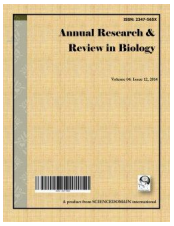

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Effect of Different Essential Oils on the Shelf Life of Concentrated Yogurt

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FAR and CE designed the study, performed the statistical analysis and wrote the protocol. Author MA wrote the first draft of the manuscript and managed the analyses of the study. Author MJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The objective of this study is to use different essential oils (cinnamon, clove, rosemary, almond sweet, sesame, wheat germ, and cedar wood) in concentrated yoghurt as antimicrobial agents to increase shelf life of concentrated yoghurt.
Place and Duration of Study: *Department of Food Technology, Faculty of Science and Technology, Al-Quds University, Palestine*, between January 2015 to August 2016.
Methodology: Essential oils were added to concentrated yogurt at a concentration of 250 µl\kg. Antibacterial activity and properties of major borne bacteria such as total aerobic count bacteria, *yeast, mold, Staphylococcus aureus*, were evaluated by plate count method, (pouring plate method).

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All yogurt samples were sensory evaluated for flavor, body and texture, and appearance. Total solid content, and titratable acidity of different yogurt samples were also determined.
Results: Total solids and pH of concentrated yogurt samples treated with essential oils were only slightly affected. The best three essential oils used in terms of influence on total bacterial viable count and mold count were found to be cinnamon, clove and rosemary. The most acceptable organoleptic properties of treated concentrated yogurt were those samples treated with sesame and rosemary oils.
Conclusion: The addition of cinnamon, clove and rosemary essential oils could increase the shelf life of concentrated yogurt.

Keywords: Concentrated yoghurt; essential oils; shelf life; dairy products; cinnamon; clove; rosemary.

1. INTRODUCTION

Dairy products are subjected to contamination by bacteria, mould, and fungi. Microorganisms such as *Staphylococcus aureus, Escherichia coli*, *Salmonella*, faecal coliform, yeasts and moulds cause undesirable reactions that can cause deterioration of flavour, odour, colour, sensory, and textural properties of food [1]. Several methods are used to prevent spoilage, and growth of microorganisms in food, such as heat treatment, salting, acidification, drying, as well as chemical treatment [2].

Plant essential oils 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 [3,4]. 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 essential oil components [5]. Usually, the compounds with phenolic groups such as oils of the glove, oregano, rosemary, thyme, sage, and vanillin are most effective [6], they are more inhibitory against Gram-negative than Gram-positive bacteria [7]. Essential oils continue to be a subject of interest among the international research community, which include lipids, terpenoids, ketones, phenols, and oxygenated derivatives and have been found for their control effects [5].

Concentrated yoghurt is a traditional fermented milk product that is widely consumed in Palestine and many Middle Eastern countries at breakfast and is called labneh. Labneh is a semi-solid fermented dairy food product produced by removing a part of the whey from yoghurt to reach a total solid level between 23 and 25 g/100 g, of which 8-11 g/100 g is fat [8]. In addition to having an acidic flavour and milky white colour, Labneh is soft, smooth, and spreadable with a consistency that resembles cultured cream. Labneh is produced by using thermophilic lactic acid bacteria which ferments lactose to lactic acid [9]. The traditional method of producing Labneh consists of straining whole milk yoghurt in a cheesecloth bag to a desired total solid level. The shelf life of Labneh is short even if stored at low temperature. This may be due to sanitary problems usually associated with the cloth bags used in the production, and due to unhygienic handling of the product which increases microbial contamination [10]. The high microbial load of Labneh, coupled with the packaging and storage conditions result in the formation of off-flavours and undesirable physicochemical changes that eventually lead to the rejection of the product [11].

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 essential oils [12,13]. Investigations of the effect of different essential oils on different microorganisms present in food have been reported, ranging from partial to complete inhibition [14]. The relatively short shelf life of cloth bag Labneh is largely responsible for the wide use of benzoates and sorbates to control the growth of spoilage microorganism [15]. The objectives of this study are therefore to use essential oils as antimicrobial agents to increase the shelf life of Labneh.

2. MATERIALS AND METHODS

Fresh cow's milk is used in the manufacture of Labneh, and the bacterial strains *Streptococcus thermophilus* and *Lactobacillus bulgaricus* used as starter cultures in the production of Labneh, were obtained from Chr. Hansen, Hoersholm, Denmark. The essential oils used in this study are: Almond sweet oil, Cedar wood oil,

Cinnamon oil, Clove oil, Rosemary oil, Sesame oil and Wheat germ oil and were obtained from *Al- Jibrini* for food industries (Hebron, West Bank, Palestine).

The main chemical composition of cinnamon oil is eugenol (4.57%), linalool (2.74%), cinnamaldehyde (68.11%). Clove oil contains mainly Eugenol (80%), beta-caryophyllene (14.7%) and acetyleugenol (0.86%). Rosmery oil contains mainly Cineole (40.3%), camphor (10.6%), camphene (5.2%), limonene (3.7%), alpha-pinene (12.7%), beta pinene (8.8%), alpha terpineol (2.4%), and borneol (4%).

2.1 Manufacturing of Labneh

Labneh was manufactured according to Robinson and Tamime [16]. 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 delbrueckii subsp. Bulgaricus)* and incubated at 40°C for 3 hours until it was completely coagulated. The coagulant was mixed with 0.5% sodium chloride. The mixture was strained using cloth bags in a refrigerated room at 5± 1°C for 18 hours, to allow whey drainage [17]. Then, essential oils: cinnamon oil, rosemary oil, almond sweet oil, sesame oil, wheat germ oil, cedar wood oil and clove were added separately, to one kilogram of labneh sample at a concentration of 250 µl\kg, with Potassium Sorbate at 150 ppm. The resulting mixture is mixed for 15 minutes and distributed to six packages of 200 gm, and stored in the fridge at 5°C for 6 weeks. Samples were analyzed either fresh (day zero) or during the storage period (7, 14, 21, 28, 35, and 42 days).

2.2 Microbiological Analysis

Antibacterial activity and properties of major labneh borne bacteria such as total aerobic count bacteria, *yeast, mould, Staphylococcus aureus*, were evaluated by plate count method, (pouring plate method). A sample of one gram 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 [18], The agar plates were incubated at 30°C for 72 hours. Mould and yeast counts were determined according to Harrigan and McConce [19]. The results were reported as the average from three replicates and the mean and standard deviation were calculated.

2.3 Organoleptic Properties

All labneh samples were sensory evaluated for flavour (50 points), body and texture (40 points), and appearance (10 points) according to Keating and Rand-white [20].

All samples were evaluated by eight panels, specialists in food science, and results were rated as a percentage.

2.4 Chemical Analysis

The methodology reported by Ling [21] was used to determine the total solid content, and titratable acidity of different Labneh samples.

2.5 Statistical Analysis

All experiments were replicated and subsampled at least once, then results were analyzed using the general linear model (GLM) procedure of the SAS system [22]. All statistical analyses were performed at 95% level of significance.

3. RESULTS AND DISCUSSION

3.1 Effect of Essential Oils on the Total Solids of Concentrated Yoghurt

Table 1 shows the changes occurring during storage in the total solids (TS) content of labneh using several types of EOs at 250 µl\kg and 150 ppm Potassium Sorbate. The TS content was only slightly increased in all treatments as the storage period increased. This is in accordance with the results of Thabet et al. [8] and Mutlag and Hassan [10] who also reported that there were no observable differences in TS of labneh produced by addition of three different essential oils. Similarly, Ismail et al. [23] also reported that there were no observable differences in TS of labneh produced by the addition of six different essential oils. The data were also similar to those of Tamime [24], Tamime and Robinson [17] and Mehaia and ElKhadragy [25] who reported that the TS of labneh ranged between 22-26%.

3.2 Effect of Essential Oils on pH of Concentrated Yoghurt

Table 2 shows the changes during storage in pH of labneh made with several types of EOs at 250 µl\kg and 150 ppm of Potassium Sorbate. Change in pH is a crucial factor as it affects 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 only slightly with the storage period. These results

were in agreement with those obtained by Abbas and Osman [26], who reported that the pH decreased gradually during storage period and titratable acidity, increased gradually during the 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 different values in the literature [27,28,29].

3.3 Microbiological Analysis

3.3.1 Total viable count of labneh with essential oils

Different types of EOs at a concentration of 250 µl\kg (almond sweet, cedar wood, cinnamon, clove, rosemary, sesame and, wheat germ) with 150 ppm potassium sorbate were used as preservatives in labneh samples and were compared to positive control samples (300 potassium sorbate as preservatives which is generally used in concentrated yogurt). Some essential oils such as cinnamon, clove and rosemary, almond sweet and cedar wood showed a clear obvious effect with reduction in bacterial count, throughout the six weeks storage, while sesame and wheat germ did not show any obvious effects.

The total viable count (TVC) decreased in the presence of essential oils compared with the negative control samples (without addition of any chemical or natural preservatives). Results (see table 3) showed that the best three essential oils were cinnamon, clove and rosemary in which the total bacterial viable count decreased to reach 12.00×10^1, 7×10^1, and 11.00×10^1 cfu/g for concentrated yogurt treated with cinnamon, clove, and rosemary, respectively compared to 100.00×10^1 cfu/g of concentrated yogurt not treated with potassium sorbate (negative control). This activity is attributed to antimicrobial effects of essential oils. Other essential oils (Almond sweet oil, Cedar wood, Sesame and Wheat germ) have no obvious effect on total viable count. Cinnamon oil, clove oil, rosemary oil, have more antiseptic, antibacterial and antifungal activities compared to other oils used in this study. This can be attributed to the phenolic content of these oils [30].

3.3.2 *Staphylococcus aureus* content

When comparing Staphylococcus aureus in concentrated yoghurt samples treated with essential oils with that of positive control, results (Table 4) showed that the bacterial count was less than negative control samples for all essential oils tested. Cinnamon oil, clove oil and wheat germ oil showed an obvious decrease in S. aureus count during the storage period (six weeks). It is noteworthy to mention that all the essential oils at 250 µl\kg showed S. aureus count less than the negative control throughout the six weeks. Different essential oils have an antimicrobial activity on the S. aureus count if present with synthetic preservative (potassium sorbate) at half concentration (150 ppm) compared to that usually used for labneh preservation (300 ppm). When E.Os were compared, the best E.O to be used to control S. aureus were: wheat germ oil after long storage time, clove oil and cinnamon oil decreased the number of *Staphylococcus aureus* during the total storage time.

3.3.3 Molds content

Quality and shelf life of labneh were also evaluated with moulds counts Table 5 showed an obvious decrease in mould content in labneh samples using cinnamon oil, clove oil and, Rosemary oil (Table 5).

It is noteworthy to mention that all the essential oils at this concentration showed mould count less than the negative control throughout the six weeks. These results showed the effectiveness of essential oils on the mould count when it is present with potassium sorbate at a half concentration (150 ppm) compared to that usually used for labneh preservation (300 ppm). When E.Os were compared, the best E.O to be used to control mould was: cinnamon oil, followed by clove oil, rosemary oil, cedar wood oil , sesame oil almond sweet oil, and wheat germ oil respectively, see Table 5.

Manso et al. [31], supported our results by demonstrating the influence of several packaging materials containing cinnamon oil (Cinnamomun zeylanicum) on the antifungal activity against *A. flavus.* Results of this work provide the best alternative to preserve labneh by using the essential oil instead of chemicals preservatives. Mihyar et al. [15] reported that more than 400 mg of sodium benzoate per Kg of labneh were needed to control the counts of yeast and molds such as *S. cerevisiae*, *Pichia farinose*, *candida blankii* and *Trichosporon brassicae* to 10^5 cfu/g after 14 days at 5oC; while 150 and 300 mg of sodium benzoate per Kg of labneh were needed for Geotrichum candidum and

Table 1. Total solids (TS) content of concentrated yogurt treated with 250 µl\kg essential oils and 150 ppm of potassium sorbate during storage time

Time	Week1		Week2		Week3		Week4		Week5		Week6	
Total solid	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**
Almond Sweet Oil	26.54	0.31	26.59	0.06	26.60	0.31	26.68	0.13	26.73	0.18	26.88	0.21
Cedar Wood Oil	27.75	0.22	27.74	0.22	27.41	0.10	27.51	0.18	27.53	0.50	27.42	0.19
Cinnamn Oil	26.82	0.05	26.58	0.38	26.53	0.10	26.73	0.03	26.75	0.21	26.83	0.27
Clove Oil	26.73	0.09	26.70	0.15	26.75	0.10	26.85	0.20	26.50	0.12	26.88	0.15
Rosemay Oil	26.63	0.13	26.77	0.10	26.24	0.23	26.74	0.10	26.92	0.03	26.01	0.08
Sesame Oil	26.81	0.13	26.47	0.08	26.87	0.23	26.89	0.30	26.84	0.16	26.91	0.32
Wheat Germ Oil	26.58	0.10	26.82	0.20	26.76	0.22	26.75	0.44	26.88	0.26	26.90	0.19
Control (300 ppm P.S)	26.18	0.14	26.35	0.18	26.47	0.19	26.56	0.21	26.78	0.11	26.87	0.22

Table 2. pH of concentrated yogurt treated with 250 µl\kg essential oils and 150 ppm of potassium sorbate during storage time

pH	Week1		Week2		Week3		Week4		Week5		Week6	
	Mean	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**
Almond Sweet Oil	4.04	0.04	4.12	0.04	4.10	0.01	3.97	0.05	3.84	0.03	3.90	0.01
Cedar Wood Oil	4.10	0.02	4.12	0.02	4.20	0.02	3.93	0.03	3.90	0.04	3.84	0.02
Cinnamon Oil	4.29	0.03	4.03	0.03	4.18	0.03	4.18	0.00	3.91	0.05	3.87	0.04
Clove Oil	4.31	0.02	4.07	0.04	4.11	0.02	4.11	0.01	3.97	0.03	3.89	0.09
Rosemary Oil	4.31	0.01	4.07	0.03	4.18	0.02	4.17	0.01	4.10	0.01	3.91	0.04
Sesame Oil	4.21	0.03	4.05	0.05	4.13	0.04	4.12	0.08	3.99	0.03	3.96	0.04
Wheat Germ Oil	4.15	0.08	4.11	0.02	4.09	0.02	3.99	0.01	3.95	0.02	3.96	0.03
Control (300 ppm P.S)	4.09	0.01	4.05	0.02	4.00	0.02	4.00	0.00	3.90	0.01	3.87	0.00

Table 3. Total viable counts of labneh with essential oils at 250 µl\kg oil concentration and 150 ppm potassium sorbate during storage

Total viable count	Week1		Week2		Week3		Week4		Week5		Week6	
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
Almond Sweet Oil	16.00	1.15	31.00	2.52	15.00	2.65	18.00	2.00	21.00	1.15	25.00	2.00
Cedar Wood Oil	31.00	1.15	10.00	1.73	28.00	1.53	18.00	4.16	32.00	2.08	35.00	2.00
Cinnamon Oil	6.00	1.53	6.00	1.15	8.00	1.53	8.00	1.00	12.00	1.53	12.00	2.00
Clove Oil	9.00	1.00	7.00	1.53	6.00	0.58	8.00	0.58	10.00	2.52	7.00	3.21
Rosemary Oil	6.00	0.58	7.00	0.58	8.00	1.53	7.00	0.58	9.00	2.52	11.00	2.00
Sesame Oil	15.00	1.53	16.00	4.04	13.00	2.00	12.00	4.93	11.00	1.00	25.00	5.57
Wheat Germ Oil	11.00	1.00	8.00	1.00	6.00	2.52	5.00	1.15	9.00	2.52	8.00	1.53
Control No 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
Control (300 ppm P.S)	8.00	2.00	9.00	0.58	9.00	1.00	8.00	0.58	9.00	0.58	13.00	2.52

The analysis was done at dilution as 1× 10^{-1} cfu /g labneh

Table 4. *Staphylococcus aureus* content of labneh at 250 µl\kg oil concentration and 150 ppm potassium sorbate during storage

*S. aureus (cfu*101/g)*	Week1		Week2		Week3		Week4		Week5		Week6	
Scale	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
Almond Sweet Oil	9.00	0.58	10.00	1.53	8.00	0.58	5.00	3.79	10.00	1.53	12.00	2.52
Cedar Wood Oil	6.00	1.53	7.00	1.53	6.00	1.53	7.00	1.53	11.00	1.73	9.00	0.58
Cinnamon Oil	2.00	2.89	4.00	0.58	4.00	1.15	7.00	1.15	7.00	0.58	4.00	1.53
Clove Oil	3.00	0.00	1.00	0.58	2.00	0.58	4.00	1.53	3.00	1.00	4.00	1.73
Rosemary Oil	5.00	0.58	5.00	1.00	4.00	2.52	5.00	1.00	5.00	1.53	8.00	2.00
Sesame Oil	13.00	1.53	10.00	1.53	10.00	1.00	10.00	1.53	12.00	2.08	13.00	1.53
Wheat Germ Oil	8.00	1.15	5.00	0.58	5.00	0.00	4.00	0.00	4.00	3.06	3.00	1.00
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

The analysis was done at dilution as 1× 10^{-1} cfu /g labneh

Tables 5. Molds content of labneh at 250 µl\kg oil concentration and 150 ppm potassium sorbate during storage

Molds	Week1		Week2		Week3		Week4		Week5		Week6	
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
Almond Sweet Oil	12.00	1.53	8.00	1.53	11.00	1.15	8.00	1.53	11.00	2.00	19.00	2.52
Cedar Wood Oil	7.00	0.58	12.00	2.52	8.00	3.06	11.00	2.08	9.00	0.58	11.00	1.53
Cinnamon Oil	1.00	0.00	3.00	0.58	3.00	0.58	3.00	1.53	3.00	1.00	2.00	0.58
Clove Oil	5.00	0.58	5.00	0.00	5.00	2.00	6.00	0.58	6.00	1.53	4.00	1.53
Rosemary Oil	5.00	0.58	7.00	1.15	4.00	1.15	3.00	0.58	4.00	1.00	5.00	2.08
Sesame Oil	10.00	0.58	8.00	1.73	9.00	1.73	8.00	1.53	8.00	0.58	11.00	2.08
Wheat Germ Oil	6.00	1.53	6.00	1.00	6.00	0.58	9.00	1.00	9.00	1.53	11.00	2.65
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	0.00	100.00	0.00

The analysis was done at dilution as 1× 10^{-1} cfu /g labneh

Table 6. Organoleptic properties of labneh treated with at 250 µl\kg oil concentration and 150 ppm potassium sorbate during storage

Essential Oil	Fresh	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Potassium sorbate l	96	96	93	91	87	82	77
Potassium sorbate	96	93	86	82	71	66	59
Almond sweet	96	93	93	90	87	83	75
Cedar wood	96	93	90	90	83	79	70
Cinnamon	96	83	82	78	76	73	72
Clove	96	80	81	76	73	72	65
Rosemary	96	90	90	91	86	83	79
Sesame	96	95	90	86	88	84	81
Wheat germ	96	94	91	83	81	72	62

**All results were evaluated as a percentage %, for flavour (50 points), body and texture (40 points), and appearance (10 points)*

Trichosporon cutaneum, respectively. This effect may be attributed to the effect of active compounds in the essential oils. It was reported that phenolic compounds in essential oils are primarily responsible for their antimicrobial properties [12].

When comparing labneh samples with different essential oils at a concentration of 250 µl\kg with the positive control (Table 5), results showed that Cinnamon, clove and rosemary oils showed an obvious decrease in bacterial count compared to positive control during the storage period. On the other hand, sesame and sweet almond and cedar wood oils didn't show obvious effect on the labneh samples compared to positive control. When E.Os were compared, the best essential oil to be used were: clove oil, cinnamon oil, and rosemary oil.

3.4 Effect of Essential oils on Organoleptic Properties of Labneh

Results given in Table 6 show the organoleptic evaluation of labneh treated with essential oil compared with the untreated control (positive control with 300ppm potassium sorbate) and with negative control (no preservative added). There were considerable and obvious differences in the flavor of these treated samples as compared with the untreated control. The total scores of the sensory evaluation decreased gradually during storage. The best oil and most acceptable is sesame oil even though it did not give the best antimicrobial activity but it decreased the mold count during storage. Cinnamon. clove and rosemary oils gave strong flavors which were not appreciated by certain panelists, and appreciated by others.

4. CONCLUSION

Essential oils represent an alternative to chemical preservatives in the food industry against spoilage bacteria, yeast, mold, and, *S. aureus*. The addition of essential oils can be used as a single substitute to potassium sorbate to increase the shelf life, or by the combination of natural preservatives and chemical preservatives leading to better results using low concentration of potassium sorbate). Cinnamon, clove and rosemary essential oils at 250 µl\kg with 150 ppm potassium sorbate can be used to increase the shelf life of concentrated yogurt for up to 6 weeks at 5 ± 1°C with acceptable taste, flavour and texture. The choice of an EO and its concentration in a particular food is important, because a small amount can cause sensory alterations. Cinnamon oil, clove oil, rosemary oil have good antiseptic, antibacterial and antifungal properties and can be used in different type of food.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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