Use of cinnamon, wheat germ, and eucalyptus oils to improve quality and shelf life of concentrated yogurt (Labneh)

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Abstract: Three essential oils, namely cinnamon, eucalyptus, and wheat germ were added to concentrated yogurt (Labneh) at a concentration of 600 μL/kg. The chemical, microbiological and organoleptic properties of freshly prepared and stored Labneh at 5°C ± 1 for 6 weeks were determined. Addition of essential oils affected slightly the pH, acidity, total solids, and dry matter values of concentrated yogurt. Total viable counts, as well as counts of Staphylococcus aureus, molds, and yeast in the treated Labneh were affected during storage period. The most acceptable organoleptic properties of treated Labneh were those samples treated with cinnamon, and eucalyptus oils, and to a lesser extent wheat germ oil. Cinnamon and eucalyptus oils were also found to inhibit yeast and mold count. It has been also found that cinnamon and eucalyptus essential oils decreased significantly the growth of S. aureus. No Coliforms or E. coli bacteria were detected in the treated Labneh, as well as in the positive control. This study showed that wheat germ oil had lesser effect compared to cinnamon, and eucalyptus. This study concluded that, addition of cinnamon and eucalyptus essential oils at 600 μL/kg could increase the shelf life of Labneh up to 6 weeks at 5 ± 1°C with acceptable taste, flavor and texture without addition of any chemical preservative.

Subjects: Food Engineering; Food Packaging; Product Development

ABOUT THE AUTHOR

Our research activity of our research group is focusing on study of antioxidant activity, antimicrobial activities of plant extracts and essential oils, and use of plant extracts and essential oils in food products, e.g., concentrated yogurt, and meat, as natural preservatives, antioxidants, and additives to increase their shelf life. The research in this manuscript is related to our research projects and research group, in which it describes the use of essential oils to improve quality and shelf life of concentrated yogurt (Labneh). In a previous study of this project our research group succeeded in reducing addition of chemical preservatives like sodium sorbate, where, in this study our research group succeeded in use of essential oils as preservative in food products.

PUBLIC INTEREST STATEMENT

Essential oils represent an alternative way to chemical preservatives in the food industry against spoilage bacteria, yeast, and mold. The addition of essential oils can be used as a substitute to chemical preservatives, e.g., potassium sorbate that’s widely used as a preservative in food industry to increase food shelf life, or by the combination of natural preservatives and chemical preservatives leading to better results.

The objective of this study is therefore to add different essential oils to concentrated yogurt as antimicrobial agents, and as natural preservative to increase shelf life of concentrated yogurt. Results showed that essential oils can be used to increase the shelf life of concentrated yogurt for 6 weeks with acceptable taste, flavor and texture. Essential oils oil has good antiseptic, antibacterial, and antifungal properties, and can be used in a variety of food types.
Keywords: essential oils; Labneh (concentrated yogurt); Eucalyptus; wheat germ; cinnamon

1. Introduction
Plant essential oils simply abbreviated as EOs, are aromatic oily liquids obtained from plant materials. Steam distillation is the most commonly used method for commercial production of EOs (Elzemni et al., 2019). It is well documented that some EOs have antimicrobial properties (Burt, 2004, Winska et al. 2019). In food industry plant EOs are gaining a wide interest for their potential as decontaminating agents, and as they are generally recognized as safe (GRAS). The active components are commonly found in the EO fractions, and it is well established that most of them have a wide spectrum of antimicrobial activity against food-borne pathogens and spoilage bacteria (Gutierrez et al., 2009, 2008). The antimicrobial activity of plants essential oils is due to their chemical structure, in particular to the groups of phenolic components and/or lipophilicity of some EO components (Dorman & Deans, 2000). Usually, the compounds with phenolic groups such as oils of glove, oregano, rosemary, thyme, sage, and vanillin are most effective (Skandamis & Nychas, 2000), they are more inhibitory against Gram-negative, than Gram-positive bacteria (Marino et al., 2001).

Microorganisms such as Staphylococcus aureus, Escherichia coli, Salmonella, Fecal Coliform, Yeast, and Mold contaminate dairy products, and cause undesirable reactions that deteriorate flavor, odor, color, sensory, and textural properties of food (Davidson & Taylor, 2007). There are several methods used to prevent spoilage, growth of microorganisms in food, such as heat treatment, salting, acidification, drying, as well as chemical treatment (Arques et al., 2008).

Concentrated yogurt is a semi solid fermented dairy food product that is widely consumed in Palestine and many other Middle Eastern countries at breakfast, and locally known as Labneh. Labneh is produced by removing part of the whey from yogurt to reach total solid level between 23 and 25 g/100 g, of which 8–11 g/100 g is fat (Thabet et al., 2014). In addition to having an acidic flavor and milky white color, Labneh is soft, smooth, and spreadable with a consistency that resembles cultured cream. Labneh is produced by strains of thermophilic lactic acid bacteria which ferment lactic acid (El Samragy, 1997). The traditional method of producing Labneh consists of straining whole milk yogurt in a cheese cloth bag to a desired total solid level. The shelf life of traditional Labneh is short, even if stored at low temperature. This may be due to the sanitary problems usually associated with the cloth bags used in its production, and due to unhygienic handling of the product, which increases microbial contamination (El Samragy, 1997).

The high microbial load of Labneh, coupled with the packaging and storage conditions result in the formation of off-flavor and undesirable physicochemical changes that eventually lead to the rejection of the product (Muir & Banks, 2000). One of the most accepted ways to extend the shelf life of perishable food products is through the use of bio-preservatives, e.g., plant EOs (Butt et al., 2000; Droughon, 2004). Investigations of the effect of different EOs on different microorganisms present in food have been reported, ranging from partial to complete inhibition (Kholeel et al., 2007). The relatively short shelf life of cloth bag Labneh is largely responsible for the wide use of benzoates and sorbates to control growth of spoilage microorganism (Mihyar et al., 1999). Recently we reported that, the addition of essential oils at a concentration of 250 μl/kg could increase the shelf life of Labneh (Elama et al., 2019). The objective of this study is to use essential oils at 600 μl/kg as antimicrobial agents to increase shelf life of Labneh without use of any preservatives.

2. Materials and methods
The method of study was basically performed as described by Elama et al. (2019), and Robinson and Tamime (1994) procedures, where, a fresh cow’s milk was used in the manufacturing of Labneh, and the bacterial strains Streptococcus thermophilus and Lactobacillus bulgaricus were used as starter cultures in the production of Labneh. The starter cultures were obtained from Chr.
Hansen, Hoersholm, Denmark. The essential oils used in this study were: cinnamon oil, eucalyptus oil, and wheat germ oil. The essential oils, and fresh cow milk were obtained from Al-Jibrini for food industries (Hebron, West Bank, Palestine).

3. Manufacturing of Labneh
Fresh cow milk (3% fat) was heated at 90°C for 20 minutes, cooled to 45°C, then incubated with 2% yogurt starter culture (Streptococcus thermophilus and Lactobacillus bulgaricus). The milk was agitated, dispersed in glass containers and incubated at 40°C for 3 h until it was completely coagulated. The coagulant was mixed with 0.5% sodium chloride. The mixture kept in cloth bags, the bags were hung in a refrigerator room at 5 ± 1°C for 18 h, to allow drainage of whey (Tamime & Robinson, 2007). Samples were taken for analysis either fresh (day zero) or during the storage period (days 7, 14, 21, 28, 35, and 42).

4. Addition of essential oils to Labneh
Addition of one of the essential oils: cinnamon, wheat germ, and eucalyptus separately, to one kilogram of Labneh sample at different a concentration of 600 μL\k{} (addition of essential oils at 300 μL\k{}, 400 μL\k{}, and 500 μL\k{} concentrations were also performed, results not shown because the 600 μL\k{} concentration was the most acceptable one). The resulting mixture is then mixed for 15 min and distributed to six packages of 200 gm, and stored in fridge at 5°C for 6 weeks without addition of potassium sorbate.

5. Microbiological analysis
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 were evaluated by plate count method, (pouring plate method). A 1 g sample of Labneh was diluted to 10 ml using 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 (1968a). The agar plates were incubated at 30°C for 72 h. Mold and yeast counts were determined according to Harrigan and Mconce (1966), while coliform bacteria were enumerated using the method described by the American Public Health Association (American Public Health Association, 1998). The colony forming units (cfu) were converted to log10 and the results were reported as the average from three replicates. Each colony can be counted and represents a single cell in Labneh. In microbiological tests, every plate was repeated three times for each type of bacteria, and the mean and standard deviation were calculated.

6. 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 Randwhite (1990).

All samples were evaluated by eight people, specialists in food science, and rated by percentage.

7. Chemical analysis
The methodology reported by Ling (1963) was used to determine the total solid content, fat content, and titratable acidity of different Labneh samples.

8. Statistical analysis
All analyses were performed triplicate samples. The averages and the standard deviations were calculated using Excel software version 11.5.1 (Microsoft, Redmond, USA). Statistical analyses were performed using JMP version 9.0 (SAS institute Inc.). The statistical analysis in pH and total solids between storage times and between different years was performed using analysis of variance (ANOVA), followed by Post hoc pairwise comparisons using the Tukey honestly significant difference test (HSD). Differences were considered significant if P values were lower than 0.05.
9. Results and discussion

9.1. Effect of essential oils on the total solids of concentrated yogurt

Table 1 shows the changes in the total solids (TS) during storage. The TS content increased slightly in all treatments as the storage period increased. Statistical analysis showed that there is no statistical difference in TS of concentrated yogurt samples treated with essential oils stored for 3 weeks. This applies also for concentrated yogurt samples treated with chemical preservative (positive control) and the one without preservative (negative control). At storage times 4–6 weeks, statistical analysis showed significant increase in TS indicated by capital letters B and C. The highest TS content at week 6 of Labneh treated with essential oils at 600 μL/kg oil observed in cinnamon 24.87%, and eucalyptus 24.84%, then by wheat germ 24.74%.

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. There were no observable differences in TS of Labneh produced by addition of three different essential oils, these results were in agreement with (Ismail et al., 2006). Tamime and Robinson (1985), also reported that the TS of Labneh ranged between 22 and 26%.

9.2. Effect of essential oils on pH of concentrated yogurt

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 Table 2, it is evident that pH values of the treated Labneh decreased with an increase in the storage period. Statistical analysis showed that there is no statistical difference in pH of concentrated yogurt samples treated with essential oils stored for 3 weeks. This applies also for concentrated yogurt samples treated with chemical preservative (positive control) but not for the one without preservative (negative control) which showed decrease in pH after two weeks indicated by capital letters A and B. At storage times 4–6 weeks, statistical analysis showed significant decrease in pH indicated by capital letters B and C.

The essential oils had a stimulatory effect on the starter culture and total viable count (Abou Dawood, 2002). These results were in agreement with that obtained by Abbas and Osman (1998), 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

| Total solid with 600 μL/kg oil concentration | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|--------------------------------------------|--------|--------|--------|--------|--------|--------|
|                                            | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D |
| Cinnamon oil                               | 24.43 ± 0.15A | 24.52 ± 0.22A | 24.60 ± 0.05A | 24.71 ± 0.09B | 24.74 ± 0.07B | 24.87 ± 0.10C |
| Eucalyptus oil                             | 24.37 ± 0.14A | 24.39 ± 0.26A | 24.48 ± 0.11A | 24.58 ± 0.09B | 24.62 ± 0.51B | 24.84 ± 0.24C |
| Wheat germ oil                             | 24.52 ± 0.30A | 24.61 ± 1.04A | 24.62 ± 0.35A | 24.65 ± 0.12B | 24.76 ± 0.07B | 24.74 ± 0.11C |
| Control 300 ppm P.S                        | 24.31 ± 0.17A | 24.49 ± 0.30A | 24.67 ± 0.16B | 24.81 ± 0.18B | 24.86 ± 0.14B | 24.91 ± 0.22B |
| Control no preservatives                   | 24.19 ± 0.06A | 24.32 ± 0.15A | 24.46 ± 0.12B | 24.58 ± 0.17C | 24.87 ± 0.30C | 25.12 ± 0.08C |

*Data are the means ± SD of three replicates. Rows with different letters indicate statistically significant differences by Tukey HSD (P < 0.05).
Table 2. Effect of some essential oils on pH degree of Labneh during storage at 600 µL/kg oil concentration*

| pH with 600 µL/kg oil concentration | Week 1  | Week 2  | Week 3  | Week 4  | Week 5  | Week 6  |
|-----------------------------------|---------|---------|---------|---------|---------|---------|
|                                   | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D |
| Cinnamon oil                      | 3.98 ± 0.01 A | 3.94 ± 0.05 A | 3.93 ± 0.07 A | 3.91 ± 0.05 B | 3.89 ± 0.04 B | 3.84 ± 0.06 B |
| Eucalyptus oil                    | 3.97 ± 0.07 A | 3.92 ± 0.04 A | 3.90 ± 0.05 A | 3.86 ± 0.09 B | 3.85 ± 0.05 B | 3.80 ± 0.05 B |
| Wheat germ oil                    | 4.01 ± 0.05 A | 3.95 ± 0.07 A | 3.92 ± 0.05 A | 3.90 ± 0.04 B | 3.90 ± 0.06 B | 3.85 ± 0.04 B |
| Control 300 ppm P.S              | 4.09 ± 0.05 A | 4.05 ± 0.07 A | 4.00 ± 0.06 A | 3.90 ± 0.05 B | 3.90 ± 0.09 B | 3.87 ± 0.07 B |
| Control no preservatives          | 4.00 ± 0.04 A | 3.92 ± 0.06 A | 3.81 ± 0.05 B | 3.74 ± 0.05 C | 3.60 ± 0.05 D | 3.45 ± 0.07 D |

*Data are the means ± SD of three replicates. Rows with different letters indicate statistically significant differences by Tukey HSD (P < 0.05).

reason, in terms of acidity and pH, there have been different values in the literature (Rosenthal et al., 1980; Guler, 2007; Ayana & Gamal El Deen, 2011; Senel et al., 2011).

9.3. Microbiological analysis

9.3.1. Total viable count of Labneh with essential oils

Cinnamon, eucalyptus, and wheat germ EOs, were used as preservatives of Labneh samples and compared to positive control (potassium sorbate, 300 ppm) which used in Labneh manufacturing in Palestine, and compared to negative control (no preservatives added). Cinnamon and eucalyptus EOs showed a clear effect with reduction in bacterial, mold, and yeast count throughout the six weeks, on the other hand, wheat germ did not show obvious effect. The total viable count (TVC) decreased in the presence of essential oils compared with the negative control samples. This activity is due to the antibacterial effect of essential oils, during storage period. As shown in Table 3, total bacterial viable count reached 13.00 × 101 cfu/g in the positive control sample. At 600 µL/kg oil concentration the TVC reached 13.00 × 101 cfu/g for Labneh treated with cinnamon and eucalyptus. Whereas, for Labneh treated with wheat germ oil TVC reached 29.00 × 101 cfu/g at the same oil concentration. This activity is due to the antibacterial effect of essential oils during per storage period. This can be attributed to phenolic content of these essential oils (Hüsnü & Gerhard, 2010).

Table 3. Microbiological analysis of total viable counts of Labneh during 6 weeks at 600 µL/kg oil concentration

| T.V.C with 600 µL/kg oil concentration | Week 1  | Week 2  | Week 3  | Week 4  | Week 5  | Week 6  |
|---------------------------------------|---------|---------|---------|---------|---------|---------|
| Scale                                 | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D |
| Cinnamon oil                          | 7.0 ± 0.58 | 7.0 ± 1.00 | 7.0 ± 2.52 | 11.0 ± 1.15 | 12.0 ± 1.53 | 13.0 ± 2.08 |
| Eucalyptus oil                        | 6.0 ± 0.58 | 8.0 ± 0.58 | 10.0 ± 1.00 | 11.0 ± 1.53 | 10.0 ± 2.52 | 13.0 ± 2.52 |
| Wheat germ oil                        | 11.0 ± 1.73 | 15.0 ± 0.58 | 16.0 ± 0.58 | 13.0 ± 4.04 | 22.0 ± 1.00 | 29.0 ± 1.53 |
| Control 300 ppm P.S                   | 8.0 ± 2.00 | 9.0 ± 0.58 | 9.0 ± 1.00 | 8.0 ± 0.58 | 9.0 ± 0.58 | 13.0 ± 2.52 |
| Control no preservatives              | 17.0 ± 3.1 | 23.0 ± 3.79 | 37.0 ± 5.00 | 50.0 ± 0.55 | 100.0 ± 059 | 100.0 ± 1.59 |
Quality and shelf life of Labneh evaluated with mold (Table 4) and yeast counts (Table 5), were detected at small number in Labneh containing cinnamon oil and eucalyptus oil throughout the storage period. At the end of the storage period molds number reached $7.00 \times 10^1$ cfu/g in positive control sample. At 600 μL/kg oil concentration, mold in treated Labneh with cinnamon reached $4.00 \times 10^1$ cfu/g, while in Labneh treated with eucalyptus, mold number reached $5.00 \times 10^1$ cfu/g. As shown in Table 5, yeast was detected at small number in Labneh containing eucalyptus oil throughout and at the end of the storage period, at least like positive control effect. At 600 μL/kg oil concentration, yeast in treated Labneh with eucalyptus reached $5.00 \times 10^1$ cfu/g, followed by cinnamon yeast number reached $6.00 \times 10^1$ cfu/g. In Wheat germ there was no obvious effect on both yeast and mold content.

The results obtained for Staphylococcus aureus indicated that bacteria detected at small number compared with positive control, in Labneh containing eucalyptus oil throughout and at the end of the storage period. At the end of the storage period S. aureus number reached $8.00 \times 10^1$ cfu/g in positive control sample. At 600 μL/kg oil concentration, S. aureus in treated Labneh with cinnamon oil reached $7 \times 10^1$ cfu/g, followed by eucalyptus that reached $8 \times 10^1$ cfu/g. While Labneh containing Wheat germ oil didn’t show obvious effect. Both coliform and E. coli were not detected in any of the Labneh prepared by addition of the respective essential oils. This effect may be attributed to an effect of active compounds in the essential oils; Burt (2004) reported that essential oils 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 essential oils, while, an increase in the oil concentrations lead to decreases in bacterial yeast and mold counts. Cinnamon and eucalyptus oils had good antiseptic, antibacterial and antifungal properties, because they contain phenols, alcohols, monoterpenes, aldehyde esters lactones, and phenylpropenes (Hüsnü & Gerhard, 2010). Phenylpropenes constitute a relatively small part of essential oils, 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 (Laekeman et al., 1990). 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, etc. (Pauli & Kubeczka, 2010).

| Table 4. Microbiological analysis of mold content of Labneh during 6 weeks at 600 μL/kg oil concentration |
|-------------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Mold with 600 μL/kg oil concentration            | Week 1         | Week 2         | Week 3         | Week 4         | Week 5         | Week 6         |
| Scale                                           | Mean ± S.D     | Mean ± S.D     | Mean ± S.D     | Mean ± S.D     | Mean ± S.D     | Mean ± S.D     |
| Cinnamon oil                                   | 2.00 ± 0.58    | 2.00 ± 0.58    | 2.00 ± 0.58    | 4.00 ± 1.15    | 3.00 ± 1.00    | 4.00 ± 1.15    |
| Eucalyptus oil                                 | 3.00 ± 1.15    | 2.00 ± 1.53    | 3.00 ± 0.58    | 2.00 ± 1.53    | 5.00 ± 1.00    | 5.00 ± 1.15    |
| Wheat germ oil                                 | 6.00 ± 0.58    | 5.00 ± 0.58    | 6.00 ± 0.58    | 7.00 ± 1.15    | 9.00 ± 0.58    | 12.00 ± 3.02   |
| Control 300 ppm P.S                            | 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 no preservatives                        | 6.00 ± 1.53    | 8.00 ± 1.53    | 11.00 ± 1.00   | 21.00 ± 2.00   | 50.00 ± 0.50   | 100.00 ± 1.50  |

The analysis was done at dilution as $1 \times 10^{-7}$ cfu/g Labneh.
Table 5. Microbiological analysis of yeast content of Labneh during 6 weeks at 600 μL/kg oil concentration

| Yeast with 600 μL/kg oil concentration | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|----------------------------------------|--------|--------|--------|--------|--------|--------|
| Scale                                  | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D |
| Cinnamon oil                           | 2.00 ± 0.58 | 2.00 ± 0.58 | 4.00 ± 0.59 | 3.00 ± 0.58 | 4.00 ± 0.58 | 6.00 ± 0.58 |
| Eucalyptus oil                         | 3.00 ± 0.58 | 3.00 ± 0.75 | 4.00 ± 0.58 | 3.00 ± 0.70 | 5.00 ± 0.58 | 5.00 ± 0.58 |
| Wheat germ oil                         | 5.00 ± 0.59 | 6.00 ± 0.58 | 7.00 ± 1.15 | 7.00 ± 1.00 | 8.00 ± 1.15 | 12.00 ± 1.53 |
| Control 300 ppm P.S                    | 2.00 ± 0.54 | 2.00 ± 0.58 | 2.00 ± 0.74 | 4.00 ± 1.15 | 5.00 ± 1.53 | 5.00 ± 2.00 |
| Control no preservatives               | 5.00 ± 0.58 | 8.00 ± 1.53 | 10.00 ± 1.15 | 15.00 ± 2.00 | 35.00 ± 5.03 | 100.00 ± 1.53 |

Table 6. Microbiological analysis of Staphylococcus aureus content of Labneh during 6 weeks at 600 μL/kg oil concentration

| S. aureus with 600 μL/kg oil concentration | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|--------------------------------------------|--------|--------|--------|--------|--------|--------|
| Scale                                      | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D |
| Cinnamon oil                               | 3.00 ± 0.58 | 3.00 ± 0.58 | 3.00 ± 0.58 | 5.00 ± 1.00 | 6.00 ± 0.58 | 7.00 ± 0.59 |
| Eucalyptus oil                             | 4.00 ± 1.15 | 5.00 ± 0.58 | 6.00 ± 0.58 | 5.00 ± 1.00 | 7.00 ± 0.58 | 8.00 ± 1.00 |
| Wheat germ oil                             | 9.00 ± 0.59 | 10.00 ± 1.00 | 11.00 ± 1.53 | 9.00 ± 1.53 | 11.00 ± 0.55 | 15.00 ± 1.53 |
| Control 300 ppm P.S                        | 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 no 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 |

Eugenol induced minor changes in the fatty acid profile of Pseudomonas fluorescens, E. coli, Brochothrix thermosphacta, S. enterica, and S. aureus, and cell damages to E. coli and B. thermosphacta cells (Di Pasquaet al., 2007).

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 (Gill & Holley, 2006a). The antifungal mode of action of eugenol needs further investigation, but it is known to depend on cell proliferation (Bennis et al., 2004). Cinnamon oil contains 68% of cinnamaldehyde, and it is the bioactive compound that responsible for antibacterial and antifungal effect, aldehyde groups are reactive and have the ability to cross-link covalently with DNA and proteins through amine groups, thereby interfering with their normal function (Feron et al., 1991). However, the mode of action of cinnamaldehyde, a phenylpropene aldehyde, is inconclusive. At least three things are believed to occur: at low concentrations, cinnamaldehyde inhibits different enzymes involved in cytokinesis, or to less important cell functions. At higher but sublethal concentrations, it acts as an ATPase inhibitor, and at lethal concentrations it perturbs cell membrane. Cinnamaldehyde was suggested to inhibit cytokinesis as a mode of action on B. cereus bacteria ecuese cells could not separate although septa were present after division (Kwon et al., 2003). At sublethal concentrations, cinnamaldehyde gains access to the periplasm and inhibits the activity of, trans membrane. ATPase Sublethal concentrations of cinnamaldehyde did not affect the integrity of the outer membrane of E. coli, but it inhibited growth and bioluminescence of...
Table 7. Organoleptic properties of Labneh treated with cinnamon, eucalyptus, and wheat germ essential oils during 6 weeks

| Oil               | Concentration | Score fresh Labneh | Score week 1 | Score week 2 | Score week 3 | Score week 4 | Score week 5 | Score week 6 |
|-------------------|---------------|--------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Positive control  | 300 ppm       | 96                 | 96           | 93           | 91           | 87           | 82           | 77           |
| Negative control  | zero          | 96                 | 93           | 86           | 82           | 71           | 66           | 59           |
| Cinnamon          | 300           | 96                 | 85           | 79           | 80           | 76           | 73           | 71           |
| Cinnamon          | 400           | 96                 | 86           | 80           | 78           | 74           | 75           | 71           |
| Cinnamon          | 500           | 96                 | 79           | 76           | 74           | 70           | 70           | 67           |
| Cinnamon          | 600           | 96                 | 76           | 74           | 75           | 70           | 68           | 64           |
| Eucalyptus        | 300           | 96                 | 83           | 80           | 77           | 73           | 72           | 67           |
| Eucalyptus        | 400           | 96                 | 81           | 78           | 76           | 76           | 71           | 68           |
| Eucalyptus        | 500           | 96                 | 78           | 74           | 71           | 68           | 63           | 66           |
| Eucalyptus        | 600           | 96                 | 71           | 72           | 70           | 67           | 68           | 64           |
| Wheat germ        | 300           | 96                 | 91           | 87           | 80           | 82           | 60           | 54           |
| Wheat germ        | 400           | 96                 | 92           | 83           | 82           | 73           | 62           | 51           |
| Wheat germ        | 500           | 96                 | 88           | 85           | 80           | 81           | 50           | 60           |
| Wheat germ        | 600           | 96                 | 86           | 84           | 77           | 81           | 56           | 48           |

*All results were evaluated as a percentage %, for flavor (50 points), body and texture (40 points), and appearance (10 points).
Photobacterium leiognathi (13.6–1362 μg/mL; Gill and Holley, 2006a). Many studies have demonstrated that cinnamaldehyde interacts with the cell membrane, but it is not yet clear how it perturbs membranes. It is not a general mode of action of cinnamaldehyde to disrupt membranes as illustrated by Di Pasqua et al. (2007). Among fungi, the primary mode of action of cinnamaldehyde has also been proposed to be inhibition of cell division. This was proposed because cinnamaldehyde inhibited the cell wall synthesizing enzymes in S. cerevisiae by functioning as a noncompetitive inhibitor of β1,3 glucan synthase and a mixed inhibitor of chitin synthase isozyms (Bang et al., 2000). Terpenoids can be sub divided into alcohols, esters, aldehydes, ketones, ethers, phenols, and epoxides. Examples of terpenoids are: thymol, carvacrol, linalool, linalyl acetate, citronellal, pipertitone, menthol, and geraniol.

9.3.2. Effect of essential oils on organoleptic properties of Labneh
The organoleptic properties of the different Labneh samples were also investigated and the results were presented in Table 7.

There were considerable and obvious differences in the flavor of these treated samples as compared with the untreated control, Labneh containing essential oils at 600 μL/kg were the most acceptable, The total scores of Labneh containing essential oils decreased with an increase in the concentration of the essential oils. In addition, in all cases the total scores of the sensory evaluation decreased gradually during storage.

9.3.3. Conclusion and recommendations
EOs have a wide spectrum of antimicrobial activity, their use as preservatives in food have 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. Essential oils represent an alternative to synthetic preservatives in the food industry against spoilage bacteria, yeast, mold and, S. aureus. Most of the selected plant extracts used in this study, have antimicrobial active compounds of that could substitute natamycin, sodium benzoate, and potassium sorbate preservatives. The addition of EOs can be used as natural preservatives at low concentration. In a previous study conducted by our group (Elama et al., 2019), we succeeded to reduce addition of chemical preservative potassium sorbate to 150 ppm instead of 300 ppm by addition of essential oils at 250 μL/kg to increase shelf life of Labneh up to 6 weeks at 5 ± 1°C with acceptable taste, flavor, and texture. In this investigation the obtained results showed that addition of cinnamon and eucalyptus essential oils at 600 μL/kg could increase the shelf life of Labneh up to 6 weeks at 5 ± 1°C with acceptable taste, flavor, and texture without addition of any chemical preservative. These results also showed that EOs lead 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 essential oils. The choice of an EO and its concentration in a particular food is important, because a small amount can cause sensory alterations. Cinnamon and eucalyptus oils have good antiseptic, antibacterial, and antifungal properties compared to wheat germ oil used in this study, because of the presence of different secondary metabolites, e.g., polyphenolic and monoterpene compounds which affect the growth of pathogenic microorganisms specially gram positive. Although the literature data about the antimicrobial effect of EOs are abundant, there are new areas of application to be discovered specially the effect of the chemical composition and its physicochemical effects.

This study succeeded in using natural preservatives without addition of any amount of chemical preservative that can be used by food manufacturers to increase shelf life of food.

Author's contribution
This work was carried out in collaboration among all authors. Authors F.A.R. designed the study, performed the statistical analysis and wrote the protocol. Author M. A. and A.C. wrote the first draft of the manuscript and managed the analyses of the study. Author M.J. and A. C. managed the literature searches. All authors read and approved the final manuscript.

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Journal of Dairy Science, 34, 59–68. doi: 10.1111/jfpp.13332
Keating, K., & Randwhite, S. (1990). Effect of alternative sweeteners in plain and fruit flavored yoghurt. Journal of Dairy Science, 37–54.
Khaleel, H., Williams, R. J., Stenbeck, G., Henderson, B., Meghji, S., & Nair, S. P. (2007). Inhibition of cell separation by Staphylococcus epidermidis. Microbes and Infection, 9(4), 460–465. https://doi.org/10.1016/j.micinf.2007.01.002
Klose, J. (1968). Harmonising der Speiseeisrechte in der EWG. Süßwaren, 14, 778–780.
Kwon, J., Yu, C., Park, H. (2003). Bacteriocidal effects and inhibition of cell separation of cinnamic aldehyde on Bacillus cereus. Lett. Appl. Microbiol. 37, 61–65 1 doi:10.1046/j.1472-765X.2003.01350.x
Loekeman, G. M., Van Hoof, L., Haemers, A., Berge, D. A. V., Herman, A. G., Vlietinck, A. J. (1990). Eugenol a valuable compound for in vitro experimental research and worthwhile for further in vivo investigation. Phytother. Res.4, 90–96. doi: 10.1002/ptr.2650040304
Ling, E. R. (1965). A text book of dairy chemistry. 2 (3rd ed.). Chapman and Hall Ltd.
Marino, M., Bersani, C., & Comi, G. (2001). Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. International Journal of Food Microbiology, 67(3), 187–195. https://doi.org/10.1016/S0168-1605(01)00447-0
Mehaia, M. A., & El Khadragy, S. M. (1999). Compositional characteristics and sensory evaluation of Labneh made from goat’s milk. Milchwissenschaft, 54(10), 567–569.
Mihyar, G. F., Yousef, A. K., & Yarnani, M. I. (1999). Determination of benzoic and sorbic acids in Labneh by high-performance liquid chromatography. Journal of Food Composition and Analysis, 12(1), 53–61. 
Muir, D., & Banko, J. (2000). The stability and shelf life of food. Wood head Publishing Limited.
Mutlag, A., & Hassan, E. (2008). Improvement of the quality and shelf life of concentrated yoghurt (Labneh) by the addition of some essential oils. African Journal of Microbiology Research, 2, 156–161.
Pauli, A., Kubeczka, K. (2010). Antimicrobial Properties of Volatile Phenylpropanes. Natural Product Communications, 5(9), 1387–1394 doi:10.1177/1934578X1000500910
Robinson, R. K., & Tamime, A. Y. (1994). Manufacture of yogurt and other fermented milks. In R. K. Robinson (Ed.), Modern dairy technology, advances in milk products (Vol. 2, pp. 1–48). Elsevier Applied Science.
Rosenthal, et al., 1980; Gulder, 2007; Abou Ayana and Gamal El Deen, 2011 and Senel et al., 2011.
Senel, E., Atamer, M., Gursoy, A., & Oztekin, F. S. (2011). Changes in some properties of strained (Suzme) goat’s yoghurt during storage. Small Ruminant Research, 99(2–3), 171–177. https://doi.org/10.1016/j.smallrumres.2011.03.042
Skandamis, P., & Nychas, G. (2000). Development and evaluation of a model predicting the survival of Escherichia coli O157:H7NCTC 12900 in homemade eggplant salad at various temperatures, pHs and oregano essential oil concentrations. Applied and Environmental Microbiology, 66(4), 1646–1653. https://doi.org/10.1128/AEM.66.4.1646-1653.2000
Tamime, A. Y. (1979a). Concentrated yoghurt “Labneh” a potential new dairy spread. The Milk Industry, 80(3), 4–7.
Tamime, A. Y. (1979b). The production of yoghurt and concentrated yoghurt from hydrolyzed milk. Cultured Dairy Products Journal, 13(3), 13–16.
Tamime, A. Y., & Robinson, R. K. (1983). Yoghurt sciences and technology, I, T Ed. Westcon and Co. Ltd.
Tamime, A. Y., & Robinson, R. K. (2007). Tamime and Robinson’s yoghurt science and technology (third ed.). Woodhead Publishing Limited.
Thabet, H., Nogaim, Q., Qasha, A., Abdoalaziz, O., & Alnsheme, N. (2014). Evaluation of the effects of some plant derived essential oils on shelf life extension of Labneh. Merit Research Journal of Food Science and Technology (2354–2527), 2(1), 008–014. http://www.meritresearchjournals.org/fst/index.htm
Wińskiego, K., Męczka, W., Łyczko, J., Grabarczyk, M., Czubaśek, A., & Szumnny, A. (2019). Essential oils as antimicrobial agents—Myth or real alternative? Molecules, 24(11), 2130. https://doi.org/10.3390/ molecules24112130
