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Full Length Research Paper

Evaluation of the effects of some plant derived essential oils on shelf life extension of Labneh

Habib M Thabet*, Qais A Nogaim, Ali S Qasha, Omar Abdoalaziz and Najib Alnsheme

Food Science and Technology Department, Ibb University, Yemen.

***Corresponding Author's E-mail: habeeb30@gmail.com**

Abstract

Concentrated yogurt (labneh) was produced by straining cow milk set yogurt in cloth bags. Three plants derived essential oils cinnamon, cumin and mint oils, were added to final concentrations of 0.3, 0.5 and 0.8% each to extend the shelf life of labneh. The chemical, microbiological and organoleptic properties of the labneh stored at 6±1ºC for up to 24 days were determined. Addition of plant derived essential oils affected the pH and total volatile fatty acid values of the prepared labneh, while total solids and fat values were only slightly affected. Total therapeutic bacterial count, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus* in the treated labneh increased and reached a maximum after 8 days of storage where after it decreased until the end of the storage period. Coliform and staphylococcus bacteria were not detected, while yeasts and moulds were detected at insignificant in some treated labneh. Labneh containing 0.3% cinnamon, cumin or mint oils were organoleptically the most acceptable and it had a good body and texture that was similar to the untreated one. From the results of this study, it can be concluded that 0.3% of cinnamon can be used in order to increase the shelf life of labneh for up to 24 days, with higher level of total volatile free fatty acid and therapeutic bacteria counts and low level of total viable, molds and yeast count.

Keywords: Labneh, cinnamon oil, shelf life, microbiological properties.

INTRODUCTION

Concentrated yogurt is popularly known as labneh in the Middle East or as strained yogurt in Greece, and the rest of Europe, or as Suzme yogurt in Turkey (Guler, 2007, Senel *et al.,* 2011). Labneh is consumed as a main dish at breakfast in many Middle Eastern countries, such as Iraq, Iran and Lebanon. Labaneh 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, of which 8–11 g/100 g is fat (Ozer and Robinson, 1999 and Hilali *et al.,* 2011). The traditional method of producing labneh consists of straining whole milk yoghurt in a cheese cloth bag to the desired total solid level. Industrially, excess liquid is removed from the yoghurt by mechanical separators (Tamime and Robinson, 1999). In the traditional process, the yogurt is not subjected to heat and, therefore, Labneh would apparently have higher counts of lactic acid bacteria and a more-complex flavor as compared to the product processed by the other procedure. On the other hand, the traditional process involves more manual handling and provides more opportunities for contamination by yeasts and molds. The differences in hygienic standards of Labneh production system are largely responsible for differential use of additives by processors in managing

the shelf-life of the product. The processing, properties, and shelf-life of Labneh have been reviewed (Nsabimana *et al.,* 2005). Essential oils are aromatic, oily liquids obtained from plant materials. Steam distillation is the most commonly used method for commercial production of essential oils. It has long been recognized that some essential oils have antimicrobial properties (Burt, 2004) and that they can be used as food flavouring agents or preservatives, and for medicinal purposes (El-Nwawy *et al.,* 1998). Results from studies regarding the effect of different concentrations of essential oils on different microorganisms present in food have been varied, ranging from partial to complete inhibition (Khaleel, 2000). The antimicrobial effect of essential oils has been attributed to the presence of phenols and polypeptides (Farag and Abo Raya 1989 ; Gould, 1996 and Ismail *et al.,* 2006). Lebneh, total plate counts of 7.5×10^7 to 1.87×10^8 cfu/g and psychrotrophic yeasts and mold and yeast counts of 2.6×10^6 and 4.4×10^6 cfu/g, respectively, have been reported for the product (Dagher and Ali, 1985; El-Samragy *et al.,* 1988 and Yamani and Abu-Jaber, 1994). A shelf life between 7 to 10 days, at refrigeration temperatures, has been recommended (Yamani and Abu-Jaber, 1994). 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 microorganisms (Mihyar *et al.,* 1999). The use of modern processing lines, that minimize manual handling during production, potentially yields a product with superior microbiological quality as compared to that produced by the cloth bag procedure. Though application of prudent measures during production and distribution, being an acid product (pH<4.5) that is kept under refrigeration (5–7°C) in retail outlets, labneh has a limited shelf –life especially as its ecology is conducive to the growth of psychrotrophic bacteria, molds and yeasts (Pitt and Hocking, 1999 and Muir and Banks, 2000). The purposes of the present study were to investigate (1) the change in the chemical, microbiological and organoleptic properties of labneh during storage (2) the effect of plant derived essential oils, cinnamon, cumin and mint oils on the shelf life extension of the labneh.

MATERIALS AND METHODS

Manufacture of labneh

Fresh cow's milk with Acidity 0.16-0.17, fat 3.5, lactose 4.4 and pH 6.11-6.14 was obtained from the farm of the animal production department, Agriculture College, Ibb University, Yemen. Spray dried skim milk powder was used then the milk was standardized to 14% total solids.

Labneh was manufactured using the method outlined by Tamime and Robinson, (1999) with some modifications. Milk was batch heated at 90 °C for 20 minutes, cooled to 45°C and then inoculated with 3% of the yoghurt starter culture (*S. thermophilus* and *L. bulgaricus*). The milk was agitated, dispensed in glass containers and incubated at 40°C until pH 4.8 it was completely coagulated. The resultant coagulant was mixed and put into cheese cloth bags, which were hung in the refrigerator at 6±1°C for 16 h, to allow drainage of the whey. 0.5% NaCl and 0.001% Tween-80 with the essential oils (cinnamon, cumin and mint) at concentrations of 0.3, 0.5 and 1.0% each, were added to contents of bags. The fresh labneh from each bag was adquoted into small plastic containers (50g) and stored at 6±1°C for 24 days. The sample was taken at day 1, 8, 16, 24 for chemical and microbial analysis and measurement of organoleptic properties.

Chemical analysis

The total solids (T.S %), Titratable acidity (T.A %) and fat contents of the different labneh samples were determined using method of Ling (1963). In addition, protein content was determined by the kjeldhl (AOAC 2000). Moreover, total volatile free fatty acids (TVFFA) expressed as ml. 0.1N NaOH/10gm, in labneh samples was estimated using method of Kosikowski (1982).

Microbiological analysis

Total bacterial count was determined according to (APHA, 1978). Enumeration of coliform, lactic acid bacteria, yeasts and molds was carried out as outlined by Marshall (1992) unless otherwise indicated. Labneh containers were wiped from the outside with aqueous ethanol and the contents thoroughly mixed by a sterile spatula. A composite sub-sample was prepared by transferring labneh into a sterile Erlenmeyer flask and blending with sterilized warm diluents, at 40°C, until a homogeneous mixture was obtained. Phosphate buffer of pH 7.2 was used as the diluents except for enumeration of lactic acid bacteria where peptone water (0.1 ml/100 ml) was used. Violet red bile agar was used to check for the presence of presumptive coliform after incubation of plates at 32 °C for 24 h. *Staphylococcus* medium MSA (DIFCO, 1974) was used to count and detect *staphylococc*i. Plate count agar, that contained 0.01 g chloramphenicol/100 g and 0.01 g chloro-tetracycline hydrochloride/100 g, was used to enumerate yeasts and molds after incubation at 25 °C for 5 days. *S. thermophilus* was enumerated on M17 selective agar medium as described by Krusch *et al*., (1987) and *L. delbrueckii* ssp. *bulgaricus* was enumerated on MRS agar medium as described by Gruev (1982). The agar plates were incubated at 42°C for 48 h. The results are reported as the average from three replicates.

Table 1. Effect of some essential oils on selected chemical properties of labneh during storage 24 days.

Property	Storage period (days)	Control	Essential oil additions								
			Mint			Cumin			Cinnamon		
			0.3	0.5	0.8	0.3	0.5	0.8	0.3	0.5	0.8
Total Solids%	0	23.30^{a}	23.50^{a}	23.40^{a}	23.50^{a}	23.50^{a}	23.45^{a}	23.30^{a}	23.50^{a}	23.30^{a}	23.45^{a}
	8	23.40^{a}	23.60^{a}	23.50^{a}	23.50^{a}	23.50^{a}	23.50^{a}	23.40^{a}	23.60^{a}	23.40^{a}	23.60^{a}
	16	23.40^{a}	23.60^{a}	23.50^{a}	23.70^{a}	23.60^{a}	23.50^{a}	23.40^{a}	23.60^{a}	23.40^{a}	23.60^{a}
	24	23.50^{a}	23.70^{a}	23.60^{a}	23.70^{a}	23.70^{a}	23.85^{b}	23.50^{a}	23.70^{a}	23.50^{a}	23.70^{a}
Acidity%	0	1.40^{a}	1.47^{b}	1.45^{a}	1.48^{b}	1.46^{c}	1.50^{b}	1.45^{b}	1.51^{c}	1.55^{d}	1.49^{d}
	8	1.57^{a}	1.57^{a}	1.51^{a}	1.57^{a}	1.50^{b}	1.51^{b}	1.46^{b}	1.55^{b}	1.58^{b}	1.53^{c}
	16	1.59^{a}	1.59^{a}	1.55^{a}	1.59^{a}	1.53^{b}	1.59^{a}	1.50^{a}	1.59^{b}	1.61^{c}	1.55^{b}
	24	1.60^{a}	1.60^{a}	1.55^{a}	1.59a	1.65^{a}	1.62^{a}	1.53^{a}	1.65^{a}	1.64^{a}	1.60^{a}
F/DM%	0	35.57^{c}	35.68^{c}	35.62^{d}	36.05^{d}	35.95^{b}	35.95^{c}	36.05^{c}	36.00^{c}	35.85^{c}	36.25^{c}
	8	35.60^{c}	35.77^{c}	36.22^{c}	36.32c	36.05^{b}	36.05^{c}	36.65^{b}	36.33^{b}	36.45^{b}	36.72^{b}
	16	35.69^{b}	36.00^{b}	36.32^{b}	36.50^{b}	36.52^{a}	36.65^{b}	36.85^{a}	36.37^{b}	36.67^{a}	36.75^{b}
	24	35.80^{a}	36.15^{a}	36.57^{a}	36.75^{a}	36.62^{a}	36.85^{a}	37.00^{a}	36.70^{a}	36.77^{a}	37.05^{a}
Protein%	0	11.12^{b}	11.28^{c}	11.52^{b}	11.51^{b}	11.21^{b}	11.52^{c}	12.28^{a}	10.87^{c}	11.25^{a}	11.69^{d}
	8	11.17^{b}	11.33^{c}	11.57^{b}	11.61^{a}	11.24^{b}	11.65^{b}	11.01^{b}	11.27^{b}	11.34^{a}	11.80^{c}
	16	11.27^{a}	11.42^{c}	11.61^{b}	11.62^{a}	11.28^{b}	11.69^{b}	12.17^{c}	11.47^{a}	11.20^{a}	12.05^{b}
	24	11.33^{a}	11.47^{c}	11.71^{b}	11.71^{a}	11.31^{b}	11.85^{a}	12.02^{d}	11.40^{a}	11.10^{a}	11.58^{a}
TVFFA	0	7.63^{d}	7.93^{d}	7.76^{d}	7.50^{d}	7.97^{d}	7.80^{d}	7.11^{d}	8.11^{d}	7.82^{d}	7.68^{d}
	8	7.80^{c}	8.13^{c}	7.93^{c}	7.76^{c}	8.23^{c}	7.91^{c}	7.23^{c}	8.63^{c}	7.99^{c}	7.80^{c}
	16	7.98^{b}	8.53^{b}	8.00^{b}	7.83^{b}	8.44^{b}	8.11^{b}	7.43^{b}	8.51^{b}	8.11^{b}	7.98^{b}
	24	8.10^{a}	8.93^{a}	8.23^{a}	7.93^{a}	8.84^{a}	8.21^{a}	7.56^{a}	8.88^{a}	8.34^{a}	8.11^{a}

TVFFA values expressed as NaOHml/10g
Titratable acidity expressed as % of lactic acid
F/DM Fat to dry matter
a, b, c, etc letters are indicated for different properties within a row between treatments at ($P \leq 0.05$).

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 (1990).

Statistical analysis

The statistical analysis of the result was carrying out using the general linear model (GLM) procedure of SASS 9.1 program. Two times for each analysis was repeated. All the statistical analysis was performed at 95% level of significance.

RESULTS AND DISCUSSION

Chemical properties

No significant differences ($P \leq 0.05$) were observed in the TS, F/DM and protein content of the different labneh, either when fresh or during the storage period. During storage, TS slightly increased and the reason could be due to moisture loss. Similarly, Mutlag and Hassan (2008) also reported that there were no observable differences in TS and F/DM of labneh produced by addition of three different essential oils.

The (TA) values of the treated labneh significantly and gradually increased in all samples during the storage period. As the change in total acidity one factors, which affect the shelf life of labneh. It was observed that the highest values of (TA) were obtained with labneh containing 0.3 % of cumin and cinnamon oils, while fresh it increased up to the 8 days of storage, suggesting that the essential oils had a stimulatory effect on the starter culture and total viable count (Abou Dawood, 2002). Generally in concentrated yogurt such as labneh, acidity and pH values varies 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 (Rosenthal *et al.,* 1980; Guler, 2007; Abou Ayana and Gamal El Deen, 2011 and Senel *et al.,* 2011). From (Table 1) that the protein in all samples gradually increased till 16 days storage of lebneh, and slightly decreased within 24 days. These results were in

Table 2. Microbiological analysis of total counts and yeasts and molds of lebneh during 24 days

	Storage period (days)	Control	Essential oil additions								
			Mint			Cumin			Cinnamon		
			0.3	0.5	0.8	0.3	0.5	0.8	0.3	0.5	0.8
Total bacterial counts	0	95	90	90	86	92	91	82	88	85	80
	8	110	105	102	92	108	106	85	107	102	86
	16	98	91	88	78	92	85	71	90	82	75
	24	92	80	77	60	81	75	62	75	71	64
Yeasts and molds	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	16	9	ND	ND	ND	ND	ND	ND	ND	ND	ND
	24	17	9	7	5	8	8	6	ND	ND	ND

Total bacterial × 10^6cfu /g labneh :yeasts and molds × 10^2cfu /g labneh: ND ,Not Detected.

agreement with that obtained by Mutlag and Hassan (2008), who reported that the percentage of protein in labneh made with the addition of the essential oils showed a gradual but significant increase in all treatments during the storage period compared to the untreated control labneh. The data also showed that labneh containing 0.8% of cumin and cinnamon oils have the highest protein content (12.30 - 12.10%), while the lowest protein content was from was from the untreated control labneh (11.10 - 11.34%) followed by the labneh manufactured with 0.3% of either the cumin, cinnamon and mint essential oils. From the same (Table 1), the total volatile fatty acids (T.V.F.F.A) contents were significantly affected by addition of essential oil during the storage period. The decrease in TVFFA was observed as the concentration of essential oils increased and may be due to the inhibitory effect of these essential oils to moulds and lipolytic bacteria, especially at higher concentrations. These results are in agreement with Ragab (2000), who reported that the TVFFA contents of labneh were affected by the type of essential oil. The highest value of TVFFA was recorded for labneh manufactured with 0.3 % of all the essential oils, whereas the lowest TVFFA values were recorded with labneh treated with 0.8% of the essential oils, followed by untreated control labneh.

Microbiological analysis

The total viable count (TVC) decreased in the presence of essential oils compared with the control samples (Table 2). The results might be due to the antibacterial effect of essential oils, during per storage period. On the other hand total bacterial viable count increased then decreased until the end (24^{th} day) to reach 92 × 10^6 cfu/g in the control sample, while in the treated lebneh the TVC ranges from 60 - 81 × 10^6 cfu/g for labneh treated with 0.8% and 0.3% of mint and Cumin oils, respectively. Sahan *et al.,* (2004) report that, the total aerobic bacteria counts decreased during the storage. Count of lactic acids bacteria (Figures 1 and 2) illustrate that the counts for *S. thermophilus* and *Lactobacillus delbruckii* ssp. *bulgaricus* that in all cases (treated and untreated) increased gradually up to 8th day of storage and then decreased thereafter. The results obtained for *S. thermophilus* indicated that the highest count was obtained for labneh treated with 0.3 and 0.5 % of cinnamon and mint oil on the 8th day of storage (27 and 25 × 10^6 cfu/g, respectively), while the lowest counts were obtained for labneh made with different concentrations of cumin oil (Figure 1). In the case of *Lactobacillus delbruckii* ssp.*bulgaricus*, the highest count was obtained from labneh treated with 0.3 % of cinnamon on the 8th day of storage (34 ×10^6 cfu/g), while the lowest count was observed in labneh treated with 0.8% of cumin 24 days of storage (6 ×10^6 cfu/g) (Figure 2). Our results indicated that these bacteria increased during the first eight days of storage then decreased till the end as well as bacteria were not inhibited by low concentrations of the different essential oils, while, increases in the oil concentrations lead to decreases in lactic acid bacterial counts. Similarly it has been reported that addition of some aromatic and essential oils to yoghurt and labneh during its manufacture had a stimulatory effect on lactic acid bacteria by enhancing their growth and acid production (Abou Ayana and Gamal El Deen, 2011 and Khaleel, 2000). El-Nawawy *et al.,* (1998) confirmed that the presence of some herbs, including thyme, in the manufacture of yoghurt increased the counts of *S. thermophilus* and *L. bulgaricus* compared to untreated controls during storage. Coliform and *Staph.aureus* bacteria counts, both coliform and *Staph. aureus* were not detected in any of the labneh prepared by addition of the respective essential oils (data not shown). The suggestion due to effect of compounds present in essential oil, Burt, (2004) reported that essential oils contain phenolic compounds that are primarily responsible for their antimicrobial properties.

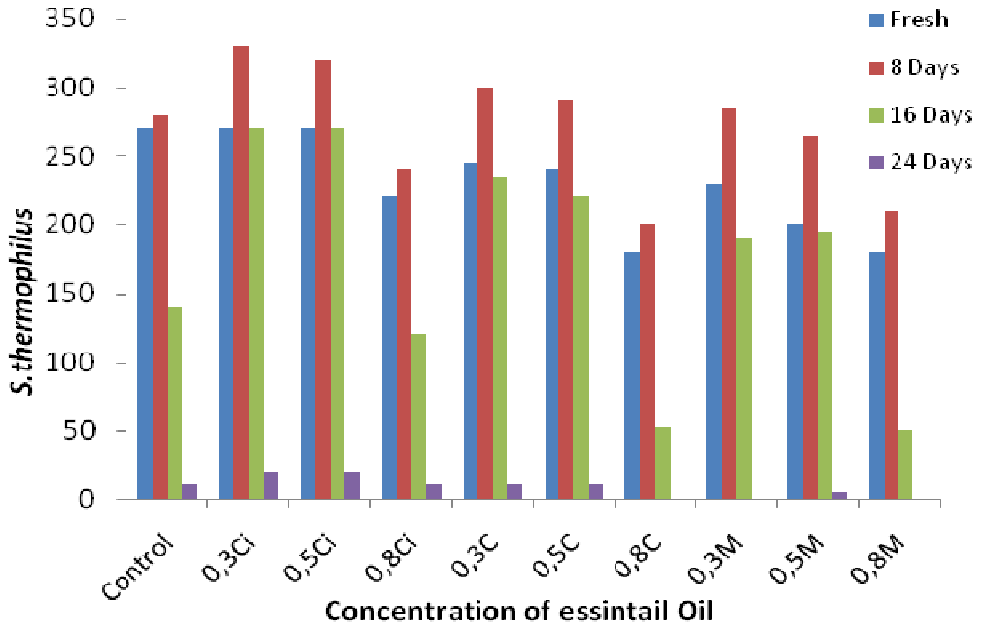

Figure 1. Effect of cinnamon (Ci), cumin (C) and mint (M) essential oils on the counts of lactic acid bacteria S. thermophilus. The count was calculated as (cfu × 10^6/g labneh).

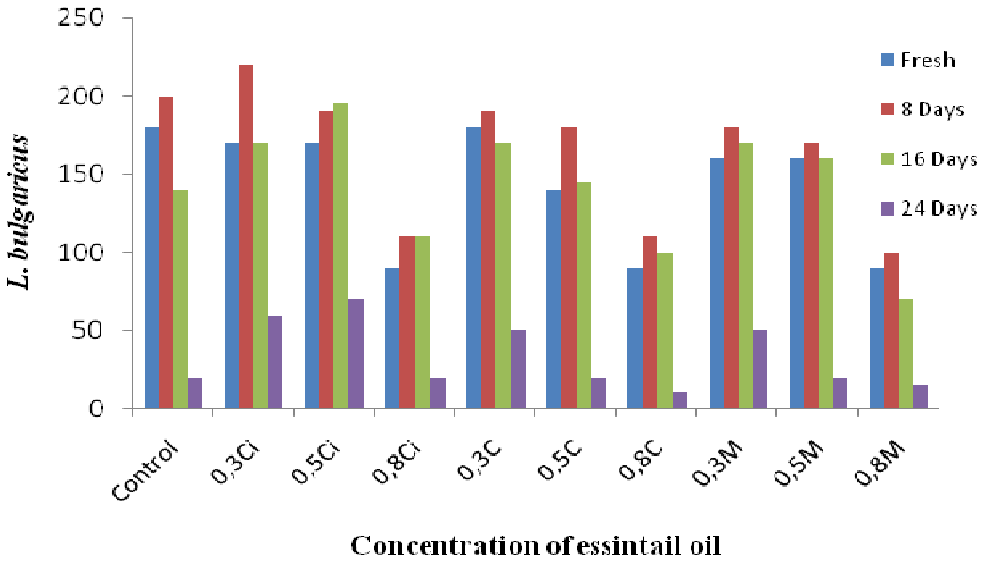

Figure 2. Effect of cinnamon (Ci), cumin (C) and mint (M) essential oils on the counts of lactic acid bacteriaL. bulgaricusof labneh during storage. The count was calculated as (cfu × 10^6/g labneh).

Yeast and mould counts

The quality and the shelf life of labneh evaluated with yeast and mould counts, so yeasts and moulds were not detected in labneh containing cinnamon oil throughout the storage period, while in labneh containing cumin and mint oils were detected at the end of storage period 24th day (Table 2). Manso *et al.,* (2013) supported our results by demonstrating the influence of the substrate of several packaging materials containing cinnamon (*Cinnamomun zeylanicum*), where essential oil as active agent on the antifungal activity against *A. flavus.* Yeasts and moulds were detected in the untreated control, only after 16 days of storage (9×10^2cfu/g), these results might be due to antimicrobial effects of essential oils with treatment labneh. These results are in agreement with Mutlag and Hassan (2008), both reported that essential oil from thyme had antifungal and antimicrobial activities. Results

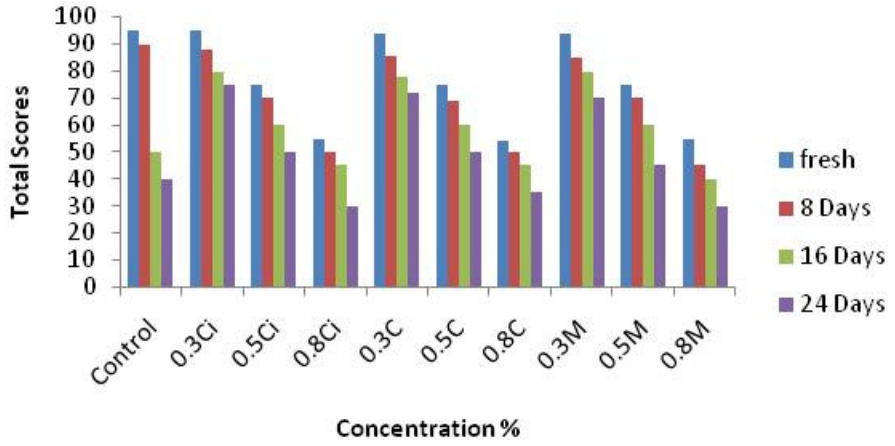

Figure 3. Organoleptic properties of labneh treated with (Ci), cumin (C) and mint (M) essential oils essential oils during 24 days.

of this work provide the best alternative to preserve the labneh by using the essential oil instead of chemicals preservatives. Mihyar *et al.,* (1999) reported that more than 400 mg of sodium benzoate per Kg of labneh were needed to control the counts of yeast and mould such a *S.cerevisiae, Pichia farinose, candida blankii and Trichosporon brassicae* to 105 cfu/g after 14 days at 5C$^{o.}$, while 150 and 300 mg of sodium benzoate per Kg of labneh were needed for *Geotrichum candidum* and *Trichosporon cutaneum,* respectively.

Organoleptic properties

Results given in Figure (3) shows the organoleptic evaluation of labneh which was treated with essential oil compared with the untreated control. The highest scored points was in labneh treated with 0.3% essential oils till the end of storage and decreased with an increase in the concentration of the essential oils, while untreated labneh till in 16 days of storage. In addition, as well as the total scores of the sensory evaluation decreased gradually during storage. The control and treated labneh samples treated with 0.8% had lowest points and decreased to reach 35 in twenty four day of storage and was not accepted and rejected by most of the plane lists. There were considerable and significant differences *(P*≤0.05) in the samples treated with either cinnamon, cumin or mint as compared with the untreated control.

CONCLUSION

The natural antimicrobial is wide and there are still a great number of possibilities to explore. The tested plant derived oils must be thoroughly described and identified in the future studies as food preservation. The results of the present study showed that, the addition of essential oils can be used to increase the shelf life of labneh, the cinnamon oil at 0.3% has shown to extend the shelf life for up to 24 day at 6 ± 1^{o}C with acceptable taste, flavor and without any microbial spoilage.

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