oil and rosemary oil at 15 days of storage. This action may be due to the effect retarding oxidation process as well as due to its antibacterial action.

Microbiological quality attributes of untreated and treated minced meat during cold storage:

Total viable bacterial count (TVBC): It is evident (Table, 7) that a slight increase in TVBC was noticed in control sample when compared with other treatments at zero time of cold storage. This may indicate that essential oils and sodium lactate caused sudden lethal effect for microorganisms . From the results in Table (7) it could be observed that control sample had always high TVBC when compared with treated samples. Moreover sample treated by thyme oil had low TVBC when compared with other treated samples during subsequent cold storage. In this concern, according to the Egyptian standard (2005), the self life of treated minced meat was 12 days except the sample treated with high concentration of thyme and marjoram oils which had self life until 15 days, corresponding only 6 days for control sample.

Psychrophilic bacteria (Ps. B): The initial numbers of (Ps. B) of untreated and treated minced meat were lower than initial number of (TVBC). During chilling storage time a similar trend of (Ps. B) was found as (TVBC). The development rate of (Ps. B) count was progressively increased more than (TVBC), ascribed to optimum temperature condition in chilling storage.

Data presented in Table (7) show the number of (CG) of various investigated samples during cold storage at 4±0.5°C. Rapidly increasing in control sample, but slowly increasing in treated sample was shown after 6 days. At end of storage periods, the CG load of treated sample not reached to the CG load of control sample. This changes were occurred as a result of essential oils and sodium lactate addition. On the other hand, *E. coli* O157:H7 was counted in all inoculated samples to study the effect of addition of essential oils and sodium lactate on retarding inoculated strain. The initial *E. coli* O157:H7 counts were $7.15x10^3$ CFU ml^{-1} in control sample. While, the treated sample had low number of *E. coli* O157:H7 as shown at zero time that could be related to the effect of essential oils and sodium lactate addition . A decremental trend during chilled storage in treated sample was occurred and return to increase in the end of storage period. But sample treated with high concentration of essential oil and sodium lactate had lower *E. coli* O157:H7 load than low concentration treated samples. The interaction between cold storage and addition treatments given low increase rate in microbial *E. coli* O157:H7 load for treated samples. While, control samples had a rapid increase rate in *E. coli* O157:H7 load. These findings are correlated with the other results of some physicochemical properties obtained in the present investigation. From all results for refrigerated minced meat there are relationship between physicochemical characters especially TVN and TBA corresponding with microbiological quality. Moreover, if any foods contaminated with *E. coli* O157:H7 the number may be lower than the number of inoculated samples. So, it is possible to recommend to treat meat or some meat products with natural additives such as essential oils in combination with sodium lactate to keep them in good microbiological and chemical quality.

Table (6): Some phsicochemical properties of minced meat stored at 4±0.5°C.

Storage period (days)	TVN						
	Control	Thyme oil+ sodium lactate		Marjoram oil + sodium lactate		Rosemary oil + sodium lactate	
		0.1+ 0.25	0.25+0.5	0.1+ 0.25	0.25+0.5	0.1+ 0.25	0.25+0.5
0	9.44± 0.65	9.00±0.52	8.80±0.13	9.47±0.43	9.37±0.33	8.64±0.7	9.31±0.42
2	12.60±0.4	-	-	-	-	-	-
3	14.70+0.44	11.74±0.63	10.83±0.34	12.17±0.3	11.63±0.37	11.77±0.42	12.36±0.23
4	17.20±0.78	-	-	-	-	-	-
6	20.74±0.22	14.34±0.18	13.3±0.40	15.77±0.20	14.42±0.21	15.92±0.41	15.02±0.54
9	-	15.60±0.45	14.72±0.21	16.80±0.57	15.04±0.47	18.43±0.62	15.74±0.58
12	-	18.90±0.40	15.67±0.27	18.20±0.16	15.67±0.08	20.95±0.12	17.80±0.59
15	-	20.25±0.17	17.97±0.17	20.63±0.31	18.67±0.12	22.54±0.63	20.30±0.40
TBA							
0	0.020±0.528	0.009±0.489	0.416±0.003	0.507±0.016	0.512±0.019	0.504±0.018	0.533±0.023
2	0.705±0.009	-	-	-	-	-	-
3	0.718±0.008	0.523±0.016	0.442±0.009	0.546±0.003	0.546±0.014	0.538±0.009	0.634±0.011
4	0.793±0.023	-	-	-	-	-	
6	0.959±0.024	0.603±0.007	0.502±0.019	0.614±0.011	0.619±0.007	0.679±0.024	0.697±0.007
9	-	0.616±.009	0.528±0.019	0.767±0.029	0.705±0.009	0.775±0.026	0.806±0.011
12	-	0.621±0.005	0.562±0.030	0.811±0.020	0.731±0.009	0.926±0.011	0.915±0.007
15	-	0.663±0.018	0.666±0.018	0.915±0.007	0.770±0.011	1.032±0.054	0.936±0.012
pH							
0	5.56±0.03	5.23±0.02	4.88±0.01	5.19±0.00	4.90±0.03	5.21±0.01	4.89±0.01
2	5.82±0.03	-	-	-	-	-	-
3	5.93+0.04	5.37±0.01	5.19±0.01	5.23±0.02	5.21±0.02	5.82±0.03	5.35±0.01
4	6.05±0.02	-	-	-	-	-	-
6	6.23±0.05	5.41±0.01	5.29±0.02	5.51±0.01	5.43±0.02	5.54±0.01	5.45±0.01
9	-	5.50±0.01	5.4±0.01	5.60±0.01	5.50±0.01	5.89±0.04	5.54±0.01
12	-	5.83±0.02	5.45±0.02	6.10±0.05	5.76±0.03	6.29±0.03	5.82±0.01
15	-	6.13±0.04	5.68±0.04	6.34±0.02	5.90±0.05	6.38±0.05	6.16±0.03
CI							
0	0.914±0.005	0.975±0.004	0.916±0.004	0.887±0.005	0.937±0.004	0.888±0.004	0.820±0.004
2	0.644±.008	-	-	-	-	-	-
3	0.567±0.017	0.819±0.002	0.892±0.001	0.788±0.003	0.818±0.004	0.754±0.005	0.733±0.005
4	0.490±0.006	-	-	-	-	-	-
6	0.319±0.004	0.744±0.004	0.771±0.006	0.635±0.012	0.769±0.005	0.675±0.013	0.570±0.002
9	-	0.668±0.026	0.710±0.008	0.572±0.028	0.677±0.048	0.519±0.002	0.442±0.012
12	-	0.502±0.029	0.523±0.011	0.532±0.010	0.505±0.011	0.413±0.026	0.383±0.004
15	-	0.386±0.012	0.458±0.021	0.401±0.012	0.404±0.012	0.319±0.007	0.308±0.009

TVN : Total volatile nitrogen (mg/100 gram)
TBA: Thiobarbituric acid (mg malonaldehyde/kg).
CI: color index as optical density

Table (7): Microbiological quality of minced meat stored at 4±0.5°C.

Storage period (days)	TVBC						
	Control	Thyme oil + Sodium lactate		Marjoram oil + Sodium lactate		Rosemary oil + Sodium lactate	
		0.1+ 0.25	0.25+0.5	0.1+ 0.25	0.25+0.5	0.1+ 0.25	0.25+0.5
0	$5.90x10^4$	$3.10x10^4$	$1.49x10^4$	$4.60x10^4$	$1.49x10^4$	$5.35x10^4$	$3.52x10^4$
2	$8.30x10^4$	--	--	--	--	--	--
3	$1.54x10^5$	$2.70x10^4$	$3.95x10^3$	$8.40x10^4$	$1.79x10^4$	$4.50x10^4$	$1.89x10^4$
4	$2.01x10^5$	--	--	--	--	--	--
6	$1.50x10^6$	$4.65x10^3$	$2.10x10^3$	$5.10x10^3$	$2.95x10^3$	$8.80x10^3$	$3.60x10^3$
9	--	$5.35x10^4$	$5.30x10^3$	$1.42x10^4$	$9.10x10^3$	$1.49x10^5$	$1.51x10^4$
12	--	$2.86x10^5$	$9.60x10^3$	$2.03x10^5$	$1.55x10^5$	$5.95x10^5$	$1.62x10^4$
15	--	$1.17x10^6$	$4.55x10^4$	$7.75x10^6$	$2.71x10^5$	$2.41x10^6$	$1.71x10^6$
Ps. B							
0	$2.10x10^3$	$3.40x10^3$	$1.90x10^2$	$7.30x10^3$	$6.15x10^2$	$3.70x10^3$	$8.90x10^2$
2	$5.50x10^3$	--	--	--	--	--	--
3	$1.55x10^4$	$2.50x10^3$	$4.00x10^2$	$5.10x10^3$	$2.70x10^2$	$3.45x10^3$	$7.00x10^2$
4	$8.55x10^4$	--	--	--	--	--	--
6	$2.90x10^6$	$6.85x10^3$	$3.25x10^2$	$7.30x10^3$	$3.05x10^3$	$5.15x10^4$	$4.05x10^3$
9	--	$7.05x10^4$	$9.50x10^3$	$8.70x10^4$	$4.55x10^3$	$4.45x10^5$	$5.35x10^3$
12	--	$2.19x10^5$	$1.26x10^4$	$2.04x10^5$	$1.61x10^5$	$1.94x10^6$	$8.25x10^5$
15	--	$5.55x10^6$	$7.10x10^4$	$6.60x10^6$	$1.86x10^5$	$7.60x10^6$	$9.05x10^6$
CG							
0	$5.60x10^3$	$5.50x10^3$	$3.10x10^3$	$5.10x10^3$	$4.00x10^3$	$6.15x10^3$	$3.55x10^3$
2	$4.10x10^4$	--	--	--	--	--	--
3	$8.90x10^4$	$4.40x10^3$	$2.65x10^3$	$8.70x10^3$	$1.70x10^3$	$4.15x10^3$	$1.00x10^3$
4	$1.31x10^5$	--	--	--	--	--	--
6	$3.35x10^5$	$2.59x10^3$	$4.00x10^2$	$6.10x10^2$	$3.45x10^2$	$1.07x10^3$	$7.60x10^2$
9	--	$6.00x10^3$	$6.00x10^2$	$9.10x10^2$	$4.00x10^2$	$3.05x10^3$	$8.90x10^2$
12	--	$2.02x10^4$	$5.15x10^3$	$3.65x10^4$	$8.15x10^2$	$8.35x10^4$	$9.20x10^3$
15	--	$5.75x10^4$	$3.20x10^3$	$1.15x10^5$	$5.40x10^3$	$5.30x10^2$	$3.75x10^4$
***E. coli* O157:H7**							
0	$7.15x10^3$	$5.95x10^3$	$4.10x10^3$	$5.35x10^3$	$4.65x10^3$	$5.70x10^3$	$6.60x10^3$
2	$5.10x10^4$	--	--	--	--	--	--
3	$6.70x10^4$	$4.95x10^3$	$2.30x10^3$	$4.80x10^3$	$4.10x10^3$	$3.50x10^3$	$1.70x10^3$
4	$8.11x10^4$	--	--	--	--	--	--
6	$2.30x10^5$	$4.17x10^3$	$1.10x10^3$	$1.40x10^3$	$3.10x10^3$	$1.02x10^4$	$1.05x10^3$
9	--	$3.00x10^3$	$3.70x10^2$	$1.25x10^3$	$3.55x10^2$	$7.20x10^4$	$4.70x10^3$
12	--	$9.30x10^3$	$5.56x10^2$	$2.81x10^3$	$3.35x10^3$	$1.55x10^5$	$4.85x10^3$
15	--	$3.10x10^4$	$1.10x10^3$	$4.65x10^5$	$1.90x10^3$	$4.60x10^5$	$1.67x10^5$

(TVBC) total viable bacterial count, (Ps. B) psychrophilic bacteria and (CG) coliform group.

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