

## Effect of some Herbs Essential Oils on Labneh

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### ABSTRACT

Labneh is one of the most popular dairy products in many Middle Eastern countries. The shelf life of labneh is short even if stored at low temperature. Therefore, chemical preservatives were used to control the activity of contaminant microorganisms. An increased awareness of the harmful chemical residues in food led to a restricted use of chemical preservatives. Recently, natural substances such as spices oil can be used to prolong the shelf life of food products, because they are antioxidant and antimicrobial agents. In the present study three essential oils namely rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*) and cumin (*Cuminum cyminum*) were characterized by means of GC-MS and their antioxidant capacity were studied. Also, their antibacterial activity against pathogens *E. coli*, *B. cereus* and *S. aureus* was determined *in vitro* by wells' agar diffusion method. The results obtained revealed that the tested essential oils exhibited noticeable antimicrobial activity, where cumin showing the highest inhibition and sage the lowest. The results were completed with the study the antibacterial effect of the tested essential oils *in vivo* (labneh). However, the previous results obtained by *in vitro* assays were confirmed by *in vivo* experiment. In addition, three batches of labneh supplemented with different essential oils were made. No noticeable differences were observed in physicochemical properties of labneh treatments in comparison with the control one. However, labneh made with 0.05% rosemary essential oil ranked the highest value of antioxidant activity after 14 days of storage, being 20.78, while plain labneh (control) possessed the lowest figure, actually 10.8. Moreover, at the end of storage period, labneh produced with 250 mg/Kg cumin essential oil was the most preferred product by the panelists and ranked the highest total score, being 87 points.

### INTRODUCTION

Concentrated yoghurt is popularly known as labneh in the Middle East or as strained Yoghurt in Europe (Guler, 2007). Labneh is consumed as a main dish at breakfast in many Middle Eastern countries; it is obtained from yoghurt after removal of a part of its whey.

In the traditional method of producing labneh, the yoghurt is not subjected to heat and involves more manual handling; therefore, resultant labneh would apparently have higher microbial contamination. Thus, the shelf life of the resultant labneh is short, even if stored at low temperatures.

Traditionally, chemical preservatives are used to control the activity of contaminant microorganisms. An increased awareness by the environmental, health agencies and consumers of the harmful chemical residues in food and environment led to a restricted use of chemical preservatives. This trend, known as green consumerism, has resulted, since 1990 s, in the increase in consumer demand for natural antimicrobial compounds (Mastromatteo *et al.*, 2010).

Natural substances such as spices oils can be used to prolong the shelf life of food products, because they are antioxidant and antimicrobial agents, so that they can be used as natural preservatives to avoid the harmful effect of synthetic preservatives on human health (El-Bastawesy *et al.*, 2009).

Recently, the use of essential oils as functional ingredients in food products is gaining momentum (Chouliara *et al.*, 2007).

Essential oils from aromatic and medicinal plants have been known since antiquity to possess biological activity, notably antibacterial, antifungal and antioxidant properties (Cosentino *et al.*, 1999 and Bounatirou *et al.* 2007), these are mainly attributed to their phenolic compounds, i.e. carvacrol, thymol and terpenes (Burt, 2004).

The objective of the present study was to performance the chemical structures of the three tested essential oils by means of GC-MC, their antioxidant and antimicrobial effects against some pathogens *in vitro* and *in vivo* experiments. Also, to evaluate their effects on the chemical, antioxidant, microbial and sensory characteristics of the resultant labneh.

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### MATERIALS AND METHODS

#### Materials:

**Essential oils:** Rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*) and cumin (*Cuminum cyminum*) essential oils were purchased from the National Research center, Giza, Egypt.

#### Tested microorganisms:

*Escherichia coli* O157: H7, *Bacillus cereus* and *Staphylococcus aureus* were obtained from Botany Dept., Fac. of Sci., Al- Azhar Univ. Assiut.

#### Starter cultures:

*Lactobacillus delbruekii subsp. bulgaricus* and *Streptococcus thermophilus* (1:1) were used.

#### Culture media:

Nutrient agar medium (Difco, 1984) was used to assay the antibacterial activity of the tested essential oils. *E.coli* count was estimated by using violet red bile agar (VRBA) medium as recommended by Klein and Fung (1978), while *Staph. aureus* count was carried out on Baird-parker's egg yolk tellurite agar medium (Baird-parker, 1962).

For total bacterial count, tryptone soya agar medium (Cook and Brown, 1960) was used. Moulds and yeasts were plated on malt extract agar medium as recommended by Difco (1984).

#### Milk:

Mixture of fresh cow and buffalo milk was obtained from the herd of the Faculty of Agriculture, Al-Azhar Univ. Assiut (Acidity 0.16% and fat 3.5%).

#### Methods:

##### Analysis of volatile compounds of essential oils:

The analysis were carried out according to Ozkan *et al.* (2010) by using GC (Agilent technologies 7890A) equipped with a polar Agilent HP-5ms (30 m x0.25 mm x 0.25 µm film thickness). Mobile phase is helium; flow rate 1 ml/min, injector and detector temperatures were 200°C and 250 °C, respectively. Split ratio 1:10, volume injected

1µl of the sample. The MS operating parameters were: ionization potential 70ev, interface temp. 250°C and acquisition mass range 50-600.

**Determination of antibacterial activity:**

Antibacterial activities of essential oils against tested microorganisms were determined by the well agar diffusion method according to NCCLS (1993). The assessment of antibacterial activity was based on measurement of inhibition zone diameter formed around the well.

**Manufacturing of herbal labneh:**

Labneh was manufactured using the method outlined by Tamime and Robinson (1999). The volatile essential tested oils were added at two portions (0.025% and 0.050%)

**Chemical analysis:**

Titrate acidity and total solids content were determined as described by AOAC (2000). While the pH values were measured by using pH meter (Model STARTER 300) USA.

**Microbiological analysis:**

In order to determine the viable bacterial count, the general plate count technique outlined in the standard Methods for Examination of Dairy products (A.P.H.A., 1978) was adopted.

**Determination of antioxidant activity on labneh:**

The antioxidant activity of labneh was determined using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay as described by Shetty et al.(2007). An aliquot of the labnehwater extract (250 µl) was added to 3 ml of DPPH (60 µM in 95% ethanol). The mixture was shaken vigorously and allowed to stand at room temperature (25 °C) for 20 min.The absorbance was then measured at 517 nm(Spectrophotometer, Shimadzu UV Mini 1240) against control, which contained 250 µl of 95% ethanol instead of the extract. The DPPH radical scavenging effect was calculated as "percentage inhibition" according to the following formula:

$$\% \text{ Inhibition} = \frac{[A_{517}^{\text{control}} - A_{517}^{\text{extract}}]}{[A_{517}^{\text{control}}]} \times 100$$

Where  $A_{517}^{\text{control}}$  absorbance of control DPPH solution at 0 min

and was  $A_{517}^{\text{extract}}$  the absorbance of test sample after 20 min.

**Organoleptic properties:**

The sensory evaluation of labneh samples was conducted according to score card suggested by Keating and Randwhite (1990).

**RESULTS AND DISCUSSION**

The antimicrobial properties of the three tested essential oils (rosemary, sage and cumin) are plotted in Figures 1-3. Bacteria susceptibility to the essential oils, as determined by agar diffusion method, showed that the inhibition zone increased with the increasing the essential oil concentration. At low concentration (1 mg/ml) the tested essential oils showed no inhibition effect against all tested organisms. While, at high concentration (10 mg/ml),

the tested essential oils exhibited a marked inhibition activity against all tested organisms.

It might be gathered from data obtained that the inhibition effect of rosemary essential oil was stronger than the rest essential oils, and possessed inhibition zones varied from 9-12 mm, followed by cumin essential oil. Also the highest inhibition effect was observed toward *B. cereus*. In this respect, it was evident as the data in Table 1 that the most abundant component in rosemary essential oil area-terpinol 13.56%, D-limonene 12.66% and γ-terpinene 11.18%. however, the antibacterial properties of these compounds are evidently associated with their lipophilic character, leading to accumulation in membranes and energy depletion (Conner, 1993 and Sikkema et al. 1995). Also, in this connection, Hufford et al. (1993) stated that terpenoid showed excellent activity against *B. subtilis*, and lesser activity against gram-negative bacteria.

In addition, cumin volatile oil appeared more active against Gram-positive organisms (*B. cereus* and *S. aureus*), our finding are in complete agreement with those reported by Dorman and Deans (2000). As shown from Table 1, the principle components detected in cumin essential oil are cumin alcohol 21.60%, cumin aldehyde 19.30% and β-pinene 14.70%. In this respect, Marino et al. (1999) stated that aldehydes, ketones and alcohols of volatile oils showed the strongest antimicrobial activity. Moreover, Andrews et al. (1980) and Uribe et al. (1985) reported that β-pinene destroy cellular integrity, inhibit respiration, ion transport processes and increase membrane permeability which accompanied with the decline in the viability of microorganisms.

Continuously, it could be noticed that either *E. coli* or *S. aureus* were resistance toward sage essential oil up to concentration of 7 mg/ml. (Fig 1 & 3). While, at 10 mg/ml concentration, sage essential oil exhibited moderate inhibition activity against all tested organisms.

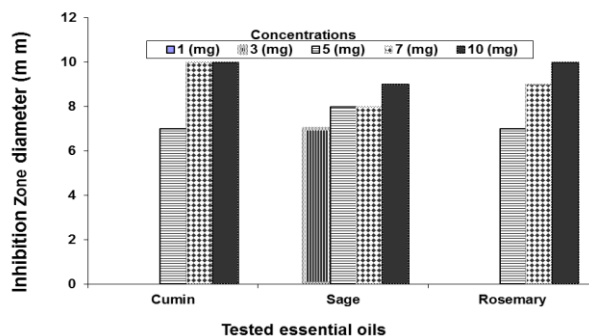


Figure1. Antibacterial activity of tested volatile oils on E.coli strain.

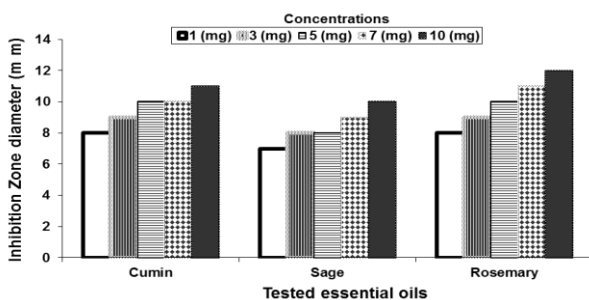


Figure 2. Antibacterial activity of tested volatile oils on B.cereus strain.

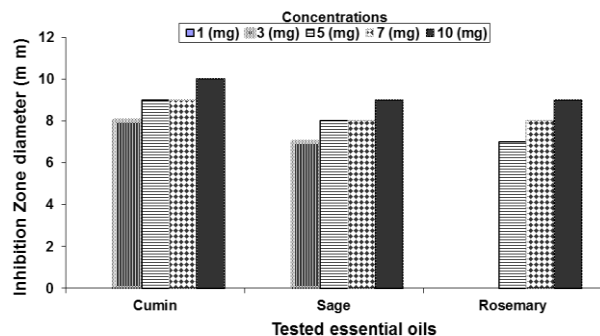


Figure 3. Antibacterial activity of tested volatile oils on *S.aureus* strain

Also, it is clear from Table 1, that the most abundant components in sage essential oil are Trans-caryophyllene 20.51%, 1,8-cineole 16.24%, camphor

Table 1. Identified components % of some essential oils (by GC/MS)

Rosemary			Sage			Cumin		
Retention time (min.)	Components	Volatile oil %	Retention time (min.)	Components	Volatile oil %	Retention time (min.)	Components	Volatile oil %
5.63	$\gamma$ -Terpinene	11.18	5.79	Ocimene	8.19	9.29	$\alpha$ -Pinene	0.80
5.97	Camphene	4.90	6.40	Camphene	5.81	9.68	$\beta$ -pinene	14.70
6.37	$\alpha$ -Pinene	4.54	6.55	$\beta$ -Pinene	8.12	10.14	<i>P</i> -Cymene	0.80
7.13	D-Limonen	12.66	6.78	$\alpha$ -Phellandrene	2.17	10.52	$\gamma$ -Terpinene	8.0
7.69	Cis-piperitol	4.58	7.11	Trans-Caryophyllene	20.51	11.26	$\alpha$ -Phellandrene	0.90
9.22	Isogeraniol	8.95	7.82	Camphor	16.19	12.19	Cumin aldehyde	19.30
9.35	$\alpha$ -Terpineol	13.56	8.14	Linalool	11.54	12.90	Thymol	1.20
10.93	Linalool	5.66	9.3	$\alpha$ -Terpineol	4.98	12.78	Cumin alcohol	21.60
11.92	Caryophyllene	4.57	9.58	Umbellulone	4.43	13.74	Acoradiene	17.20
13.11	Camphor	7.83	10.23	1,8-Cineole	16.24	14.19	$\beta$ -Caryophyllene	15.40

It was evident from data presented in Table 2 that the antibacterial effect of rosemary essential oil possessed the highest antagonistic effect against either *B. cereus* or *S. aureus*, with inhibition % of 5.97% and 3.46%, respectively, at 0.5% oil concentration. While, cumin essential oil showed the highest inhibition effect against *E. coli* at both tested essential oil concentrations, with inhibition figures of 4.64% and 5.22%, respectively.

According to the foregoing results, it could be concluded that the obtained results (in vivo) are confirmed those previously recorded in vitro assessment.

In order to give full consideration of resultant products (labneh) incorporated with different essential oils (0.25% and 0.50%) new batches were made and their chemical, antioxidant, microbiological properties and organoleptic assessment had been carried out.

The results presented in Table 3 revealed that no observable differences in total solids (TS) contents between the control and different treatments. The same statement was previously reported by Ismail et al. (2006).

Moreover, the change in total acidity (TA) is very important factor, since it affects the shelf-life and acceptability of resultant labneh. Based on results in Table 3, it may be gathered that the TA% were nearly the same in fresh labneh samples from different treatments. The acidity values of the treated labneh slightly increased with advanced storage period up to 21 days. A similar trend of result was previously found by Mutlag and Hassan (2008). However, at

16.19% and linalool 11.54%. However, the antibacterial effect of sage essential oil has been attributed to the presence of phenols and polypeptides (Gould, 1996 and Ismail et al. 2006). Also, Rota et al. (2004) mentioned that the bactericidal properties of sage essential oil may be associated with high levels of carvacrol and linalool.

In general, the different performance of essential oils in this study can be linked to their chemical compositions such as their contents of phenolic, aldehydes and alcohols (Bruni et al. 2003 and Sacchetti et al. 2005)

On the other hand, to study the antimicrobial effect of the tested essential oils in vivo, a small batches of labneh artificially contaminated with different pathogens ( $10^4$ cfu/ml) and two concentrations of essential oil (0.25% and 0.50%) were produced.

the end of storage period, different treatments possessed the same value for TA%, actually 1.4%, while slightly low value attained in plain labneh (control) being 1.35%.

Table 2. Effect of tested essential oils on the viability of some pathogens in labneh during storage

Pathogens	Storage period (days)	control	Essential oils					
			Rosemary		Sage		cumin	
			T1	T2	T1	T2	T1	T2
Log cfu/ml								
<i>E.coli</i>	0	4.75	4.72	4.64	4.61	4.55	4.53	4.41
	4	4.74	4.67	4.54	4.56	4.49	4.44	4.32
	7	4.72	4.61	4.46	4.52	4.43	4.32	4.18
G.inh%		0.63	2.33	3.88	1.94	2.64	4.64	5.22
<i>B.cereus</i>	0	4.81	4.69	4.69	4.77	4.71	4.67	4.57
	4	4.80	4.64	4.55	4.72	4.68	4.62	4.47
	7	4.78	4.56	4.41	4.69	4.60	4.55	4.36
G.inh%		0.62	2.77	5.97	1.68	2.34	2.57	4.60
<i>S.aureus</i>	0	4.76	4.69	4.62	4.69	4.56	4.49	4.44
	4	4.72	4.63	4.56	4.61	4.51	4.44	4.38
	7	4.72	4.59	4.46	4.58	4.40	4.43	4.32
G.inh%		0.84	2.13	3.46	2.35	3.51	1.34	2.70

T1: Treatment 1, 0.25% volatile oil      Cfu: Cell forming unit  
T2: Treatment 2, 0.50% volatile oil      G.inh%: Growth inhibition%

In contrast, the pH values of different treatments followed an opposite trend to acidity throughout storage period. However, labneh made with either 0.025% or 0.050% cumin essential oil possessed the lowest pH values at the end of storage period, being 3.58 and 3.54, respectively.

Whereas, labneh with rosemary oil ranked the highest pH value, actually 4.14.

Since antioxidants are essential for human health and dietary antioxidants play an important role in controlling

oxidative stress, therefore, this part of the present work was undertaken to evaluate the effect of the tested essential oils on antioxidant activity of labneh during storage period.

**Table 3. Effect of tested essential oils on some chemical and microbiological properties of labneh**

properties	Storage period (days)	Control	Rosemary		Sage		Cumin	
			T1	T2	T1	T2	T1	T2
Total solids %	0	27.35	27.03	27.26	27.28	27.91	27.81	27.94
	7	27.47	27.22	27.27	27.83	27.46	27.87	27.98
	14	27.53	27.23	27.27	28.65	28.75	28.02	28.05
	21	27.58	27.23	27.28	28.71	28.78	28.11	28.20
Acidity %	0	1.20	1.20	1.30	1.20	1.30	1.20	1.30
	7	1.30	1.40	1.40	1.30	1.30	1.30	1.30
	14	1.35	1.40	1.40	1.40	1.40	1.40	1.40
	21	1.35	1.40	1.40	1.40	1.40	1.40	1.40
pH	0	4.37	4.22	4.20	4.43	4.41	4.49	4.44
	7	4.09	4.15	4.25	3.99	3.98	3.93	3.90
	14	4.02	4.14	4.14	3.67	3.89	3.78	3.68
	21	3.60	4.14	4.14	3.66	3.61	3.58	3.54
Total bacterial count	0	8.15	8.14	8.14	8.16	8.17	8.15	8.15
	7	8.16	8.15	8.16	8.17	8.18	8.18	8.18
	14	8.19	8.16	8.17	8.20	8.21	8.19	8.19
	21	8.21	8.19	8.20	8.20	8.21	8.22	8.21
Yeasts and Moulds	0	ND	ND	ND	ND	ND	ND	ND
	7	ND	ND	ND	ND	ND	ND	ND
	14	2.8	ND	ND	ND	ND	ND	ND
	21	3.17	2.78	2.48	2.70	2.48	2.70	2.70

T1: 0.025% volatile oil

T2: 0.050 % volatile oil

cfu: cell forming unit

ND: not detected

It could be observed from data summarized in Table 4 that values of antioxidant activity were gradually increased in both treatments (T1 and T2) by prolongation storage period up to 14 days and then reduced during the third week of storage.

Also, it might be gathered that rosemary essential oil ranked the highest values for antioxidant activity, being 19.8 and 20.78 after 14 days of storage at 0.025% and 0.050% concentrations, respectively. Badee *et al.* (2013) stated that rosemary essential oil is a rich source of polyphenols which are known as natural antioxidant.

On contrast, plain labneh (control) possessed the lowest figure for antioxidant activity after 14 days of storage, actually 10.8, and then decreased on the third week of storage to 2.63.

Generally, viewing the previous results, it might be deduced that rosemary and sage essential oils have a good antioxidant effect and this is mainly attribute to their phenolic contents. However, the same conclusion was previously reported by Fecka and Turek (2008). Also, Elena *et al* (2009) mentioned that phenolic compounds are well known as radical scavengers, reducing agents, metal chelators and hydrogen donors. Therefore, natural antioxidants can protect the human body from free radicals and could retard the progress of many chronic diseases (Robbins and Bean, 2004, Arts and Hollman, 2005).

From Table 3 it could be noticed that the bacterial populations were not affected by either low or high concentrations of different tested essential oils. However, this conclusion is consistent with previous finding by Mutlag and Hassan (2008).

Moreover, yeasts were completely absent in all treated samples till 14 days of storage, while plain labneh (control) showed relatively low number of yeasts after 14 days of storage, being 2.80-log cfu/ml, this figure increased up to 3.16 log cfu/ml as storage period extending to 21 days. On the other hand, yeasts were detected in all treated samples at the end of storage period (21 days). Labneh supplemented with 0.05% of either rosemary or sage attained the lowest yeast counts, actually 2.48 log cfu/ml. In this respect, Bruni *et al.* (2003) and Sacchetti *et al.* (2005) were previously reported that yeasts and fungi are markedly inhibited by oils rich in phenolics, aldehydes and alcohols. However, our results are in complete agreement with those reported by Hassan *et al.* (2001), Schelz *et al.* (2006), Mutlag and Hassan (2008).

**Table 4. Effect of essential oils on antioxidant activity of labneh**

Storage period (days)	Control	Rosemary		Sage		Cumin	
		T1	T2	T1	T2	T1	T2
0	3.46	6.09	7.48	7.62	12.74	6.23	9.28
7	9.27	18.84	19.53	17.04	18.42	13.43	13.99
14	10.80	19.81	20.78	18.56	19.67	14.40	15.10
21	2.63	8.17	9.42	3.74	6.51	3.05	5.82

T1: Treatment 1, 0.025% volatile oil

T2: Treatment 2, 0.050% volatile oil

Organoleptic assessment of resultant labneh treated with different essential oils had been carried out, the data obtained summarized in Table 5.

According to the total score points of different fresh tested samples, it was found that labneh treated with 250 mg/kg cumin essential oil (T1) gained the highest score

points, actually 92 points. While those made with 0.05% rosemary essential oil (T2) attained the lowest value, being 85.2 points.

Additionally, after 7 days of storage slight increases in total score points of all tested samples were detected, while extending storage period up to 21 days reduced the values of different parameters and thus lowered its total scoring. Also, it could be noticed that throughout storage period labneh produced with either 0.025% or 0.05% cumin essential oil ranked the highest figures for flavor and body & texture. In contrast, labneh made with 0.05% rosemary essential oil possessed the lowest figures.

However, at the end of storage period (21 days), labneh supplemented with 250 mg/kg cumin essential oil was the most preferred product by the panelists and ranked the highest total score, being 87 points.

Finally, it could be concluded from the foregoing results that cumin, sage and rosemary essential oils were effective as antibacterial and antioxidant agents and they could be used as natural preservative agents and good sources of antioxidant in making labneh with healthy benefits and good sensory acceptability.

**Table 5. Effect of tested essential oils on some organoleptic properties of labneh**

Properties	Storage period (days)	Control	Rosemary		Sage		cumin	
			T1	T2	T1	T2	T1	T2
Appearance (10)	0	9.3	85	7.1	9.1	80	9.0	85
	7	9.2	85	7.1	9.1	78	9.0	85
	14	9.2	82	6.5	9.0	78	8.8	80
	21	9.1	80	6.2	8.8	75	8.6	80
Body & texture (40)	0	36.2	35.8	36.0	36.0	36.5	36.5	36.8
	7	38.4	36.1	36.1	37.0	37.0	38.6	38.5
	14	37.5	36.0	35.1	36.5	37.0	37.0	37.1
	21	35.0	35.0	34.8	36.0	36.0	35.4	34.0
Flavor (50)	0	45.0	43.5	42.1	44.5	42.3	46.5	44.2
	7	46.2	43.8	42.0	45.1	43.0	46.8	44.0
	14	45.0	42.3	40.3	42.6	41.1	46.0	43.1
	21	42.0	41.5	39.8	42.1	40.0	43.0	43.0
Overall score (100)	0	90.5	87.8	85.2	89.6	86.8	92.0	89.5
	7	93.8	88.4	85.1	91.2	87.8	94.4	91.0
	14	91.7	86.5	81.9	88.1	85.9	91.8	88.2
	21	86.1	84.5	80.8	86.9	83.5	87.0	85.0

T1: Treatment 1, 0.025% volatile oil.  
T2: Treatment 2, 0.050% volatile oil.

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## تأثير بعض الزيوت الطيارة للأعشاب على اللبنة

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تعتبر اللبنة من أكثر منتجات الألبان إنتشاراً في العديد من دول الشرق الأوسط. وفترة حفظها قصيرة حتى مع التخزين على درجة حرارة منخفضة، ولذلك يتم استخدام المواد الحافظة الكيماوية للتحكم في نشاط الكائنات الحية الدقيقة الملوثة. وبالعلم بأضرار المتبقيات من المواد الحافظة الكيماوية في الأغذية، يرشدنا إلى منع استخدام المواد الحافظة الكيماوية. حديثاً تستخدم المواد الطبيعية مثل زيوت التوابل وذلك لإطالة مدة حفظ الأغذية، لأن بها مواداً مضادة للأكسدة وأخرى مضادة لنمو الميكروبات. في هذه الدراسة تم استخدام ثلاثة زيوت أساسية هي: حصالبان والمرمية والكمون، التي تم تحليلها باستخدام (GC/MS) وكذلك دراسة سعة نشاط المواد المضادة للأكسدة بها. وكذلك دراسة نشاطها المضاد للبكتيريا ضد سلالات *B. cereus* و *E. coli* و *S. aureus* في المعمل بواسطة استخدام طريقة Well agar diffusion. وأظهرت النتائج المتحصل عليها معملياً *In vitro* أن الزيوت الأساسية المستخدمة أحدثت تأثيراً مضاداً للميكروبات وكان زيت الكمون العطري الأعلى تثبيطاً، والأقل تثبيطاً كان زيت المرمية. تتكامل نتائج هذه الدراسة على التأثير المضاد للبكتيريا للزيوت الأساسية معملياً *In vitro*، مع الدراسة التطبيقية على اللبنة (*In vivo*). بالإضافة إلى ذلك، تم تصنيع ثلاث دفعات من اللبنة مدعمة بالثلاثة زيوت الأساسية، حيث لم تلاحظ أي فروق في الخواص الكيماوية الطبيعية في معاملات اللبنة بالمقارنة مع عينة الكنترول. وجد أن اللبنة المصنعة من زيت حصالبان أخذت أعلى قيمة في نشاط المواد المضادة للأكسدة؛ وذلك بعد 14 يوماً من التخزين، بقيمة 20,78، بينما عينة الكنترول سجلت أقل قيمة حيث كانت 10,80، علاوة على ذلك، وجد أن اللبنة المصنعة بنسبة 250 ملليجرام/كجم من زيت الكمون في نهاية مدة التخزين؛ كانت الأكثر تميزاً حيث حصلت عيناتها على أعلى قيمة كلية وبلغت 87 نقطة.