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Effect of Lemon Basil and Marjoram Essential Oils on Shelf Life of Refrigerated Chicken Minced Meat.

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Abstract

This research was carried out to identify the chemical components of lemon basil and marjoram volatile oils. Also, study the effect of different essential volatile oils and their mixture at 300 ppm on total bacterial count, *E. coil* and *B. cereus* counts of inoculated chicken minced meat with *E. coil* and *B. cereus* during cold storage at 4ºC. Results indicated that the major components of lemon basil volatile were E-citral (27.20%), Z-citral (25.65%), β-myrcene (11.39%) and Phthalic acid, hept-4-yl isobutyl ester (9.85%), meanwhile the major components of marjoram volatile oil were l, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R) (24.47%), Cis-Sabinene hydrate (17.05%), and γ.-terpinene(14.40%). Also, addition of different volatile oils at 300 ppm to inoculated chicken minced meat with *E coli* and *bacillus cereus* led to decreased total bacterial count, *E. coli* and *B. cereus* counts when compared with control sample during cold storage period. Also, lemon basil volatile oil more effective in reduction of the above bacteria during cold storage followed by mixture of volatile oils and finally marjoram volatile oil.

Key words: basil, marjoram, chicken meat**.**

Introduction

Chicken meat is one of the most popular meats and commonly consumed worldwide for being considered as healthier and is frequently more affordable than other meats **(Farrell, 2019 and OECD , 2019)**. So that, it contains a high amount of water, proteins, and fats that enables the growth of microorganisms on it (**FAO, 2019)**. Spoilage of food is easily by microorganisms and further has a relatively short shelf life. For prolong its shelf life and minimize the risk of foodborne illness and food poisoning, a preservation process is required **(Dave and Ghaly, 2011)**. Food spoilage and pathogenic bacteria can contaminate meat products and lead to public health hazard and economic losses **(Salem, *et al*., 2010)**. So that it become today a great interest in replacing the synthetic preservatives with aromatic compounds like essential oils in foods. The food industries researchers headed for superseded sources of antibacterial and chemical preservatives against inroad of bacteria and lipid oxidation in foods **(Guimaraes, *et al*., 2010)**.

On the other hand, it use of chemical antioxidants with high activity, such as tertiary butyl hydroquinone with carcinogenic property can threaten consumers' health **(Ayoughi, *et al*., 2011)**.

Now, the extracts and essential oils of medicinal plants have been considered not only for protection **(Baradaran *et al*., 2012)** or treatment **(Asgary, *et al*., 2014)** of various diseases, but also as natural food preservatives. It found the most of these effects have been attributed to their antioxidant activities **(Rafieian-Kopaei, 2012)**. Essential oils are mixtures of secondary metabolites of plants that have volatile and aromatic products, and can be stored in various plant organs, usually in leaf, fruit, or bark **(Angane, *et al*., 2022)**. The most of essential oils are composed of terpenic substances, plus smaller molecules such as alcohols, esters, aldehydes and short-chain ketones **(Amor, *et al*., 2021)**.

Several essential oils have antimicrobial properties and can act as bio preservers, reducing or eliminating pathogenic bacteria and increasing the microbiological quality of food products of animal and plant origin **(Angane, *et al*., 2022)**. Basil is one of these medicinal plants and is called "Reyhan" in Iran. This genus is of the *Lamiaceae* family and its dried leaves as well as its essential oil are used in food industry as aromatic and flavoring ingredients. Also, basil is commonly used to treat fever, stomach ache, inflammatory, flatulence, constipation and is also used as an antibacterial and antifungal agent **(Rafieian- Kopaei and Hosseini-Asl, 2005, Ozcan and Chalchat, 2002)**. Previous studies have demonstrated the antibacterial and antioxidant activities of essential oil on different kinds of plants as basil in vitro **(Bunrathep, *et al*., 2007, Kordali, *et al*., 2005, Lee, *et al*., 2005, Sokmen, *et al*., 2004, Ozcan and Chalchat, 2002)**.

Marjoram essential oil is a natural product classified as generally recognized as safe and known to possess antimicrobial and antioxidant activities (**Burt, 2004, Chan, *et al.*, 2012)**. In Egypt many essential oils including marjoram essential oil (MEO) are produced and some are exported for increasing income from foreign currency **(El-Aeshmawiy, *et al.*, 2009)**. Marjoram herb of happiness‖ is cultivated for use of its aromatic leaves and their essential oil for flavoring, industrial uses (natural components of these plants are used as food additives, herbal tea, pigments, insecticides, dyes, perfumes, cosmetics and natural preservatives), as well as medicinal and other culinary purposes **(Tapsell, *et al.*, 2006 and Buchbauer, 2010)**. Marjoram *(Origanum majorana L.),* a member of the Lamiaceae family is one of the most familiar kitchen herbs, which contains up to 3% of volatile oil, other compounds like flavonoid, arbutin, tannins, caffeic acid, labiatic acid, rosmarinic acid, ursolic acid, carnosic acid and carnosol can be found in the herb **(Shan, *et al.*, 2005)**. The aim of this study was determined antibacterial effects of lemon basil (*Ocimum basilicum*), marjoram (*Origanum marjorana L.*) essential oils and their mixtures in vitro and vivo. This research was carried out to identify the chemical component of lemon basil and marjoram essential oils by GC mass and study the antibacterial activity of these essential oils and their mixture against *S. typhimurium*, *E. coli*, *S. aureus and B. cereus* by disc diffusion assay. Also, study the effect different essential volatile and their mixture at 300 ppm on total bacterial count, *E coil* and *B cereus* counts of chicken minced meat inoculated with *E coil* and *B cereus* during storage at 4ºC

Materials and methods

2.1. Materials:

2.1.1. Raw material:

Whole chicken carcasses (14 kg) from fresh slaughtered broilers with average weight (1.5-2 kg) were obtained from local market, Qalubia Governorate, Egypt, and transported in icebox to the Meat and Fish lab., Food Tech. Res. Inst., A.R.C., Giza. Limon Basil (*Ocimum citriodorum*) and Marjoram (*Marjorana hortensis*) volatile oils were obtained from (PHATRADE) Company, Obour City, Qalubia governorate, Egypt.

2.1.2. Bacterial strains:

Four bacterial strains representing two gram-negative bacteria (*E. coli* ATCC 25922 and *S. typhimurium* ATCC14028) and two gram positive bacteria (*B. cereus* ATCC 9634 and *S. aureus* ATCC 12600) were obtained from Department of Chemistry of Natural and Microbial product, N. R. C. ,Cairo, Egypt. These bacterial strains were checked for purity and recultivated to obtain active cultures.

2.1.3. Microbiological media:

Nutrient agar medium, violet red bile agar medium (VRBA) and Manitol egg yolk polymyxin agar medium (MYP) and *B. cereus* selective supplement were imported by Al-Badr Engineering Company, which is the Egyptian agency of Italian Biolife Company.

2.2. Methods:

2.2.1. Preparation of Chicken minced meat and inoculation with bacterial strain:

Upon arrival to the laboratory, chicken carcasses were gutted, cleaned, deboning manually and chopped in mincer under aseptic conditions. The standard methods of **(AOAC, 2005)** and the International Commission for the Microbiological Specifications of Foods **(Tompkin, 2002)** were adopted for preparation and microbiological analyses of chicken minced meat samples. Fresh chicken minced meat was divided into three batches the first kept without any treatment (C), the second and the third batches were inoculated by *B. cereus* (B) and *E. coli* (E) strains approximately (6 log^{10} CFU/ml) using micropipettes on chicken minced meat sample to gain a final concentration of 4 log^{10} CFU/g and each of them was divided into four groups, the first was untreated (B1 and E1), the second group was treated with 300 ppm lemon basil essential oil (B2 and E2), the third group was treated with marjoram essential oil (B3 and E3) and the fourth group treated with 300 ppm mixture of basil and marjoram essential oils (1:1, v:v) (B4 and E4) as shown in Table (1).

Table 1. Experimental treatments.

Treatments	Description
C	Chicken minced meat sample (control sample).
B1	Chicken minced meat sample inoculated by *B. cereus*
B2	Chicken minced meat sample inoculated by *B. cereus* and treated with basil essential oil at 300ppm.
B3	Chicken minced meat sample inoculated by *B. cereus* and treated with marjoram essential oil at 300ppm.
B4	Chicken minced meat sample inoculated by *B. cereus* and treated with mixture of basil and marjoram essential oil (1:1, v:v) at 300ppm.
E1	Chicken minced meat sample inoculated by *E. coli.*
E2	Chicken minced meat sample inoculated by *E. coli* and treated with basil essential oil at 300ppm.
E3	Chicken minced meat sample inoculated by *E. coli* and treated with marjoram essential oil at 300ppm.
E4	Chicken minced meat sample inoculated by *E. coli* and treated with mixture of basil and marjoram essential oil (1:1, v:v) at 300ppm.

Each sample was packaged in polyethylene bags, labeled, and stored at 4±1˚C. Minced meat was sampled at 3 days intervals during storage for analysis **El-Desouky, *et al.,* (2006):**

2.2.2. Identified the essential oils components by Gas mass spectrometry analysis.

The GC-mass system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, N.R. C., Egypt according to the method described by **(Abd El-Motaleb, *et al*., 2021)**.

2.2.3. Microbiological examination:

The bacterial examination of chicken minced meat inoculated by *E. coil* and *B. cereus* and treated with different essential oils such as total bacterial count, *E coil* and *B cereus* counts were determined according to the following steps:

2.2.3.1. Sample preparation

Ten grams of chicken minced meat were mixed with 90 ml of sterile peptone water 1.0% by using a vortex under sterilized conditions, to give 1/10 dilution. Serial dilutions were prepared to be used for counting total bacterial count, *E coil* count and *B cereus* count.

2.2.3.2 Total bacterial count:

Total bacterial count was analyzed using nutrient agar media. Incubation was carried out at 37°C for 48 hrs. The counts were then calculated per gram of samples (**ISO, 4833-1, 2013)**.

2.2.3. 3. *Bacillus cereus* count:

Bacillus cereus was analyzed using Mannitol Egg Yolk Polymyxin Agar and *B. cereus* selective supplement. Incubate plates for 18-24 h at 30°C and observe colonies surrounded by precipitate zone, which indicates that lecithinase is produced. *B. cereus* colonies are usually pink on (MYPA) and may become more intense after additional incubation (**FDA, 2021)**.

2.2.3. 4. *Escherichia coli* count:

E. coli was analyzed using violet red bile agar. Incubation was at 37°C for 48 hrs. The counts were then calculated per gram of samples (**ISO, 21528-2 2017)**.

2.2.4. Statistical analysis:

The obtained results were statistically analyzed least significant different (LSD) at the 5% level of probability by **Snedecor and Cochran (1994)**.

Results and Discussion

3.1. Chemical components of basil and marjoram essential oils:

The components of lemon basil and marjoram volatile oils were identified by GC mass spectrometer analysis. The components percentages calculated as the ratio of peak area to the total chromatographic peaks area as listed in **Table (2)**. From these data it could be identified that twenty five and nineteen volatile components were fractionated from basil and marjoram volatile oils, which represented 99.99 and 99.96 %, respectively. The most abundant chemical compounds of basil volatile oil were E- citral, Z- citral, beta-Myrcene and phthalic acid which represented 74.09% of the total identified chemical compounds. E- citral (27.20%) was the main component of basil essential oil followed by Z- citral (25.65%), while phthalic acid (9.85%) few were among the most abundant chemical compounds of basil essential oil.

Also, isogeranial (3.16%), 1,2- benzene di-carboxylic acid, bis (2-methylpropyl) ester (2.94%), geraniol (2.92%), isoneral (2.47%), L-Linalool (1.98%), 5-Hepten-2-one, 6-methyl (1.43%) and Selin-6-en-4.alpha.-ol (1.37%) were found in moderate amount of basil volatile oil which represented 16.27 % of the total identified chemical compounds.

Moreover, basil volatile oil contained fourteen components in small or trace amounts which recorded 9.63 % of the total identified chemical compounds such as citronellol (0.97%), 2, 6-octadienoic acid, 3, 7-dimethyl-(E) (0.78%), geranyl acetate (0.88%), trans -caryophyllene (0.83%), Isogeranial (0.43% of the total identified chemical compound) was the lowest chemical compounds in basil volatile oil.

These results are in agreement of these reported by **Avetisyan, *et al*., (2017)** who found that E-citral and Z- citral considered the major compounds in basil volatile oil. Also, **Hartanti, *et al.,* (2019)** found that the main constituents of lemon basil were estragol, linalool, E-citral and Z-citral. **Hamad, *et al*., (2021)** noticed that lemon basil contained 14.6% neral (Z citral) and 22.79% geranial (E citral).

Also, E-citral and Z-citral were the principal components of lemon basil essential oil collected in Chiang Mai, Thailand **(Tangpao, *et al*., 2018)**. Moreover, citral (55.0-75.5%), γ-bisabolene (2.6-9.5%), nerol (1.7-8.9%), geraniol (1.5-6.5%), linalool (1.10 - 6.0%) and β-caryophyllene (0.7-3.2%) were identified as the characteristic constituents of lemon basil essential oil originated from northern India **(Padalia, *et al*., 2018).**

The different percentage of each compound in lemon basil essential oil was mentioned following the different harvesting and post-harvest drying methods of lemon basil aerial parts.

From the same table, it could be observed that, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R), Cis-Sabinene hydrate, γ -Terpinene, α –Terpinene and Bicyclo [3.1.0]hexane, 4-methylene-1-(1-methylethyl) were the most predominant chemical compounds in marjoram volatile oil which represented 74.72 % of total chemical compounds.

Table 2. Chemical compositon of basil and marjoram essential oils (%) identified by GC mass.

Componends	RT (min)	Basil essential oil	Marjoram essential oil
		Area %	Area %
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)	9.42	-	8.67
α -Terpinene	9.42	-	10.03
β -Pinene	9.47	-	0.55
β-Myrcene	10.04	-	2.27
α-Phellandrene	10.44	-	0.65
5-Hepten-2-one, 6-methyl	11.30	1.43	-
D-Limonene	11.33	-	4.02
Eucalyptol	11.39	-	0.40
beta.-Myrcene	11.45	11.39	-
γ.-Terpinene	12.49	-	14.50
α- Terpinenolene	13.48	-	3.75
Myroxide	14.66	0.39	-
L-Linalool	14.86	1.98	-
1- Terpinenol	15.36	-	1.15
Isogeranial	16.02	0.43	-
6-Octenal, 7-methyl-3-methylene	16.23	0.54	-
trans-Chrysanthemal	16.34	0.78	-
7-Octenal, 3,7-dimethyl	16.47	0.73	-
Isoneral	16.83	2.47	-
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)	16.85	-	24.47
Alpha -Terpinenol	17.24	-	4.11
Isogeranial	17.36	3.16	-
Piperitol isomer I (cis)	17.37	-	0.56
Estragole	17.44	-	0.70
Citronellol	18.74	0.97	-
Z-Citral	19.14	25.65	-
1,6-Octadien-3-ol, 3,7-dimethyl-, formate	19.45	-	2.08
Geraniol	19.49	2.92	-
E-Citral	20.02	27.20	-
2,6-Octadienoic acid, 3,7-dimethyl-, (E)	22.30	0.78	-
3,6-Heptadien-2-ol, 2,5,5-trimethyl-, (E)	22.66	0.67	-
Geranyl acetate	22.89	0.88	-
trans-Caryophyllene	23.83	0.83	-
Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)	24.22	0.67	-
Caryophyllene	24.79	-	3.82
cis-sesquisabinene hydrate	25.68	0.52	-
Caryophyllene oxide	27.81	0.53	-
Selin-6-en-4.alpha.-ol	28.63	1.37	-
Phthalic acid, hept-4-yl isobutyl ester	29.29	9.85	-
1,2-Benzene di-carboxylic acid, bis(2-methylpropyl) ester	33.91	2.94	-
Neric acid	45.10	0.91	-
Cis-Sabinene hydrate	46.05	-	17.05
Bis (2-ethylhexyl) phthalate	46.17	-	0.88
5A-Methyl -3,8-di methylene-2-oxododecahydrooxireno [2',3':6,7]naphtho [1,2-B]furan-	47.50	-	0.30
RT= Retention time.		**99.99**	**99.96**

The 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl) was the major component (24.47%) of marjoram volatile oil followed by Cis-Sabinene hydrate (17.05%). On the other hand, Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl) was the trace one (8.67%) among the most permanent chemical compounds in marjoram essential oil.

Moreover, alpha-terpinenol (4.11%), D-limonene (4.02%), caryophyllene (3.82%), α-terpinenolene (3.75%), β-myrcene (2.27), 1,6-Octadien-3-ol, 3,7-dimethyl-, formate (2.08%) and 1-Terpinenol (1.15%) were present in moderate amount of marjoram essential oil which recorded 21.20 % of the total identified chemical compounds. Other identified compounds in marjoram volatile oil such as β –pinene, α-phellandrene, eucalyptol, Piperitol isomer I (cis) and estragole were found in trace amounts. These results are in agreement with **Badee, *et al.*, (2013)** who recorded that the major components in Egyptian marjoram were γ-terpinene (23.20%), α-terpinene (19.71%) and terpinen-4-ol (12.64%), followed by α-terpinolene (6.76%),sabinene hydrate (6.56%), p-cymene (5.81%), α-phellandrene (5.43%) and Limonene (5.40%),they constituted 85.51% of total essential oil, indicating that oil belonged to terpinen-4-ol /sabinene-hydrate chemotype. These results were similar with those obtained by **Yasar *et al.* (2022), Ouedrhiri *et al.* (2017), Busatta *et al.* (2008) and Abdel-Aziz and Morsy (2014).**

3.2. Effect of basil and marjoram essential oils and their mixture (1:1 v:v) on total bacterial count of chicken minced meat inoculated with *E. coli* during cold storage at 4 ±1°C for 12 days.

Data in **Table (3)** noticed that the initial total bacterial count (TBC) of control sample was 4.68 log CFU/g, this count increased in (E1) sample to 5.97 CFU/g. Also, the initial TBC in (E2), (E3) and (E4) samples were 5.96 log CFU/g, 5.96 log CFU/g and 5.95 log CFU/g, respectively.

Total bacterial counts in (E1) sample increased by increasing the cold storage periods at 4 ±1°C. These results are in agreement with those obtained by **Ntzimani, *et al.*, (2011)** who illustrated that total count of untreated chicken meat was increased by increasing the cold storage periods. Also, similar results were obtained by **Majdinasab, *et al.*, (2020).**

Table 3. Effect of basil, marjoram essential oils and their mixture on total bacterial counts (Log CFU/g) of chicken minced meat inoculated with *E. coli* during cold storage at 4 ±1°C for 12 days.

Treatments	Storage period (days)									
	Zero time		3^{th} day		6^{th} day		9^{th} day		12^{th} day	
	TC	Log	TC	Log	TC	Log	TC	Log	TC	Log
C	4.8×10^4	4.68	7.8×10^4	4.89	4.3×10^5	5.63	1.1×10^7	7.04	7.6×10^7	7.88
E1	9.31×10^5	5.97	2.7×10^6	6.43	4.6×10^7	7.66	3.4×10^8	8.53	2.9×10^9	9.46
E2	9.1×10^5	5.96	2.8×10^5	5.45	9.5×10^3	3.98	4.4×10^3	3.64	2.19×10^4	4.34
E3	9.1×10^5	5.96	7.1×10^5	5.85	4.7×10^4	4.67	2.13×10^5	5.33	6.8×10^5	5.83
E4	8.9×10^5	5.95	6.2×10^5	5.79	1.7×10^4	4.23	7.4×10^3	3.87	5.4×10^4	4.73

Log: Logarithm CFU: Colony Forming Unit g: Gram
TC: Total bacterial count.
E1: Chicken minced meat sample inoculated by *E. coli.*
E2: Chicken minced meat sample inoculated by *E. coli* and treated with basil essential oil 300ppm.
E3: Chicken minced meat sample inoculated by *E. coli* and treated with marjoram essential oil 300ppm.
E44: Chicken minced meat sample inoculated by *E. coli* and treated with mixture of basil and marjoram essential oils (1:1, v:v) at 300ppm.

On the contrary, total bacterial counts of chicken minced meat inoculated with *E. coli* and treated with 300 ppm of different essential oils (basil and mixture of basil and marjoram) were decreased by cold storage period increment up to the 6th day of cold storage for (E3) and up to 9^{th} day for (E2) and (E4) after that TBC of above samples were increased.

The highest decrement of total bacterial count was recorded (E2) sample which decreased from 5.96 log CFU/g at zero time to 3.64 log CFU/g after 9 days of cold storage, followed by (E4) sample which decreased from 5.95 log CFU/g at zero time to 3.87 log CFU/g after 9 days of cold storage. While, the lowest decrement of TBC was recorded in (E3) sample which decreased from 5.96 log CFU/g at zero time to 4.67 log CFU/g after 6 days of cold storage. These results are agreement with those obtained by **Barbosa, *et al.*, (2009)** who found that basil essential oil had higher antimicrobial than marjoram essential oil. Generally, the decrement of TBC of chicken minced meat inoculated with *E coli* and treated with different essential oils (basil, marjoram and mixture of them) compared with untreated sample with essential oils (E1) during cold storage period indicate the antimicrobial effect of these essential oils. Similar results were obtained by **Barbosa, *et al.*, (2009); Poonkodi, (2016)** and **Schuh, *et al.*, (2022)** who reported that basil and marjoram had antimicrobial effect.

3.4. Effect of basil and marjoram essential oils and their mixture (1:1 v:v) on *E. coli* counts (Log CFU /g) of chicken minced meat during cold storage at 4 ±1°C for 12 days.

Basil and marjoram essential oils and their mixture of them at 300 ppm were evaluated for their effectiveness against *E. coli* when inoculated in chicken minced meat during cold storage at 4 ±1°C to 12 days as shown in **Table (4)**.

The initial population of *E. coli* in C sample was 1.70 log CFU/g, this value increased to 5.68 log CFU/g E1 sample. The *E coli* counts of above-mentioned treatments were increased from 1.7 to

5.13 log CFU/g for (C) and from 5.68 to 8.15 log CFU/g for E1 sample by increasing cold storage period from zero time to 12 days of storage respectively. Similar results were reported by **Majdinasab, *et al*.,(2020) and Ntzimani, *et al*., (2011)**.

Table 4. *E. coli* counts (Log CFU /g) of inoculated chicken minced meat with *E. coli* and treated with basil and marjoram essential oils and their mixture during cold storage at 4 ±1°C for 12 days.

Treatments	Storage period (days)									
	Zero time		3^{th} day		6^{th} day		9^{th} day		12^{th} day	
	TEC	Log	TEC	Log	TEC	Log	TEC	Log	TEC	Log
C	5 x 10	1.70	9 x 10	1.95	1.6×10^{2}	2.20	1.4×10^{3}	3.15	1.35×10^{5}	5.13
E1	4.8×10^{5}	5.68	1.7×10^{6}	6.23	8.3×10^{6}	6.92	1.2×10^{7}	7.08	1.41×10^{8}	8.15
E2	4.9×10^{5}	5.69	5.8×10^{4}	4.76	8.3×10^{3}	3.92	8.1×10^{2}	2.91	5.8×10^{3}	3.76
E3	4.7×10^{5}	5.67	9.3×10^{4}	4.97	2.1×10^{4}	4.33	1.7×10^{5}	5.23	5×10^{5}	5.70
E4	5×10^{5}	5.70	6.6×10^{4}	4.82	1.3×10^{4}	4.11	3.8×10^{3}	3.58	4.4×10^{4}	4.64

C: Chicken minced meat sample (control sample).
TEC: Total *E. coli* count.
E1: Chicken minced meat sample inoculated by *E. coli.*
E2: Chicken minced meat sample inoculated by *E. coli* and treated with basil essential oil 300ppm.
E3: Chicken minced meat sample inoculated by *E. coli* and treated with marjoram essential oil 300ppm.
E4: Chicken minced meat sample inoculated by *E. coli* and treated with mixture of basil and marjoram essential oils (1:1, v:v) at 300ppm.

From the same table, it could be noticed that addition of different essential oils (basil and marjoram) or mixture of them at 300 ppm to chicken minced meat inoculated by *E. coli* led to decrease the counts of *E. coil* during cold storage up to the 9^{th} day for both basil (E2) and mixture of volatile oils (E4) and to the 6^{th} day of cold storage for marjoram volatile oil (E3), after that counts of *E coli* tend to increase.

The highest decrease of *E coli* was recorded in (E2) which decreased from 5.69 log CFU/g at zero time to 2.91 log CFU/g after 9 days of cold storage, followed by E4 which decreased from 5.70 log CFU/g at zero time to 3.58 log CFU/g at the 9^{th} day of cold storage, but (E3) sample decreased from 5.67 log CFU/g at zero time to 4.33 log CFU/g at the 6^{th} day of cold storage and increased after that. These results reflect the basil volatile oil more effective against *E. coli* than marjoram. Similar results were obtained by **Marques, *et al*., (2015**) and **Barbosa, *et al*., (2009)** who reported that basil essential oil had higher antimicrobial activity than marjoram essential oil.

3.5. Effect of basil, marjoram essential oils and their mixture on total bacterial count of inoculating chicken minced meat with *Bacillus cereus* during cold storage at 4 ±1°C for 12 days:

Inoculation of chicken minced meat by *Bacillus cereus* increased the initial total bacterial count from 4.68 log CFU/g (C) to 5.71, 5.73, 5.72 and 5.71 CFU/g for B1, B2, B3 and B4 (mixture of basil and marjoram volatile oils), respectively as shown in Table (5). At zero time, the TBC of chicken minced meat inoculated with *Bacillus cereus* not affected by addition of different volatile oils at 300 ppm.

Table 5. Effect of basil, marjoram and their mixture essential oils on total bacterial counts (Log CFU/g) of inoculated chicken minced meat with *Bacillus cereus* during cold storage at 4±1°C for 12 days.

Treatments	Storage period (days)									
	Zero time		3^{th} day		6^{th} day		9^{th} day		12^{th} day	
	TC	Log	TC	Log	TC	Log	TC	Log	TC	Log
C	4.8×10^{4}	4.68	7.8×10^{4}	4.89	4.3×10^{5}	5.63	1.1×10^{7}	7.04	7.6×10^{7}	7.88
B1	5.1×10^{5}	5.71	3.9×10^{6}	6.59	5.1×10^{7}	7.71	1.62×10^{8}	8.26	7.9×10^{9}	9.90
B2	5.4×10^{5}	5.73	7.2×10^{4}	4.86	6.5×10^{3}	3.81	3.1×10^{3}	3.49	6.8×10^{4}	4.83
B3	5.2×10^{5}	5.72	3.9×10^{5}	5.59	3.4×10^{4}	4.53	1.29×10^{5}	5.11	9.5×10^{5}	5.98
B4	5.1×10^{5}	5.71	3.2×10^{5}	5.51	1.3×10^{4}	4.11	5.1×10^{3}	3.71	9.5×10^{4}	4.98

Log: Logarithm CFU: Colony Forming Unit g: Gram
C: Chicken minced meat sample (control sample).
TC: Total bacterial count.
B1: Chicken minced meat sample inoculated by *B. cereus.*
B2: Chicken minced meat sample inoculated by *B. cereus* and treated with basil essential oil 300ppm.
B3: Chicken minced meat sample inoculated by *B. cereus* and treated with marjoram essential oil 300ppm.
B4: Chicken minced meat sample inoculated by *B. cereus* and treated with mixture of basil and marjoram essential oils (1:1, v:v) at 300ppm.

Total bacterial counts of (C) and (B1) progressively increased by increasing cold storage periods at 4 ±1°C. The increment rate was higher for (B1) than (c) sample. Similar results were obtained by **Ntzimani, *et al*., (2011)** and **Majdinasab, *et al*.,(2020)** who illustrated that total count of untreated sample (control) was increased with increasing cold storage at 4^0C. On the contrary, total bacterial counts of chicken minced meat inoculated with *B. cereus* and treated with different essential oils decreased by increasing cold storage period up to the 6^{th} day for (B3) and 9^{th} day of cold storage for (B2 and B4) after that TBC of above samples were increased. The highest decrement of total bacterial count after 9 days of storage was recorded for (B2) which decreased from 5.73 to 3.49 log CFU/g log CFU/g at zero time to after 9th of cold storage respectively, followed by (B4) which decreased from 5.73 log CFU/g at zero time to 3.71 log CFU/g after 9 days of storage.

On the contrast, the lowest decrement of TBC during cold storage period was recorded in (B3) which decreased from 5.72 log CFU/g at zero time to 4.53 log CFU/g after 6 days of storage and increased after that.

3.6. Effect of addition of basil, marjoram essential oils and their mixture on *Bacillus cereus* counts of chicken minced meat during cold storage at 4 ±1°C for 12 days.

Essential oils of basil and marjoram and their mixture at 300 ppm were also evaluated for their effectiveness against *B. cereus* when inoculated in chicken minced meat during cold storage at 4 ±1°C for up to 12 days as shown in **Table (6)**. The initial count of *B. cereus* in control sample (1.78 log CFU/g) was increased to 5.94 log CFU/g in (B1). The counts of *B. cereus* at zero time was increased from 1.78 to 5.32 log CFU/g for (C) to 12 th day and from 5.94 to 7.63 log CFU/g in B1 by increasing of cold storage. In this concern, **Sharma, *et al*., (2017)** who noticed that total count of fresh chicken sausage had increased more in control sample than treated samples through storage at 4^0C. Moreover, addition of different essential oils to chicken minced meat inoculated by *B. cereus* led to decrease the counts of *B. cereus* during cold storage up to the 6^{th} day in (B3) and the 9^{th} day for both B2 and B4, after that the count of *B. cereus* were increased. The highest decrement of *B. cereus* was recorded in (B2) which was decreased from 5.92 to 2.81 log CFU/g at zero time to the 9^{th} day of cold storage, followed by B4 which recorded 5.92 CFU/g at zero time to 3.53 CFU/g at 9 the day of cold storage, but the count of *B. cereus* in sample treated with marjoram essential oil (B3) was decreased from 5.93 to 4.89 log CFU/ g at zero time to the 6^{th} day of cold storage. These result reflect the basil essential oil more effective against *B. cereus* than marjoram. According to **Barbosa, *et al*., (2009)** who reported that basil essential oil had higher antimicrobial activity than marjoram essential oil. Also, **Sharafati, *et al*., (2015)** reported that basil essential oil at concentrations of 0.0625, 0.125 and 0.25% reduced the count of *S. aureus* for 12 days of storage at 4^0C in beef burger.

Table 6. Effect of *B. cereus* count (Log CFU /g) of inoculated chicken minced meat with *B. cereus* and treated with basil, marjoram essential oils and their mixture during cold storage period at 4 ±1°C for 12 days.

Treatments	Storage period (days)									
	Zero time		3^{th} day		6^{th} day		9^{th} day		12^{th} day	
	TBC	Log	TBC	Log	TBC	Log	TBC	Log	TBC	Log
C	6 x 10	1.78	1.4×10^2	2.15	1.1×10^3	3.04	8.9×10^3	3.95	2.1×10^5	5.32
B1	7.8×10^5	5.94	1.6×10^6	6.20	7.2×10^6	6.86	1.5×10^7	7.18	4.3×10^7	7.63
B2	8.3×10^5	5.92	9.3×10^4	4.97	5.2×10^3	3.72	6.5×10^2	2.81	6.6×10^3	3.82
B3	8.5×10^5	5.93	2.18×10^5	5.34	7.8×10^4	4.89	1.2×10^5	5.08	1.2×10^5	5.63
B4	8.3×10^5	5.92	1.35×10^5	5.13	5.1×10^4	4.71	3.4×10^3	3.53	3.4×10^4	4.53

Log: Logarithm CFU: Colony Forming Unit g: Gram

C: Chicken minced meat sample (control sample).

TBC: Total *Bacillus cereus* count.

B1: Chicken minced meat sample inoculated by *B. cereus.*

B2: Chicken minced meat sample inoculated by *B. cereus* and treated with basil essential oil 300ppm.

B3: Chicken minced meat sample inoculated by *B. cereus* and treated with marjoram essential oil 300ppm.

B4: Chicken minced meat sample inoculated by *B. cereus* and treated with mixture of basil and marjoram essential oils (1:1, v:v) at 300ppm.

Conclusions

This research was demonstrated the potential add of lemon basil and marjoram essential oils and their mixture to control the *E. coli* and *B. cereus* in chicken minced meat at 4°C. The results, also indicated that lemon basil essential oil at 300 ppm had the best effect on inhibition the *E. coli* and *Bacillus cereus* in chicken minced meat when used, followed by their mixture of basil and marjoram essential oils and finally marjoram essential oil with the same concentration, compared to the control sample. The producer and consumer preferred the use of natural additives in food, it is suggested that volatile oils should be practically applied in chicken meat in order to increase its safety against pathogenic bacteria**.**

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تأثيرزيوت الريحان الليمونى والبردقوش العطرية على فترة صلاحية مفروم لحم الدجاج المبرد.

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إستهدف البحث التعرف على المركبات الكيميائية لكل من زيت الريحان الليمونى وزيت البردقوش بواسطة جهاز كرماتوجراف مطياف الكتلة وتم أيضا دراسة تاثير هذه الزيوت ومخلوطهاعند تركيز 300 جزء فى المليون على العد الكلى للبكتريا وعد الإيشريشيا كولاى والباسيلس سيريوس فى مفروم لحوم الدجاج الملقح بالإيشريشيا كولاى والباسيلس سيريوس أثناء التخزين بالتبريد على 4 ^{o}م. أظهرت النتائج أن المركبات الكبرى لزيت الريحان الليمونى كانت E−citral (27.20%), Z−citral (25.65%), β−myrcene (11.39%) and Phthalic acid, hept−4−yl isobutyl ester (9.85%)

بينما المركبات الكبرى لزيت البردقوش كانت I, 3−Cyclohexen−1−ol, 4−methyl−1− (1−methylethyl)−, (R) (24.47%), Cis−Sabinene hydrate (17.05%), and γ.−terpinene(14.40%).

أيضا أدت إضافة الزيوت المختلفة حتى 300 جزء فى المليون لمفروم الدجاج الملقح بالإيشريشيا كولاى والباسيلس سيريوس إلى إنخفاض العد الكلى للبكتريا وعد الإيشيريشيا كولاى والباسيلس سيريوس عند مقارنتها بعينة الكنترول أثناء التخزين بالتبريد وكان لزيت الريحان الليمونى تأثير فعال فى خفض عدد البكتريا أثناء التخزين يتبعه المخلوط منهما ثم زيت البردقوش.