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EFFECT OF SOME PLANT ESSENTIAL OILS AS NATURAL ANTIMICROBIAL IN WHITE CHEESE

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ABETRACT

Fifty samples of home-made cheese were investigated in 2006/2007 autumn for their microbiological quality immediately after made (fresh) and after storage for various duration periods and at different conditions. Spearmint, Clove and Basil essential oils (*EOs*) were purchased from Egyptian markets. The activity of those essential oils against some white cheese pathogenic bacteria was investigated. Spearmint *EO* had the highest organoleptic score (fresh white cheese) among all tested *EOs*, followed by Basil and Clove, especially in low concentration.

The results showed significant differences ($p < 0.05$) between control samples (free from *EOs*) and supplemented samples with different doses of *EOs* "0.25, 0.5 and 1.0 %" at different temperatures (about 22 and 5°C) in aerobic plate count. Control white cheese samples which stored for 2 days or more at room temperature were unsafe. However, all white cheese samples added with *EOs* at the 0.5, and 1.0 % and examined after storage at room temperature and chill temperature for 7 days were satisfied bacteriological quality. The microbiological quality for white cheese with *EOs* additives after cooling of milk "37°C" during preparation of cheese was better than those of it before heating of milk "72°C".

INTRODUCTION

Dairy products are considered a very important sector in the food industry (George, 1994). In dairy products processing, the most common type of microbial spoilage is bacteria and moulds and in many cases it is the major factories governing shelf-life (Ismail *et al.*, 2006). Dairy products are classified as perishable or semi-perishable food and require protection from microbial spoilage during their shelf life (Nasr, 2002). Milk and milk products contain many types of microorganisms such as *(Staph. aureus. Bacillus cereus, E.coli,* and *S. entridis)* coming from different sources (Potter *et al.*, 1993).

Microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety (Tauxe, 1990). Currently, there is a growing interest to use natural antibacterial compounds, like extracts of herbs and spices for the preservation of foods (Smid and Gorris, 1999).

In dairy products some synthetic chemical compounds such as sodium nitrate, sodium or potassium sorbate, formalin, hydrogen peroxide and phenolic compounds are used. Those compounds have a high effective and stability for preservation. But, the recent researches indicated that, synthetic chemical compounds aren't safes enough to consume by peoples because they can cause some danger disease such as cancer and toxic (Johson and Cart, 1985). Therefore, there is a clear need for new methods of preserving

dairy products using natural additives such as Spearmint *(Mentha spicata)*, Clove *(Syzygium aromaticum)* and Basil *(Ocimum basilicum)* essential oils (EOs) as antimicrobial additives, because they are rich sources of biologically active compound (volatile aromatic compounds) (Fandohan *et al.* 2004, Lopez *et al.* 2005 and Sacchetti *et al.* 2005).

The antimicrobial properties of essential oils have been known for many centuries. From the period between 1987 to 2001; a large number of essential oils such as (Spearmint, Clove and Basil) and their constituents have been investigated for their antimicrobial properties against some bacteria and fungi (Kalemba and Kunicka, 2003). Many authors, in fact, have reported antimicrobial and antifungal properties by species and essential oils (Madsen and Bertelsen, 1995 & Hirasa and Takemasa, 1998). An essential oil (*EO*) of some plant and some species has been shown to possess bactericidal activity against *Listeria* spp. and *Staphylococcus aureus* isolated from dairy products (Gill *et al.*, 2002).

This study aim, in general, at finding safe methods to resist the microbial activity of pathogenic bacteria *(Staph. aureus., Bacillus cereus, E.coli , S. entridis*) and some moulds and extended the shelf life of white cheese by using some essential oils (EOs) such as Spearmint (*Mentha spicata*), Clove (*Syzygium aromaticum*) and Basil (*Ocimum basilicum*) at different concentration.

The current study had three specific aims:

Firstly, to investigate the pure *EOs* activities with some pure pathogenic types of bacteria and fungi to evaluate their effectiveness when there is no direct contact with food.

Secondly, to evaluate the overall acceptability of white cheese supplemented with different concentrations of essential oils (*EOs*) under study.

Thirdly, to evaluate the microbiological quality of home-made white cheese by:

1- Check the effectiveness of selected *EOs* against total aerobic plate count and total fungal count presented in fresh white cheese.
2- Investigate the effect of different storage temperatures and various duration of times on microbial profile for white cheese treated with *EOs*.
3- Check the effectiveness of selected *EOs* against some pathogenic types of bacteria presented in fresh and stored white cheese.

MATERIALS AND METHODS

Materials:

- Spearmint, Clove and Basil essential oils *(EOs)* were obtained from Kato Aromatic Company, Giza, Egypt.
- Fresh cow's and buffalo's milk were collected from retail markets around Cairo city during autumn (2006/2007).
- The pure cultural of *B. cereus, E. coli, S. enteritis* and *Staph. aureus*), and *A. flavus, P. corylophilum, E. repens* were obtained from National Research Institutes, El-Doky, giza, Egypt.

- Nutrient agar media, potato-dextrose agar media and Peptone water were purchased from El-Gomhoria pharmaceutical Company, Cairo, Egypt.
- Selective agar media (Mannitol-egg Phenol-Red-Egg-Yolk polymyxin *(PREY)* and Agar Base media were purchased from El-Badr El-Hanasyia Company (Biolife, Milan, Italy).

Methods:

Preparation of home-made white Cheese:

Fresh cow's and buffalo's milk were mixed at a ratio of 1 : 1 to obtain cheese milk of 5.1% fat, 14.53 % total solids and 0.18 % acidity as average. The mixed milk was heated to 72°C for 30 seconds to 1 min. then cooled to 37°C. Sodium chloride was added at the ratio of 8% (w/v). The starter was added at the ratio of 0.25 ml/l of mixed milk. The mixture has been swelling well for approximately five minutes at room temperature. To obtain the coagulation, the previous mixture will leave at room temperature for 4 hours. Coagulum has been transferred to matting, and hung till have the heavy coagulum *(Rowida, 2001)*. *EOs* for Spearmint, Clove and Basil were added before heating (72°C) and/or after cooling (37°C) of milk in the concentration of 0.25, 0.50 and 1.0 %. Control white cheese was manufactured from the same milk without *EOs* addition (free of *EOs*).

Three samples were taken from each group (Control, Spearmint, Clove and Basil groups) and put into plastic box. Fresh sample and other two samples were stored at room temperature (22°C±3°C) and/or at refrigerator temperature (5°C±2°C) for 2, 4 and 7 days, and then examined.

Microbiological examination:

Antimicrobial activity (solid diffusion test):

A glass Petri dish (90 *mm* diameter) containing the appropriate solidified medium was inoculated with 100µl of a physiological solution containing 10^5 colony forming units (*CFU/ml*) of the microorganisms under study. Different concentration of undiluted *EOs* (from 0.25 to 1.5%) added to 5 mm sterile column disc, placed on top of the cultural media. After incubation at optimal condition (temperature and time), the average diameter of free zone (where no growth of microorganisms) was measured. All analyses were carried out in triplicate (Adguzel *et al.*, 2005 and Lopez *et al.*, 2005).

The tested microorganisms were selected on the basis of their potentiality as spoilage microorganisms causing undesirable changes in food, or for their pathogenic activity. The pure cultural selected microorganisms were: (*B. cereus, E. coli, S. enteritis* and *Staph. aureus*), and *A. flavus, P. corylophilum, E. repens*. Bacterial strains were maintained on nutrient agar media, while yeast and mold cultures were maintained on potato-dextrose agar media.

Organoleptic evaluation of home-made white cheese:

The obtained results from control and supplement fresh white cheese samples with different doses of *EOs* "0.25, 0.5, 1.0 and 1.5 %" were compared with Egyptian standards for sensory evaluation. The white cheese samples of different treatment by *EOs* were organoleptically scored, out side color (15 points), inside color (15 point), odor (20 points), taste (20 points), softness (15 points) and texture (15 points) according to the score card suggested by (Rowida, 2001). Samples were judged by 10 staff members.

Preparation of cheese for microbiological analysis:

About 50 *gm* of cheese were aseptically weighed and grinded in sterilized mortar. One gram of the grinded cheese was transferred into another sterilized mortar for microbiological analysis where nine ml of sterilized saline solution was added and thoroughly mixed with the cheese and this represents 10 dilutions which were then used making further dilution.

Determination of total aerobic bacterial and fungal counts:

Aerobic plate count (*APC*) and total fungal count (*TFC*) were determined using nutrient agar media and potato-dextrose agar media, respectively. Peptone water was used to make serial dilution before plating. Total bacteria and fungal were counted according to Bulletin of International Commission on Microbiological Specifications for Foods (*ICMSF*, 1987).

Total *Bacillus cereus* count:

Bacillus cereus selective agar media (Mannitol-egg Phenol-Red-Egg-Yolk polymyxin *(PREY)* were incubated for 72 h at 30°C and confirmed by establishing certain key morphological and biochemical characteristics as described by (Rowan *et al.*, 1997).

Total *Staph. aureus* count:

The total *Staph. aureus* count was determined according to the method of the International Commission on Microbiological Specifications for Foods *(ICMSF,* 1987*)*. Samples were shaken with 0.1% mixture of peptone water. Dilutions of 10^{-1} - 10^{-4} were made and 0.1 ml was plated on Baird Parker Agar Base media.

Statistical analyses:

Pathogen incidences were reported as the percentage of samples that tested positive for each pathogen. Microbiological plate count was transformed into base-10 logarithms (log 10 *CFU* g^{-1}) before computing and performing statistical analyses. Minimum detection limits for *APC* and *TFC* were 1 log *CFU* g^{-1}, based on the maximum sensitivity of the test with samples dilatation by 10^{-1}. Average of triplicates was analyzed. The effects of storage conditions on microbial numbers were examined using a Duncan-Test analysis using *SPSS* 10.0 Program. All significances were reported as the 95 % level of confidence ($p < 0.05$) (Kurtz, 1983).

RESULTS AND DISCUSSION

First: Antimicrobial activities of pure essential oils (*EOs*):-

Many plant essential oils, compounds isolated from the oils, and phenolic compounds are reported to be antibacterial and antifungal against several pathogens (Friedman *et al.*, 2003). To further facilitate the application of these oils, we selected three active oils (Spearmint, Clove and Basil) for evaluation as antibiotic-resistant against four organisms (*B. cereus, E.coli, S. entridis and Staph. aureus*). Those pathogens were selected to explore the potential of the selected *EOs* to prevent the growth of the resistant bacteria. Moreover, the ability of several quantities of pure essential oils as antimicrobial were tested (Table 1). In a first series of assays, the antimicrobial activities of the pure *EOs* under study were screened by means

of total solid diffusion test (total inhabitation zone (*mm*). The current data declared that the Spearmint, Clove and Basil *EOs* at different amounts tended to have a good inhibitory affect against all of the tested organisms, especially molds (*A. flavus, P. corylophilum, E. repens)*. In most cases, the antimicrobial activities of the Clove and Basil *EOs* were the highest, among all tested essential oils. On contrast, Spearmint had the latest inhibition for all tested organisms among all selected *EOs*. for *B. cereus, E. coli* and *Staph. aureus*. The Basil essential oil (*EO)* gave a stronger inhabitation than the other tested *EOs*. This result are in general within many studies which reported that, the oil of Basil showed strong exhibited antimicrobial activity against Gram positive bacteria and Gram negative bacteria (Meena and Sethi, *1994;* Nyein *et al., 1999* & Khafagi *et al., 2000)*.

The current study indicated that, Gram-negative bacteria are generally more resistant than Gram-positive bacteria in solid diffusion test. It may be due to the external lipopolysaccharide wall that surrounds the peptidoglycan cell wall of the former (Sacchetti *et al.*, 2005).

Table (1): Antimicrobial activity of pure essential oils, solid diffusion test: total inhibition (clear zone, mm)*.

Essential oils (EOs)	Con. of *EOs* %	Gram positive		Gram negative		Moulds		
		E. coli	*S. entridis*	*B. cereus*	*Staph. aureus*	*A. flavus*	*P. corylophilum*	*E. repens*
Spearmint	0.25	13	11	10	10	90	90	85
	0.50	13	15	10	12	90	90	80
	1.00	15	17	10	13	90	90	82
	1.50	25	19	13	16	90	90	90
Clove	0.25	15	12	12	12	90	90	85
	0.50	15	18	12	12	90	90	85
	1.00	15	18	16	13	90	90	90
	1.50	25	20	16	13	90	90	90
Basil	0.25	14	12	12	12	90	90	90
	0.50	22	16	15	12	90	90	90
	1.00	24	20	18	15	90	90	90
	1.50	27	21	18	14	90	90	90

***Total inhibition or clean zone means no growth of microorganism. An inhibition of 90 mm represents total inhibition (Petri dish diameter). Inhibition diameter is the mean of three different observations taken from three different experiments.**

The different performances offered by essential oils, in fact, can be linked to their different chemical composition (Bruni *et al.*, 2003 and Sacchetti *et al.*, 2005). According to this test, Spearmint, Clove and Basil *EOs* were selected for further microbiological and organoleptic evaluation for white cheese.

Second: Organoleptic properties evaluation:

Data illustrated in (Table 2) showed the average score of outside colures, inside colure, odor, taste, softness and texture of white Cheese (fresh) supplemented with different concentrations of *EOs*. In most cases, white cheese supplemented with high concentration of *EOs* had the lowest

organoleptic score among all tested *EOs* concentrations .In addition, a little difference between control sample and samples supplemented by 0.25 %, 0.5 % of Spearmint essential oil, even insignificant differences ($p < 0.05$) has been observed between them. Following by white cheese samples supplemented with 0.25 % of Clove and Basil. Odor, out side colour, taste and softness of white cheese added with 0.25 % of Spearmint *EO* had record approximately similar to control samples. It confirmed that, increasing the additive rates with Clove and/or Basil *EOs* equal 1.0 % of total weight caused sharply decrease of odor and taste score compared to control sample.

In general, white cheese added by Spearmint *EO* at a rate of 0.25 % and 0.50 % was not affecting significantly the score of organoleptic properties. White cheese treated with different concentration of Spearmint *EO* tended to have approximately overall acceptable score higher than white cheese treated with the same concentration of other essential oils (Clove and Basil).

Table (2): Organoleptic evaluation of white cheese (Fresh) treated with essential oils.

Factors	degree	Control	Spearmint				Clove				Basil			
			0.25	0.50	1	1.5	0.25	0.50	1	1.5	0.25	0.50	1	1.5
Out side colour	15	13	13	12	10	8	10	8	7	7	10	8	7	7
Inside colour	15	13	12	12	11	8	11	7	5	5	11	7	5	5
Odor	20	17	18	16	14	12	15	8	3	2	16	11	7	5
Taste	20	18	18	16	12	12	16	8	5	2	17	12	10	7
Softness	15	12	13	13	14	14	12	12	12	10	13	12	12	10
texture	15	12	11	11	10	10	10	12	12	11	10	12	12	11
Total	100	85^{d}	85^{a}	80^{ab}	71^{c}	64^{d}	74^{c}	55^{cd}	44^{f}	37^{g}	77^{bc}	62^{d}	53^{e}	45^{f}

Data followed by different letters are significantly different ($p < 0.05$).
Statistical analyses were done among all the treatment.

Third: Microbiological quality of home-made white cheese:-

1. Microbiological quality of home-made white cheese (*EOs* has been added before heating process of milk (72°C) during preparation of white cheese).

A. White cheese examined immediately after made (Fresh cheese):

Fifty samples of white cheese prepared in laboratory were examined for their microbiological quality. It should be noted that, *EOs* has been added before heating of milk during preparation of white cheese. The mean aerobic plate count (*APC*), the effect of different storage condition on the microbiological quality and the mean total fungal count of examined white cheese are shown in (Tables 3, 4 and 5), respectively.

The mean aerobic plate counts (*APC*) were presented in (Table 3). All samples of white cheese added with Basil *EO* were of satisfactory bacteriological quality. They had total aerobic plate counts (*APC*) lower than the Recommended Safety Limit of (10^5 *CFU* g^{-1}) proposed by Egyptian Organization for Standardization and Quality Control (*EOSQC*, 2005).

Control samples (*EOs* free) examined immediately after made could be safe enough, as compared to Egyptian standards. They had total aerobic.

plate counts (*APC*) equal to the upper recommended safety limit. The *APC* showed a significant difference ($p < 0.05$) between control samples (free from *EOs*) and added samples with different quantities of *EOs*. The *APC* of white cheese found in the current study was in agreement with data obtained by Chami *et al.*, 2005.

It is clear that all white cheese samples supplemented with *EOs* and investigated immediately after making were of satisfactory bacteriological quality, having *APC* lower than 10^6. Accordingly, it could be safe enough for human consumption. It should be noted that, samples added with low doses of *EOs* tended to have *APC* higher than its counterparts added with high doses of *EOs*, even statistical significant differences were observed.

Table (3): Total Aerobic Plate Counts (*APC*) exhibited of home-made white cheese examined immediately after made*.

Sample	Con. of *EOs* %	No. units with total aerobic count (log *CFU* g^{-1}) in the range				Total aerobic count (*log CFU* g^{-1})
		<4.0	≤4.0-5.0	≤5.0-6.0	≤6.0-7.0	
Control	0.00	-	1	3	1	$9.9 \times 10^{5\,d}$
Spearmint	0.25	-	1	2	2	$8.4 \times 10^{5\,c}$
	0.50	-	1	2	2	$7.3 \times 10^{5\,bc}$
	1.00	-	-	4	1	$6.6 \times 10^{5\,b}$
Clove	0.25	-	-	2	3	$9.9 \times 10^{5\,d}$
	0.50	-	-	3	2	$8.8 \times 10^{5\,c}$
	1.00	-	1	2	2	$7.7 \times 10^{5\,bc}$
Basil	0.25	-	-	4	1	$8.7 \times 10^{5\,c}$
	0.50	-	-	3	2	$5.2 \times 10^{5\,b}$
	1.00		1	2	2	$2.4 \times 10^{5\,a}$

Data followed by different letters are significantly different ($p < 0.05$).
Statistical analyses were done among all the treatment.
*** *EOs* has been added before milk heating "72°C" (values are means of 5 replications).**

B. Microbiological quality of white cheese examined after storage at different conditions for various duration of time (temperature about, 22°C and 5°C for 2, 4 and 7 days):

Dairy products have to be produced in such a way as to protect them against microorganisms infection. However, in every day practice very often a white cheese is not consumed immediately after preparing or purchased and stays for longer time sometimes till it is completely ripened. During this period the white cheese is usually critically exposed to microorganisms from different sources such as air, handling, equipment and package (David *et al.*, 1995). In the following investigations, samples of home-made white cheese were stored after making and supplemented with different concentration of *EOs* at different temperatures: 5 and 22°C and their total aerobic counts were determined after 2, 4 and 7days.

The mean *APC* (*log CFU/g*$^{-1}$) for home-made white cheese samples examined after storage were presented in Table (4). Control white cheese sample (*EOs* free) which stored for 2 days or more at room temperature could be unsafe for human consumption. They had *APC* higher than the recommendation safety limit. However, all white cheese samples added with *EOs* at the 0.5 and 1.0 % and examined after storage at chill temperature and

room temperature for 2, 4 and 7 days were of satisfied bacteriological quality. They had *APC* lower than the recommended safety limit of (10^6 *CFU* g^{-1}) proposed by Egyptian standards (*EOSQC*, 2005).

Also, with increasing the concentration of *EO* the number of *APC* has been sharply decreased (Table 4). Home-made white cheese stored at room temperature exhibited the highest counts of *APC*. Whereas home-made white cheese stored at cold temperature exhibited the lowest ones. It may be due to chill temperature not providing appropriate temperature for most aerobic bacteria to grow as compared to elevated temperatures (35°C) (Richard *et al.*, 2000).

Home-made white cheese that supplemented with a high concentration of *EOs* (1.0 %) tended to have *APC* lower than its counterparts added with low doses of *EOs*. In most cases, there were no statistically significant differences ($p < 0.05$) in *APC* between control samples and supplemented samples with *EOs* at low concentration (0.25 %) examined after storage for 2 days.

Table (4): Total Aerobic Plate Counts (*APC*) exhibited by home-made white cheese examined after storage at various duration time and temperature*.

Sample	Con. of *EOs* %	Mean total aerobic count: *log CFU* g^{-1} for 50 samples of examined white cheese stored for:					
		2 days		4 days		7 days	
		Room temp.	Ref. temp.	Room temp.	Ref. temp.	Room temp.	Ref. temp.
Control	0.00	9.2×10^{6a}	2.1×10^{6a}	1.3×10^{7i}	4.0×10^{6f}	3.9×10^{7i}	8.3×10^{6fi}
Spearmint	0.25	8.8×10^{6a}	1.9×10^{6ab}	6.1×10^{6a}	4.1×10^{6a}	9.1×10^{6h}	6.9×10^{6h}
	0.50	7.3×10^{5b}	7.1×10^{5b}	1.5×10^{6ab}	8.8×10^{5b}	6.9×10^{6ah}	6.3×10^{6a}
	1.00	4.3×10^{5c}	3.1×10^{5c}	5.2×10^{5g}	6.8×10^{5g}	3.2×10^{6ah}	1.9×10^{6ab}
Clove	0.25	3.9×10^{6a}	1.1×10^{6ab}	5.3×10^{6h}	3.5×10^{6h}	6.2×10^{6h}	6.0×10^{6h}
	0.50	7.2×10^{5b}	7.9×10^{5ab}	3.1×10^{6a}	1.8×10^{6a}	4.2×10^{6ah}	2.2×10^{6a}
	1.00	5.2×10^{5e}	4.1×10^{5e}	1.7×10^{5c}	1.2×10^{5c}	1.5×10^{6c}	9.6×10^{5c}
Basil	0.25	4.1×10^{5c}	7.4×10^{5c}	5.5×10^{5g}	8.5×10^{5g}	4.9×10^{6ab}	2.6×10^{6ab}
	0.50	3.9×10^{5d}	1.4×10^{5d}	7.2×10^{5cd}	4.2×10^{5d}	1.2×10^{6b}	6.1×10^{5bc}
	1.00	3.3×10^{5e}	8.3×10^{4e}	7.0×10^{5c}	4.5×10^{4c}	9.5×10^{5c}	4.2×10^{5c}

Data followed by different letters are significantly different ($p < 0.05$).
Statistical analyses were done for control sample with *EOs* of each treatment.
*** *EOs* has been added before milk heating "72°C" (values are means of 5 replications).**

Table (5) showed that the total fungal count of home-made white cheese examined immediately and after various duration of times and temperature. *EOs* has been added before heating of milk. The current results indicated that, all investigated samples of satisfactory fungal quality. They had total fungal count lower than the recommendation consumable safety limit of 10^3 proposed by (*IDAEC*, 1999). With the exception, control sample had total fungal count higher than recommendation consumable safety limit after storage for equal or more than 4 days. In general, these results are in agreement with those reported by Velluti *et al.*, 2003.

The results showed strong exhibited antifungal activity; even all examined home-made white cheese added with high concentration of *EOs* (1.0 %) and stored at room or refrigerated temperature for 2 days were free from mould. While, its control samples counterparts had total fungal count higher than recommendation safety limit proposed by the Egyptian standard.

It is clear from (Table 5) that home-made white cheese supplemented with Clove *EO* at different concentration had the lowest total fungal count among all another counterparts (Velluti *et al.*, 2003). On contrast, samples supplemented with Spearmint *EO* even at high concentration had the highest total fungal count among all another added counterparts. Also, this result was consistent with a similar study stated that Clove and Basil *EOs* had strongest effect against most of moulds (Soliman and Badeoa, 2002).

Table (5): Total fungi exhibited of home-made white cheese examined immediately after made (Fresh) and after various duration time and temperature*

Sample	Con. of *EOs* %	Mean total fungi: *log CFU g*$^{-1}$ for 50 samples of white cheese stored for:						
		Fresh	2 days		4 days		7 days	
			Room temp.	Ref. temp.	Room temp.	Ref. temp.	Room temp.	Ref. temp.
Control	0.00	3.2×10^{1}	9.8×10^{2}	6.6×10^{2}	5.5×10^{4}	3.3×10^{4}	3.2×10^{5}	7.1×10^{4}
Spearmint	0.25	1.1×10^{1}	3.1×10^{2}	8.2×10^{1}	7.2×10^{2}	1.4×10^{2}	6.1×10^{3}	5.3×10^{3}
	0.50	1.3×10^{1}	1.1×10^{2}	3.1×10^{1}	3.5×10^{2}	3.3×10^{2}	2.3×10^{3}	8.7×10^{2}
	1.00	-	0.3×10^{1}	-	8.5×10^{1}	3.7×10^{1}	6.4×10^{2}	3.1×10^{2}
Clove	0.25	1.6×10^{1}	2.1×10^{2}	9.2×10^{1}	4.9×10^{2}	1.6×10^{2}	5.3×10^{3}	6.6×10^{2}
	0.50	0.9×10^{1}	1.6×10^{1}	1.1×10^{1}	2.2×10^{2}	9.5×10^{1}	2.6×10^{3}	5.9×10^{2}
	1.00	-	-	-	8.8×10^{1}	4.1×10^{1}	8.1×10^{2}	2.6×10^{2}
Basil	0.25	1.1×10^{1}	3.1×10^{1}	2.9×10^{1}	2.5×10^{2}	6.2×10^{1}	5.2×10^{3}	2.3×10^{2}
	0.50	-	1.1×10^{1}	-	8.8×10^{1}	6.4×10^{1}	2.1×10^{3}	9.8×10^{1}
	1.00	-	-	-	3.7×10^{1}	1.2×10^{1}	6.5×10^{1}	4.2×10^{1}

* *EOs* has been added before milk heating "72°C" (values are means of 5 replications).

2. Microbiological quality of white cheese (*EOs* has been added after milk cooling "37°C" during preparation of white cheese).

A. white cheese examined immediately after made (Fresh cheese):

In the following investigations samples of home-made white cheese were examined immediately after made, and part of it stored after made at different temperatures: 5 and 22°C and their total aerobic counts and total fungal count were determined after 2, 4 and 7 days (Tables 6 to 8) .

Data present in (Table 6) explained the total aerobic plate counts (*APC*) of home-made white cheese examined immediately after made. Control samples showed the highest *APC*, which was 9.3×10^{6} *CFU* g^{-1} while the mean total aerobic plate (*APC*) count for all home made white cheese analyzed was 1.6×10^{6}

CFU g^{-1}. Accordingly, all examined samples investigated directly after made were of satisfied microbiological quality, they had APC lower than or equal 10^5 proposed by Egyptian norms (*ESOQC*, 2005).

The *APC* showed insignificant difference between investigated supplemented samples ($p < 0.05$) at the same concentration. It should be noted that, there were slightly differences for APC between results obtained with samples supplemented with high concentration of *EOs* and those obtained with samples added with low concentration of *EOs*. It may be due to the fact that active antimicrobial components *EOs* needs period of times to inhibit the growth of bacteria (Velluti *et al.*, 2003).

Table (6): Total aerobic plate counts (*APC*) exhibited by home-made white cheese examined immediately after made*.

Sample	Con. of *ESo* %	No. units with total aerobic count (*log CFU* g^{-1}) in the range:				Total aerobic count (*log CFU* g^{-1})
		<4.0	≤4.0-5.0	≤5.0-6.0	≤6.0-7.0	
Control	0.00	-	1	1	3	$9.3 \times 10^{6\,D}$
Spearmint	0.25	-	-	3	2	$8.4 \times 10^{5\,c}$
	0.50	-	-	4	1	$5.5 \times 10^{5\,b}$
	1.00		1	3	1	$3.3 \times 10^{5\,a}$
Clove	0.25	-	-	3	2	$8.1 \times 10^{5\,c}$
	0.50	-	-	3	2	$6.1 \times 10^{5\,b}$
	1.00	-	-	4	1	$2.5 \times 10^{5\,a}$
Basil	0.25	-	-	3	2	$6.4 \times 10^{5\,b}$
	0.50	-	-	3	2	$4.8 \times 10^{5\,c}$
	1.00	-	1	2	2	$2.4 \times 10^{5\,a}$

Data followed by different letters are significantly different ($p < 0.05$).
Statistical analyses were done among all the treatment.
*** *EOs* has been added after milk cooling "37°C" (values are means of 5 replications).**

B. Bacteriological quality of white cheese examined after storage at different conditions and for various duration of times (temperature about, 22°C and 5°C for 2, 4 and 7 days):

In the following investigations samples of home-made white cheese were stored after making at different temperatures. Their total aerobic counts and total fungi count were determined after 2, 4 and 7 days of storage.

Mean *APC* (log CFU/g) for samples of home-made white cheese stored for various duration of periods at different conditions were presented in (Table 7). It should be noted that, *EOs* has been added after milk cooling "37°C" during the preparation of cheese. Control samples examined after storage at chill temperature for 4 days or more as well at room temperature for 2 days or more were of unsatisfactory bacteriological quality. They had *APC* higher than recommended by the (*ESOQC*, 2005). While, added samples with high doses of different *EOs* examined after storage at chill or room temperature had *APC* lower than or equal 10^5, as well supplemented samples with low doses of different *EOs* had APC higher in the rang of 10^6. It is clear that, with increasing the concentration of *EOs* the number of *APC* has been decreased. The *APC* obtained in this Table is a rather low compared to

APC reported in (Table 4). Accordingly, the antimicrobial activity for EOs that added after milk cooling is better than the activity of *EOs* that added before heating of milk during preparation of white cheese. This expectation was in general agree (Richard *et al.*, 2000).

Table (7): Total Aerobic plate Counts (*APC*) exhibited by home-made white cheese examined after storage at various duration time and temperature*

Sample	Con. of *EOs* %	Mean total aerobic count: *log CFU g*$^{-1}$ for 50 samples of white cheese stored for:					
		2 days		4 days		7 days	
		Room temp.	Ref. temp.	Room temp.	Ref. temp.	Room temp.	Ref. temp.
Control	0.00	1.2×10^{7a}	9.1×10^{6b}	3.1×10^{7b}	6.6×10^{6b}	6.9×10^{7b}	6.2×10^{6a}
Spearmint	0.25	4.2×10^{6a}	3.5×10^{6a}	5.3×10^{6a}	4.9×10^{6a}	8.9×10^{5a}	6.3×10^{6a}
	0.50	8.8×10^{5a}	8.1×10^{5a}	1.5×10^{6a}	1.2×10^{6a}	5.2×10^{6a}	4.4×10^{6a}
	1.00	3.5×10^{5a}	7.1×10^{5a}	5.8×10^{5a}	4.2×10^{5a}	2.2×10^{6a}	9.3×10^{5a}
Clove	0.25	2.7×10^{6a}	3.3×10^{6a}	6.2×10^{6a}	4.4×10^{6a}	7.7×10^{6a}	4.5×10^{5a}
	0.50	8.0×10^{5a}	7.2×10^{5a}	2.6×10^{6a}	9.3×10^{5a}	2.9×10^{6a}	2.2×10^{6a}
	1.00	6.3×10^{5a}	6.1×10^{5a}	5.3×10^{5a}	6.6×10^{5a}	2.9×10^{6a}	6.5×10^{5a}
Basil	0.25	1.9×10^{6a}	8.5×10^{5a}	2.1×10^{6a}	8.1×10^{5a}	5.5×10^{6a}	3.1×10^{6a}
	0.50	6.2×10^{5a}	3.9×10^{5a}	6.3×10^{5a}	2.7×10^{5a}	1.4×10^{6a}	5.1×10^{5a}
	1.00	2.1×10^{5a}	7.9×10^{4a}	4.0×10^{5a}	3.4×10^{5a}	1.7×10^{6a}	6.1×10^{5a}

Data followed by different letters are significantly different ($p < 0.05$).
Statistical analyses were done for control sample with *EOs* of each treatment.
*** *EOs* has been added after milk cooling "37°C" (values are means of 5 replications).**

Fifty samples of home-made white cheese were examined for their fungal quality (Table 8). All examined white cheese supplemented with tested *EOs* examined after storage for 2, 4 and 7 days had total fungal count lower than control sample counterparts. Control samples examined after storage for more than 4 days at room or refrigerated temperature had total fungal count (*TFC*) high er than or equal the recommendation consumable safety limit proposed by (*EOSQC*, 2005). However, all white cheese added with *EOs* examined after storage at refrigerated and at room temperatures for 2, 4 and 7 days were of satisfied bacteriological quality. Fungi were not detected in any of the investigated white cheese samples immediately after made. This could be due to the heat processing of milk during preparation of cheese and the effect of *EOs* were enough to kill all fungi and its' spores (Bibek, 2001). All examined white cheese supplemented with *EOs* and stored at room or refrigerated temperature for 2 days as well as samples added with high doses of *EOs* (1.0 %) and stored at refrigerated temperature for 4 days were free from mould. In a conclusion, All tested *EOs* were active against fungal especially at high concentration.

It could be concluded that white cheese (*EOs* has been added after cooling of milk) stored at different conditions have *APC* and *TFC* lower than white cheese (*EOs* has been added before heating of milk). It may be due to the fact that most of antimicrobial compounds of essential oils were sensitive to heat treatment (Leena and Achaleshwar, 2006).

Table (8): Total fungi exhibited by home-made white cheese examined immediately after made (Fresh) and after storage for various duration time and temperature*.

Sample	Con. of *EOs* %	Mean total fungi: log *CFU* g^{-1} for 50 samples of white cheese stored for:						
		Fresh	2 days		4 days		7 days	
			Room temp.	Ref. temp.	Room temp.	Ref. temp.	Room temp.	Ref. temp.
Control		2.4×10^{1}	3.2×10^{3}	5.9×10^{2}	6.5×10^{3}	3.3×10^{4}	1.7×10^{5}	4.1×10^{4}
Spearmint	0.25	-	2.8×10^{2}	2.3×10^{2}	3.0×10^{2}	3.0×10^{2}	6.6×10^{3}	3.7×10^{3}
	0.50	-	9.6×10^{1}	1.1×10^{1}	2.2×10^{2}	9.5×10^{1}	6.3×10^{2}	5.1×10^{2}
	1.00	-	-	-	8.8×10^{1}	-	2.1×10^{2}	7.2×10^{1}
Clove	0.25	1.0×10^{1}	2.0×10^{1}	2.9×10^{1}	2.5×10^{1}	1.0×10^{1}	5.2×10^{3}	2.3×10^{2}
	0.50	-	0.9×10^{1}	-	1.2×10^{1c}	1.4×10^{1}	3.8×10^{2}	3.1×10^{2}
	1.00	-	-	-	2.2×10^{1c}	-	4.9×10^{1}	3×10^{2}
Basil	0.25	-	1.5×10^{1}	8.2×10^{1}	3.0×10^{1}	1.4×10^{2}	3.3×10^{2}	3.3×10^{2}
	0.50	-	0.7×10^{1}	3.1×10^{1}	1.5×10^{1}	3.3×10^{1}	5.2×10^{2}	3.7×10^{2}
	1.00	-	-	-	1.9×10^{1}	-	2.0×10^{1c}	1.9×10^{1}

* *EOs* has been added after milk cooling "37°C" (values are means of 5 replications).

3- Effectiveness of selected *EOs* against some pathogenic types of bacteria presented in white cheese.

This part was designed to study the effect of the studied essential oils on the growth of some common undesirable and spoilage microorganisms such as *Bacillus cereus* and *Staph. aureus* which may contaminate and spoil white cheese (Adams and Moss, 1997).

Tables 9 and 10 reveal the effect of Spearmint, Clove and Basil *EOs* on freshly white cheese and/or the end of storage period (7 days) at different condition (before heating "72°C" and after cooling "37°C" of milk).

Total *Bacillus cereus* (*log CFU/g*$^{-1}$) for white cheese samples were presented in (Table 9). The highest number of *B. cereus* detected in freshly white cheese was 2.1 x 10^{2} *CFU* g^{-1}, for control samples. In most cases, no statistical significant difference ($p < 0.05$) was observed between control sample and white cheese samples supplemented with Spearmint, Clove and Basil *EOs* at concentration 0.25 %. It was noticeable that, in all cases with increasing the concentration of *EOs* the number of total *B. cereus* has been significantly decrease. These observations are consistent with those obtained in the first part of this study and are in agreement with Eneroth et al., 2001.

In most cases, there are statistically significant differences ($p < 0.05$) in total *Bacillus cereus* (*TBC*) between samples stored at room and refrigerate temperature for 7 days. There were insignificant differences ($p < 0.05$) in *TBC* among samples of white cheese supplemented with high doses (1.0 % of different *EOs*).

Table (9): Total *Bacillus cereus* (TBC) counts exhibited by home-made white cheese examined immediately after made and the end of storage period at different temperature.

Sample	Con. of *EOs* %	*Bacillus cereus*					
		Fresh		Storage for 7 days at::			
		Before milk heating-72°c-	After milk cooling-37°c-	Before milk heating-72°c-		After milk cooling-37°c-	
				Room temp.	Ref. temp.	Room temp.	Ref. temp.
Control	0.00	2.1×10^{2C}		4.1×10^{3FG}	9.3×10^{2E}	5.2×10^{3G}	9.3×10^{2E}
Spearmint	0.25	1.1×10^{2BC}	1.1×10^{2C}	4.2×10^{3FG}	2.8×10^{3F}	2.0×10^{3F}	6.6×10^{2D}
	0.50	7.3×10^{1B}	7.9×10^{1B}	4.2×10^{3FG}	6.6×10^{2CD}	6.1×10^{2D}	4.8×10^{2CD}
	1.00	5.5×10^{1A}	3.1×10^{1A}	6.1×10^{2CD}	5.5×10^{2D}	4.8×10^{2CD}	3.3×10^{2CD}
Clove	0.25	1.2×10^{2BC}	1.6×10^{2C}	2.6×10^{3F}	5.5×10^{3G}	2.2×10^{3F}	7.2×10^{2D}
	0.50	7.6×10^{1B}	9.2×10^{1BC}	7.7×10^{2D}	5.2×10^{2CD}	5.2×10^{2D}	2.8×10^{2CD}
	1.00	4.4×10^{1A}	4.6×10^{1A}	5.1×10^{2CD}	4.9×10^{2C}	2.7×10^{2BCD}	1.3×10^{2C}
Basil	0.25	8.8×10^{1BC}	3.2×10^{2CD}	3.3×10^{3F}	3.0×10^{3F}	3.2×10^{3FG}	2.9×10^{3F}
	0.50	8.5×10^{1B}	6.7×10^{1B}	6.9×10^{2D}	2.3×10^{2C}	6.2×10^{2D}	1.2×10^{2C}
	1.00	5.1×10^{1A}	4.4×10^{1A}	6.6×10^{2CD}	1.9×10^{2BC}	6.0×10^{2DE}	1.1×10^{2C}

Data followed by different letters are significantly different ($p < 0.05$).
Statistical analyses were done for control sample with *EOs* of each treatment.
Values are means of 5 replications.

The lowest number of *TBC* among all tested oils was observed especially with 1.0 % concentration. This result is completely agreed with many studies that reported that Clove *EO* had strongest effect inverse *B. cereus* (Krishma *et al.*, 1999 & Leuschner and Ielsch, 2003). A lso were confirmed with a recent study which indicated that essential oils of Clove and Basil gave the strongest inhibition against *Bacillus cereus* (Lopez *et al.*, 2005).

TBC for control samples was approximately the same with those obtained with samples supplemented with low doses (0.25%) of different *EOs*, even insignificant differences ($p < 0.05$) were observed. It may be due to the fact that *EOs* needs duration of times to appear their antimicrobial activity (Ponce *et al.*, 2004). The afore-mentioned results were in general agreement with (Ozcan and Erkmen, 2001). Consequently, investigated samples in the current study were unacceptable under Egyptian Legislation, which confirmed that white cheese should not contain more than 1 cell of *B. cereus* per g *(ESOQC,* 2005).

In (Table 10) noticed that all tested *EOs* were active against *Staph. aureus* especially at high concentration. This result is in agreement with the observation that the ethanol, methanol and hexane extracts from Ocimum basilicum (*Sweet basil*) showed antibacterial activities against *Staphylococcus aureus* (Adguzel *et al.*, 2005). In addition, essential oil (*EO*) of Basil gave an inhibition against *Staphylococcus aureus* (Lopez *et al.*, 2005). Moreover, In vitro anti-*Staphylococcus aureus* activities of the extracts Clove (*Syzygium aromaticum*) and Spearmint (*Mentha piperita*) was confirmed by (Betoni *et al.*, 2006).

Samples supplemented with Spearmint *EO* at different concentration in fresh white cheese had the lowest total *Staph. aureus* count among all tested

EOs (Table 10). This is consistent with the observation that Spearmint essential oil had a good effective against *Staph. aureus (Ozcan and Erkmen, 2001).*

In most cases, there are a slightly differences for total *Staph. aureus* between fresh samples (examined immediately after made) and the samples investigated at the end of storage period (7 days) at different concentration. It may be due to the fact that *EO* is able to inhibit the growth of most gram-negative and positive bacteria and achieving adequate shelf-life for foods *(Ozcan and Erkmen, 2001).* In addition, the gain results in this part were consistent with those obtained in the first part of the current study (Table 1).

In general, white cheese stored at chill temperature had *Bacillus cereus* and *Staph. aureus* lower than those stored at room temperature.

Table (10): Total *Staph. aureus* counts exhibited by home-made white cheese examined immediately after made and the end of storage period at different temperature.

Sample	Con. of *EOs* %	*Staph. aureus*					
		Fresh		Storage for 7 days at::			
		Before milk heating-72°C-	After milk cooling-37°C-	Before milk heating-72°C-		After milk cooling-37°C-	
				Room temp.	Ref. temp.	Room temp.	Ref. temp.
Control	0.00	$2.7\times10^{2\,E}$		$5.1\times10^{2\,G}$	4.3×10^{2F}	4.5×10^{2F}	3.7×10^{2EF}
Spearmint	0.25	$2.9\times10^{1\,B}$	1.2×10^{1A}	$4.1\times10^{2\,G}$	9.6×10^{1E}	2.5×10^{2E}	8.3×10^{1CD}
	0.50	$1.3\times10^{1\,A}$	1.5×10^{1A}	$8.2\times10^{1\,D}$	4.8×10^{1BC}	7.3×10^{1CD}	4.4×10^{1B}
	1.00	$1.8\times10^{1\,A}$	0.8×10^{1A}	$8.1\times10^{1\,D}$	3.3×10^{1B}	6.6×10^{1C}	2.1×10^{1AB}
Clove	0.25	$9.0\times10^{1\,D}$	7.5×10^{1CD}	$3.1\times10^{2\,F}$	8.9×10^{1DE}	2.9×10^{2E}	7.6×10^{1CD}
	0.50	$7.1\times10^{1\,C}$	6.1×10^{1C}	9.0×10^{1DE}	8.8×10^{1DE}	8.3×10^{1CD}	5.6×10^{1BC}
	1.00	3.7×10^{1BC}	4.1×10^{1B}	7.9×10^{1D}	6.3×10^{1C}	4.4×10^{1B}	3.6×10^{1AB}
Basil	0.25	7.4×10^{1AB}	2.6×10^{1AB}	1.3×10^{2EF}	7.6×10^{1D}	9.6×10^{1D}	7.3×10^{1CD}
	0.50	$4.9\times10^{1\,C}$	1.3×10^{1A}	$6.6\times10^{1\,C}$	5.2×10^{1C}	5.9×10^{1BC}	4.2×10^{1B}
	1.00	$2.4\times10^{1\,A}$	0.9×10^{1A}	$5.1\times10^{1\,C}$	2.5×10^{1AB}	3.1×10^{1B}	1.9×10^{1A}

Data followed by different letters are significantly different ($p < 0.05$).
Statistical analyses were done for control sample with *EOs* of each treatment.
Values are means of 5 replications.

In conclusion, these results indicated that, Spearmint, Clove and Basil *EOs* at different concentrations inhibited all of the tested organisms. The Basil EO gave a strongest inhabitation for *B. cereus* and *Staph. aureus* than the other tested *EOs*. As described from this investigation that antimicrobial effect of Spearmint, Clove and Basil *EOs* more potent on moulds than Gram positive and Gram negative bacterial.

The microbiological quality of white cheese with *EOs* additives after cooling of milk during preparation of cheese was better than those of it before heating of milk. It may be due to the fact that, *EOs* has some sensitive components to heat such as volatile components.

Basil *EO* at different concentration had the strongest effect for total bacterial count and total fungal count among all another tested *EOs*, especially at 1.0 % concentration. By increasing the fortification ratio of *EOs* in white cheese the number of bacteria has been decreased. Unfortunately,

the organoleptic properties, especially for taste and odor were decreased by the increasing the doses of *EOs*. Therefore, more attention should be paid to produce essential oils with low flavorings.

From the microbial point of view, it could be concluded that examined white cheese were suitable and safe for human's feeding and this applies to white cheese properly treated with *EOs*, stored at chill temperature equal to or less than 5°C and under hygienic conditions.

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تأثير بعض الزيوت النباتية العطرية كمضادات ميكروبية طبيعية علـــى الجبن الأبيض

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تم دراسة ٥٠ عينة جبن أبيض مصنع محليا لتقييم الجودة الميكروبيولوجية بعد صناعتها مباشرة ، وبعد تخزينها لفترات مختلفة وعند ظروف مختلفة – خلال فصل خريف ٢٠٠٦/٢٠٠٧ م. حيث استخدمت زيوت عطرية من النعناع والقرنفل والريحان والتي تم شرائها مـــن الأســـواق المصرية لدراسة نشاط هذه الزيوت ضد الميكروبات الممرضة بالجبن الأبـــيض. وســـجل زيـــت النعناع أعلى درجات التقييم الحسي (على الجبن الأبيض الطازج) يليه زيوت الريحان والقرنفـــل وخصوصا عند التركيزات المنخفضة.

وأضحت النتائج وجود فروق معنوية بين العينات الخالية من الزيوت العطرية "الكنترول" والعينات المدعمة بجرعات مختلفة من هذه الزيوت " ٠,٢٥ ، ٠,٥ ، ١,٠ % " وعنـــد درجـــات حرارة مختلفة " ٢٢ ، ٥°م ". حيث أن العينات الكنترول المخزنة لمدة يومين أو أكثر على درجـــة حرارة الغرفة كانت غير امنة صحيا. بينما عينات الجبن الأبيض المختبرة والمدعمـــة بتركيـــزات ٠,٥ ، ١,٠ % من الزيوت العطرية كانت جودتها البكتيريولوجية مرضية عند تخزينها على درجة حرارة التبريد ودرجة حرارة الغرفة لمدة ٧ أيام. ودلت النتائج على أن اضافة الزيوت العطريـــة للبن بعد المعاملة الحرارية وتبريده " ٣٧°م " الحصول على نتائج أفضل عما اذا أضيفت اليه قبـــل التسخين " ٧٢°م ".