USE OF DIFFERENT ESSENTIAL OILS IN CONCENTRATED YOGURT AS NATURAL PRESERVATIVE*

Mohanad ALAYOUBI[1*], Avni ÇAKICI[1], Fuad RIMAWI[2]

[1] *Department of Food Engineering, Faculty of Engineering, İstanbul Aydın University,*

[2] *Department of Chemistry and Chemical Technology, Faculty of Science and Technology, Al-Quds University, P.O.Box 20002, Jerusalem, Abu Dies, Palestine.*

**Corresponding author's email: mohannadalayoubi@stu.aydin.edu.tr*

ORCID Number: 0000-0002-9980-4724

ABSTRACT

The main method of producing labneh consists of straining whole milk yogurt in a cheesecloth bag to the desired total solid level, it is a critical step in labneh manufacturing, due to the sanitary problems usually associated with the cloth bags used, which increases microbial contamination. In this study, essential oils (EOs) are used to increase the shelf life of labneh from 4 weeks to at least 6 weeks with decrease in the concentration of synthetic antimicrobial agent used. Measurement of the antimicrobial activity of EOs is done using total plate count method, on mold, yeast, *Staphylococcus aureus*, coliforms, and *Escherichia coli* O157:H7 .

The EOs used in this study are namely clove, rosemary, sweet almond oil. They were added to labneh without any synthetic preservative. EOs were added at

* Received: 23.05.2020 – Accepted: 21.06.2020
DOI: 10.17932/IAU.IJFER.2015.003/ijfer_v06i2001

concentrations 600 µl/kg without addition of the synthetic preservative. Total solids of labneh sample, treated with EOs, were only slightly affected. EOs affect the pH. In the presence of synthetic preservative, in terms of influence a total bacterial viable count, the best EOs used were found to be clove and rosemary. The mold count for EOs, the best EOs used were found to be clove and rosemary. yeast decreased, where the best EOs were found to be cinnamon, clove, rosemary, sweet almond. However, clove, rosemary, sweet almond when added to labneh significantly decreased the growth of *S. aureus* and even better than positive control. However, for EOs used the best essential oil that significantly decreased the growth of *S. aureus* was found to be rosemary at concentration of 600 µl/kg. No coliform bacteria or *E. coli* were detected in the treated labneh as well as in the positive control. The most acceptable organoleptic properties of treated labneh were rosemary oils followed by sweet almond. Organoleptic properties in these groups were better than positive control. In this study, it can be concluded that the addition of rosemary and clove EOs at (600 µl/kg), could increase the shelf life of labneh for up to 6 weeks instead of 4 weeks.

Keywords: *Concentrated yogurt (Labneh), Essential oils, Dairy products.*

INTRODUCTION

Many food products are perishable by nature and require protection from spoilage during their preparation, storage, and distribution to give them desired shelf life, especially dairy product. Food products can be subjected to contamination by bacteria and fungi. Many of these microorganisms can cause undesirable reactions that deteriorate flavor, odor, color, sensory and textural properties of food. Illness can be caused because of the consumption of foods contaminated with pathogens such as *Staphylococcus aureus*, *Escherichia coli* O157, *Salmonella*, Fecal coliform, total coliform, yeast, and mold. To prevent growth of spoilage and pathogenic microorganisms in foods, several preservation

techniques, such as heat treatment, salting, acidification, and drying have been used in the food industry **[1, 2]**. In addition, a chemical method can be used which involved the use of chemical preservatives and artificial antimicrobials to inactivate or inhibit growth of spoilage and pathogenic microorganisms **[3, 4].** Numerous efforts are conducted to find natural alternatives to prevent bacterial and fungal growth in foods. In recent years, because of the great consumer awareness and concern regarding synthetic chemical additives, foods preserved with natural additives have become very popular. To inhibit growth of undesirable microorganisms in food, the antimicrobials can be directly added into the product formulation, coated on its surface, or incorporated into the packaging material. Direct incorporation of active agents into food results in an immediate but short-term reduction of bacterial populations, while the antimicrobial films can maintain their activity for a long period of time **[5, 6].**

Natural antimicrobials are derived from animal, plant, and microbial sources. There is considerable potential for utilization of natural antimicrobials in food. However, methods and mechanisms of action, as well as the toxicological and sensory effects of natural antimicrobials, are not completely understood **[7, 8].** Main natural compounds are EOs derived from plants (e.g., cinnamon, clove, rosemary, sweet almond, sesame, wheat germ, sandalwood, basil, thyme, eucalyptus and oregano), enzymes obtained from animal sources (e.g., lysozyme, lactoferrin), bacteriocins from microbial sources (nisin, natamycin), organic acids (e.g., sorbic, propionic, citric acid, benzoic), and naturally occurring polymers (chitosan).

Most plant EOs are gaining a wide interest in the food industry for their potential as decontaminating agents, as they are Generally Recognized as Safe (GRAS). The active components are commonly found in the essential oil fractions and it is well established that most of them have a wide spectrum of antimicrobial activity, against food-borne pathogens and spoilage bacteria **[9, 10].**

The antimicrobial activity of plant EOs is due to their chemical structure, to the presence of hydrophilic functional groups, such as hydroxyl groups of phenolic components and/or lipophilicity of some essential oil components **[11].** Usually, the compounds with phenolic groups such as oils of clove, oregano, rosemary, thyme, sage, and vanillin are the most effective **[12].** They are more inhibitory against gram-positive than gram-negative bacteria **[13, 14].**

Many reviews focus on the use of natural compounds to control microbiological and physicochemical shelf life of main food categories, such as meat, fish, dairy products, minimally processed fruit and vegetables and cereal-based foods. The information is mostly based on case-studies dealing with application of active compounds to prevent microbial proliferation occurring in packaged food during storage.

EOs are very interesting natural plant products and among other qualities, they possess various biological properties. The term "biological" comprises all activities that these mixtures of volatile compounds (mainly mono-and sesquiterpenoids, benzenoids, phenylpropanoids, etc.) exert on humans, animals, and other plants **[7, 8].**

Milk the main component of labneh, a concentrated fermented yogurt, is a good media for many bacterial growths including pathogens. Labneh is a semisolid food that results from the concentration of yogurt using different methods. The most important is the use of cloth bags and draining the yogurt for 14 hours. The total solid of the resulting labneh is approximately 23 g/100g and the product has a cream white color and a flavor that is slightly acidic, the texture is soft and smooth. The high microbial load of labneh, coupled with the packaging and storage conditions, result in the formation of off-flavors and undesirable physicochemical changes that eventually lead to rejection of the product **[15].**

One of the most accepted methods to extend the shelf life of perishable food products is using bio-preservatives **[7, 16].**

Concentrated yogurt is popularly known as labneh in the Middle East or as strained yogurt in Greece, and the rest of Europe, or as süzme yogurt in Turkey. Labneh is a semisolid fermented dairy food produced by removing part of the whey from yogurt to reach total solid levels between 23 and 25 g/100 g **[17].**

Labneh preparation is carried out as follows: Fresh cow's milk (3% fat) was heated at 90°C for 20 minutes, cooled to 45°C and then inoculated with 2% of the yogurt starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). The milk was agitated, dispensed in glass containers, and incubated at 40°C for 3 hours until it was completely coagulated. The resultant coagulant was mixed thoroughly with 0.5% sodium chloride. The mixtures were then put into cheesecloth bags, which were hung in the refrigerator room at 5 ± 1°C for 18 hours, to allow drainage of the whey **[18, 19].**

EOs are not simple compounds or even simple mixtures of several individual compounds. They may contain up to approximately 100 components, although many contain about 20 to 60. The compounds found in EOs are from a variety of chemical classes, predominantly terpenes, but phenylpropanoids and other compounds also occur although at a lesser frequency and often, but not always, in smaller proportions. They are all hydrocarbons and their oxygenated derivatives, and they may also contain nitrogen or sulfur. They are generally low-molecular-weight compounds with limited solubility in water **[20].**

The classification and nomenclature of essential oil compounds are complicated by the fact that many were isolated and studied before the instigation of systematic chemical nomenclature. Consequently, many are known by nonsystematic or trivial or common names. These are sometimes but not always

based on their source, such as eucalyptol, limonene, pinene and thymol, names which hint at historical botanical origins of these compounds.

In terms of shedding light on their chemistry, the long history and widespread use of these nonsystematic names further obfuscates the chemical nature and characteristics of EOs and their components **[21].**

EOs are a group of terpenoids, sesquiterpenes and possibly diterpenes with different groups of aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters, or lactones **[22].** EOs and other plant extracts are principally responsible for antimicrobial activities in plants, herbs, and spices. These extracts can be obtained from plants and spices by various methods, such as steam, cold, dry, and vacuum distillation. These plant compounds, including glucosides, saponins, tannins, alkaloids, EOs, organic acids and others, are present as parts of the original plant defense system against microbial infection **[23, 24].** Generally, phenolic compounds of EOs such as citrus oils extracted from lemon, olive oil (oleuropein) and tea-tree oil (terpenoids), orange and bergamot have broader antimicrobial effects and are not categorized as spices. Meanwhile, there are increasing reports of nonphenolic compounds of oils, which are effective against both gram-positive and gram-negative groups of bacteria, from oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, purple (cultivar Ison) and bronze (cultivar Carlos) muscadine seeds and sage **[25, 26, 27].** Little information is available on interaction among constituents in essential oil sweet almond and the effects they have on antimicrobial activity.

Phenolic components are responsible for antimicrobial action and other constituents are believed to have little activity. Dependability of EOs as antimicrobials could be improved if their content of active agents should be standardized by distillation **[28].**

MATERIALS AND METHOD

Materials

EOs such as Clove (*Syzygiumarom aticum*) oil, Sweet almond (*Prunus dulcis*) oil, Cedarwood (*Cedrus spp*) oil and Rosemary (*Rosmarinus officinalis*) oil used in this study were obtained from Al-Jibrini for food industries (Hebron, West Bank, Palestine).

All EOs were stored at cold temperature 5°C until analysis. Labne used in the analysis prepared from fresh and pasteurized milk and stored +4°C until analysis.

Ethanol, Water, Microbiological media (Plate count agar for the detection viable bacterial growth in labneh, Violet Red Bile Agar recommended for the detection of coliforms in labneh, Eosin Methylene Blue for the detection of E. Coli in labneh, Oxytetra Glucose Yeast Agar base for the detection of yeast and mold in labneh, Baird–Parker agar for the detection of Staphylococcus aureus in labneh), peptone water. All these used in this study were obtained from Himedia Laboratories (Mumbai – India).

Method

Antimicrobial activities of EOs

The antimicrobial activity of EOs will be evaluated against major microorganisms that can be present in labneh such as Coliforms*, Escherichia coli O157:H7*, yeast, mold, *Staphylococcus aureus* and total count bacteria.

Experiments will involve the evaluation of the effect of the addition of EOs each type separately, rosemary oil, sweet almond oil, cedarwood oil, clove oil at different concentrations, 600 µl/kg, on the microorganisms that present in labneh.

Addition of EOs to labneh

Addition of one of the EOs: rosemary oil, sweet almond oil, clove separately, to one kilogram of labneh sample at concentrations 600 µl/kg, without addition of synthetic preservative (potassium sorbate). The resulting mixture is then mixed for 15 minutes and distributed to six packages of 200 mg and stored in fridge at 5°C for 6 weeks.

Chemical analysis

The methodology reported by Ling (1963) **[29]** was used to determine the total solid content, and pH of the different labneh samples.

Microbiological analysis

Evaluated antibacterial activity and properties against major labneh borne bacteria such as, coliforms, *Escherichia coli O157:H7*, yeast, mold, *Staphylococcus aureus* and total aerobic count bacteria by plate count method, (pouring plate method) is used for counting microorganisms in labneh.

1 g sample of labneh was diluted in 9 ml of peptone water yielding a 10^{-1} dilution. Serial dilutions were subsequently prepared and viable numbers were enumerated using the pour plate technique. Total viable counts (TVC) were determined according to Klose (1968) **[30],** the agar plates were incubated at 30°C for 72 hours. Mold and yeast counts were determined according to Harrigan and McConce (1966) **[31],** while *coliform* bacteria were enumerated using the method described by the American Public Health Association (1978) **[32].** The colony forming units (CFU) were converted to log10 and the results are reported as the average from three replicates, each colony can be counted and represents a single cell in the labneh. When labneh sample is mixed with liquefied agar, then must be used dilution to obtain accurate quantitative analyses of cell number. In

microbiological tests, every plate was repeated three times for each type of bacteria, and calculates the mean, then the standard deviation.

Organoleptic properties

All labneh samples were sensory evaluated for flavor (50 points), body and texture (40 points), and appearance (10 points) according to Keating and Rand-white (1990) **[33]**. All samples were evaluated by eight people, specialists in food science, and rated by percentage.

RESULT AND DISCUSSION

Effect of EOs in labneh on total viable counts of bacteria

Different types of EOs such as sweet almond, clove, eucalyptus, rosemary oil, were used as preservatives of labneh sample and compared to positive control (potassium sorbate, 300 ppm) which used in labneh manufacturing in Palestine and compared to negative control (no preservatives added). Some EOs such as clove and rosemary showed a clear effect with reduction in bacterial, mold and yeast count throughout the six weeks, and others such as sweet almond did not show obvious effect.

TVC decreased in the presence of EOs compared with the positive control samples. This activity is due to the antibacterial effect of EOs, during storage period. On the other hand, total bacterial viable count reached 13.00×10^{1} CFU/g in the positive control sample, while in the best EOs clove, rosemary the total bacterial viable count, at 600 µl/kg oil concentration the TVC reached 12.00×10^{1} CFU/g in rosemary labneh. This activity is due to the antibacterial effect of EOs, during per storage period.

Quality and shelf life of labneh are evaluated with mold and yeast counts, so molds were detected at small number in labneh containing clove oil, rosemary oil

throughout the storage period. At the end of the storage period molds number reached 7.00×10^1 CFU/g in positive control sample, at 600 µl/kg oil concentration the best EOs were clove, rosemary, mold in treated labneh with clove labneh mold number reached 6.00×10^1 CFU/g, and in rosemary.

Yeasts were detected at small number in labneh containing rosemary throughout and at the end of the storage period, at least similar to positive control effect. At 600 µl/kg oil concentration the best EOs clove, yeast in treated labneh with clove reached 5×10^1 CFU/g, followed by rosemary yeast number reached 6.00×10^1 CFU/g. In Sweet almond there was no obvious effect on yeast content.

The results obtained for *Staphylococcus aureus* indicated that bacteria detected at small number compared with positive control, in labneh containing rosemary throughout and at the of end the storage period. At the end of the storage period *S. aureus* number reached 8.00×10^1 CFU/g in positive control sample, at 600 µl/kg oil concentration the best essential oil is rosemary, *S. aureus* in treated labneh reached 6×10^1 CFU/g, followed by clove 8×10^1 CFU/g. While in labneh containing sweet almond were not show obvious effect.

Both coliform and *E. coli* were not detected in any of the labneh prepared by addition of the respective EOs. This effect may be attributed to an effect of active compounds in the EOs; Burt (2004) **[7]** reported that EOs contain phenolic compounds that are primarily responsible for their antimicrobial properties. Our results indicated that these bacteria show a few inhibits at low concentrations of the different EOs, while an increase in the oil concentrations leads to decreases in bacterial, yeast and mold counts.

Clove oil and rosemary oil has good antiseptic, antibacterial and antifungal properties, because contain phenols and monoterpene, alcohols, monoterpene, aldehydes esters, lactones and phenylpropenes **[20].**

The phenylpropenes constitute a relatively small part of EOs, and those that have been most thoroughly studied are eugenol, isoeugenol, vanillin, safrole, and cinnamaldehyde. The comparison of the molecules that are chemically similar to eugenol and isoeugenol indicated that the free hydroxyl groups are important for their activity against bacteria **[34].** Furthermore, the antimicrobial activity of phenylpropenes depends on the kind and number of substituents on the aromatic ring, selected microbial strains, and the experimental test parameter such as choice of growth medium, temperature **[35].**

Clove oil contains 80% of eugenol, 4.5% in cinnamon oil and it's the bioactive compound that responsible for antibacterial and antifungal effect. And its antimicrobial activity is linked to its ability to permeabilize the cell membrane and interact with proteins. Eugenol's action on membranes occurs mainly by a non-specific permeabilization **[36, 37].**

Eugenol induced minor changes in the fatty acid profile of *Pseudomonas fluorescens*, *E. coli*, *Brochotrix thermosphacta*, *S. enterica*, and *S. aureus*, and cell damages to *E. coli* and *B. thermosphacta* cells **[38, 39].** Consistent with this, eugenol has proven to inhibit the activity of the following enzymes: ATPase, histidine decarboxylase, amylase, and protease. Inhibition of the ATPase may be important for cell killing at high Eugenol concentrations because energy generation needed for cell recovery is impaired **[36].** The antifungal mode of action of eugenol needs further investigation, but it is known to depend on cell proliferation **[40].**

Total viable counts of labneh at 600 µl/kg oil concentration

When comparing the positive control and negative control, with labneh samples at a concentration of 600 µl/kg EOs, sweet almond oil did not show obvious effect on the labneh sample compared to positive control (Table 1). Concerning clove

oil and rosemary results showed that there was relative obvious decrease in bacterial count, because the bacteria count was a slightly higher than positive control especially in the last week and because bacteria did not multiply very quickly compared with samples without preservatives because of essential oil (Table 1).

Table 1. Total viable counts of labneh during 6 weeks at 600 µl/kg oil concentration

	Total Viable Counts of Labneh					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Sweet almond oil	9.00 ± 2.52	11.00 ± 1.00	13.00 ± 3.51	19.00 ± 2.52	23.00 ± 2.52	27.00 ± 2.08
Clove oil	8.00 ± 1.00	8.00 ± 0.58	9.00 ± 0.58	10.00 ± 2.00	12.00 ± 1.53	16.00 ± 1.15
Rosemary oil	9.00 ± 2.00	6.00 ± 2.08	10.00 ± 0.58	10.00 ± 1.15	10.00 ± 4.93	12.00 ± 1.53
Control 300 ppm potassium sorbate	8.00 ± 2.00	9.00 ± 0.58	9.00 ± 1.00	8.00 ± 0.58	9.00 ± 0.58	13.00 ± 2.52
Control without preservatives	17.00 ± 3.61	23.00 ± 3.79	37.00 ± 6.00	50.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Mold content in labneh at 600 µl/kg oil concentration

When comparing the positive control and negative control, with labneh samples at a concentration of 600 µl/kg EOs, sweet almond oil, did not show obvious effect on the labneh sample compared to positive control. The mold count was less than negative control (Table 2).

When rosemary oil and clove oil was used and compared with the positive control, results showed that there was relative obvious decrease in mold count, mold content approximately constant from the first week until the last week as well mold content in the last week less than positive control, this is an evidence of the effect of oil throughout the six weeks (Table 2).

It is noteworthy to mention that all the EOs at this concentration showed mold count less than the negative control throughout the six weeks. This is a promising result showing the effectiveness of EOs on the mold count when the absence of synthetic preservative (potassium sorbate) compared to that usually used for labneh preservation (300ppm). This showed the effect of EOs in labneh preservation.

When all EOs were compared with the positive control, the best essential oil was rosemary oil, followed by clove oil (Table 2).

Table 2. Mold content of labneh during 6 weeks at 600 µl/kg oil concentration

	Mold Content of Labneh					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Sweet almond oil	5.00 ± 1.00	8.00 ± 0.58	7.00 ± 2.52	16.00 ± 2.08	16.00 ± 5.86	18.00 ± 5.03
Clove oil	2.00 ± 0.58	2.00 ± 0.58	4.00 ± 1.00	5.00 ± 1.00	5.00 ± 0.00	6.00 ± 0.58
Rosemary oil	2.00 ± 1.00	2.00 ± 1.00	2.00 ± 1.53	5.00 ± 1.15	5.00 ± 1.53	5.00 ± 1.15
Control 300 ppm potassium sorbate	1.00 ± 0.58	1.00 ± 0.58	2.00 ± 0.58	3.00 ± 1.15	5.00 ± 1.53	7.00 ± 1.53
Control without preservatives	6.00 ± 1.53	8.00 ± 1.53	11.00 ± 1.00	21.00 ± 2.00	50.00 ±0.00	100.00 ± 0.00

Yeast content in labneh at 600 µl/kg oil concentration

When comparing the positive control and negative control, with labneh samples at a concentration of 600 µl/kg EOs, sweet almond oil did not show obvious effect on the labneh sample compared to positive control. The bacterial count was less than negative control (Table 3).

Concerning clove oil when compared with the positive results showed that there was obvious decrease in bacterial count, because bacteria count is a bit higher than positive control in the sixth week in the labneh sample, also multiplication of yeasts slow compared with normal multiplication due to the oil effect of, and the effect of oil similar to positive control effect until the end of period (Table 3).

It is noteworthy to mention that all the EOs at this concentration showed yeast count less than the negative control throughout the six weeks. This is a promising result showing the effectiveness of EOs on the yeast count when the absence of synthetic preservative (potassium sorbate) compared to that usually used for labneh preservation (300ppm). This showed the effect of EOs in labneh preservation.

When all EOs were compared with the positive control, the best essential oil was clove oil and followed by rosemary oil (Table 3).

Table 3. Yeast content of labneh during 6 weeks at 600 µl/kg oil concentration

	Yeast Content of Labneh					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Sweet almond oil	6.00 ± 0.00	6.00 ± 1.00	10.00 ± 1.00	16.00 ± 2.08	16.00 ± 3.79	17.00 ± 1.00
Clove oil	5.00 ± 0.58	6.00 ± 0.58	9.00 ± 0.58	16.00 ± 1.15	18.00 ± 0.58	22.00 ± 2.52
Rosemary oil	3.00 ± 0.58	2.00 ± 0.00	2.00 ± 0.58	2.00 ± 1.00	4.00 ± 0.58	5.00 ± 2.00
Control 300 ppm potassium sorbate	4.00 ± 1.15	2.00 ± 0.58	3.00 ± 1.00	4.00 ± 0.58	5.00 ± 0.58	6.00 ± 0.58
Control without preservatives	2.00 ± 0.58	2.00 ± 0.58	2.00 ± 0.00	4.00 ± 1.15	5.00 ± 1.53	5.00 ± 2.00

***S. aureus, E. coli* O157:H7 and coliform content in labneh at 600 µl/kg oil concentration**

When comparing the positive control and negative control, with a labneh sample at a concentration of 600 µl/kg EOs, sweet almond oil did not show obvious effect on the labneh sample compared to positive control. The bacterial count was less than negative control (Table 4).

Clove oil when compared with the positive control, results showed that there was relative obvious decrease in bacterial count, bacteria multiply slow compared to normal multiplication due to the oil effect, the effect was approximately similar to positive control effect (Table 4).

When rosemary oil was used results showed that there was relative obvious decrease in bacterial count, because bacteria multiply slowly compared to normal multiplication due to the oil effect, there was a difference in the number of bacteria from the first week until the sixth week, the number of bacteria decreases continuously until the end of the period (Table 4).

It is noteworthy to mention that all the EOs at this concentration showed that *S. aureus* count less than the negative control throughout the six weeks. This is a promising result showing the effectiveness of EOs on the *S. aureus* count when the absence of synthetic preservative (potassium sorbate) compared to that usually used for labneh preservation (300ppm). This showed the effect of EOs in labneh preservation. When all EOs were compared with the positive control, the best essential oil was rosemary oil, followed by clove oil (Table 4).

E. coli and coliform bacteria were not detected at 600 µl/kg oil concentration in all samples.

Tables 4. *Staphylococcus aureus* content of labneh during 6 weeks at 600 µl/kg oil concentration

	Staphylococcus aureus Content of Labneh					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Sweet almond oil	7.00 ± 1.00	6.00 ± 2.08	8.00 ± 1.15	9.00 ± 1.53	9.00 ± 1.53	12.00 ± 2.52
Clove oil	5.00 ± 0.58	4.00 ± 0.58	5.00 ± 1.00	7.00 ± 0.58	8.00 ± 0.58	8.00 ± 1.15
Rosemary oil	6.00 ± 0.58	6.00 ± 1.00	8.00 ± 0.58	5.00 ± 1.00	6.00 ± 1.00	6.00 ± 0.58
Control 300 ppm potassium sorbate	5.00 ± 0.58	3.00 ± 0.58	5.00 ± 0.58	4.00 ± 0.58	6.00 ± 1.53	8.00 ± 1.15
Control without preservatives	10.00 ± 1.53	14.00 ±1.15	15.00 ± 0.58	16.00 ± 2.00	32.00 ± 2.00	44.00 ± 6.00

Effect of EOs on total solids content of labneh

Table 5 show the changes in the total solids (TS) during storage. The TS content increased slightly in all treatments as the storage period increased. Clove labneh at week 6 had the highest TS content (600 µl/kg oil; 25.86%).

All samples were similar to the positive control at all concentrations in all weeks; the proportion of solids slightly increased during storage period; this increase could be described by moisture loss. Similarly, Ismail *et al*. (2006) **[41]** also reported that there were no observable differences in TS of labneh produced by addition of six different EOs. The data is also similar to literature **[42, 43, 44]** those of who reported that the TS of labneh ranged between 22 - 26%.

Effect of EOs on pH

Table 6 show the changes during storage in pH of labneh made with several types of EOs in the absence of synthetic preservative potassium sorbate. The change in pH is a very important factor since it affects the shelf life and the acceptability of labneh. Based on the results presented in tables, it is evident that pH values of the treated labneh decreased with an increase in the storage period. These results agreed with that obtained by Abbas and Osman (1998) **[45],** who reported that the pH decrease gradually during storage period and Titratable acidity increased gradually during storage period. Generally, in concentrated yogurt such as labneh, acidity and pH values vary depending on the starter culture and draining conditions. For this reason, in terms of acidity and pH there have been main different values in the literature **[46, 47, 48, 49].**

Table 5. Changes in the total solids (TS) content of labneh during storage at 600 µl/kg oil concentration

	Total Solid Content of Labneh					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Sweet almond oil	24.38 ± 0.23	24.47 ± 0.08	23.58 ± 0.07	23.62 ± 0.48	23.73 ± 0.22	23.83 ± 0.13
Clove oil	25.15 ± 0.34	25.24 ± 0.23	25.31 ± 0.13	25.59 ± 0.37	25.73 ± 0.29	25.86 ± 0.24
Rosemary oil	24.50 ± 0.15	24.56 ± 0.13	24.63 ± 0.04	24.78 ± 0.12	24.86 ± 0.09	24.84 ± 0.35
Control 300 ppm potassium sorbate	24.31 ± 0.17	24.49 ± 0.30	24.67 ± 0.16	24.81 ± 0.18	24.86 ± 0.14	24.91 ± 0.22
Control without preservatives	24.19 ± 0.06	24.32 ± 0.15	24.46 ± 0.12	24.58 ± 0.17	24.87 ± 0.30	25.12 ± 0.08

Table 6. Effect of some EOs on pH values of labneh during storage at 600 µl/kg oil concentration

	pH Values of Labneh					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Sweet almond oil	4.05 ± 0.01	3.94 ± 0.01	3.87 ± 0.01	3.83 ± 0.02	3.79 ± 0.01	3.70 ± 0.01
Clove oil	3.98 ± 0.01	3.95 ± 0.00	3.95 ± 0.02	3.93 ± 0.01	3.84 ± 0.01	3.80 ± 0.01
Rosemary oil	3.99 ± 0.01	3.95 ± 0.01	3.92 ± 0.01	3.90 ± 0.00	3.85 ± 0.00	3.80 ± 0.01
Control 300 ppm potassium sorbate	4.09 ± 0.00	4.05 ± 0.00	4.00 ± 0.01	4.00 ± 0.00	3.90 ± 0.01	3.87 ± 0.01
Control without preservatives	4.00 ± 0.01	3.92 ± 0.01	3.81 ± 0.01	3.74 ± 0.01	3.60 ± 0.01	3.45 ± 0.00

Effect of different concentrations of EOs in the absence of synthetic preservatives on organoleptic properties of labneh

The organoleptic properties of the different labneh samples were also investigated and the results were presented in Table 7. The total scores of labneh containing EOs decreased with an increase in the concentration of the EOs. In addition, in all cases the total scores of the sensory evaluation decreased gradually during storage. The best oil and most acceptable oil were rosemary at followed by sweet almond. It is noted that the sweet almond oil does not have the strong taste or distinctive taste, but the evaluation was not very good, especially in the last weeks because the taste of acidity in labneh sample.

Table 7. Organoleptic properties of labneh treated with sweet almond, clove, eucalyptus, rosemary EOs for 6 weeks

Oil	Concentration(ppm)	Fresh labneh	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Positive Control	300	96	96	93	91	87	82	77
Negative Control	0	96	93	86	82	71	66	59
Sweet almond	300	96	92	90	83	76	77	72
Sweet almond	400	96	90	91	82	79	75	73
Sweet almond	500	96	89	90	87	83	80	75
Sweet almond	600	96	87	86	83	76	73	70
Clove	300	96	80	82	77	75	71	68
Clove	400	96	73	70	68	63	58	52
Clove	500	96	70	67	67	60	61	54
Clove	600	96	66	62	58	52	50	50
Rosemary	300	96	90	88	86	82	80	78
Rosemary	400	96	91	91	91	84	81	75
Rosemary	500	96	88	90	84	80	76	70
Rosemary	600	96	86	83	79	80	75	73

CONCLUSION

EOs have a wide spectrum of antimicrobial activity, their use as preservatives in food has not yet been extended. In the last few decades, consumers are demanding healthy safe food with least concentration of synthetic food additives and least heat treatment. EOs represent an alternative to synthetic preservatives in the food industry against spoilage bacteria especially *coliforms*, *E. coli O157:H7*, yeast, mold, *S. aureus* which were tested in this study. Most of the selected plant extracts used in this study, have antimicrobial active compounds of that could substitute natamycin, sodium benzoate and potassium sorbate.

Labneh is a middle eastern fermented milk, that is highly consumed but with a major problem in its short shelf life due to contamination during processing, leading to use of synthetic potassium sorbate at different concentrations. The addition of EOs can be used as a single substitute to potassium sorbate to increase the shelf life, or by the combination of natural preservatives and synthetic preservatives leading to better results using low concentration of synthetic antimicrobial agents (150ppm of potassium sorbate). According to our study, there are two possibilities either using natural plant extracts as substitutes and /or use in combination with synthetic antimicrobial agent. Our results showed that clove and rosemary essential oil at 600 µl/kg can be used to increase the shelf life of labneh for up to 6 weeks at 5 ± 1ºC with acceptable taste, flavor, and texture.

Rosemary and clove EOs at concentrations of, 600 µl/kg can be used to increase the shelf life of labneh for up to 6 weeks without any synthetic preservatives. An increase in the EOs concentrations leads to a decrease in bacterial, yeast and mold counts. Both coliform and E. coli were not detected in any of the labneh samples prepared by addition of the respective EOs. The choice of an essential oil and its concentration in a particular food is important, because a small amount can cause sensory alterations. Clove oil and rosemary oil have good antiseptic, antibacterial

and antifungal properties compared to other oil used in this study, because of the presence of phenols, monoterpene, alcohols, aldehydes esters and lactones which affect the growth of pathogenic microorganisms especially gram positive. Although the literature data about the antimicrobial effect of EOs are abundant, there are new areas of application to be discovered especially the effect of the chemical composition and its physicochemical effects. Extraction of the active ingredients of these oils or other oils and their applications as preservatives or antioxidants on food may give appreciable results.

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