

Full Length Research Paper

Improvement of the quality and shelf life of concentrated yoghurt (labneh) by the addition of some essential oils

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Three essential oils, namely thyme, marjoram and sage, were added to concentrated yoghurt (labneh) at concentrations of 0.2, 0.5 and 1.0 parts per million (ppm). Subsequently, the chemical, microbiological and organoleptic properties of freshly prepared labneh and of the labneh stored at $5^{\circ}\text{C} \pm 1$ for up to 21 days were determined. Addition of essential oils affected the pH, soluble nitrogen -to-total nitrogen, total volatile fatty acid and acetaldehyde values of the prepared labneh. On the other hand, total solids and fat-to-dry matter values were only slightly affected. Total viable counts, as well as counts of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in the treated labneh increased and reached a maximum after 7 days of storage where after it decreased until the end of the storage period. Yeasts and moulds, coliform bacteria and spore-forming bacteria were not detected in the treated labneh. Of the different treated labneh, labneh containing 0.2 ppm thyme, marjoram or sage oils were organoleptically the most acceptable, and it had a good body and texture that was similar to that of the untreated control. From the results of this study, it can be concluded that 0.2 ppm of thyme, marjoram or sage can be used in order to increase the shelf life of labneh for up to 21 days.

Key words: Labneh, essential oils, shelf life, chemical properties, microbiological properties

INTRODUCTION

Labneh, a traditional fermented milk product that is consumed in Middle Eastern countries, is obtained from yoghurt after removal of part of its whey. 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 strains of thermophilic lactic acid bacteria (LAB), which ferment the lactose present to produce organic acids, mainly lactic acid (El-Samragy, 1997). 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).

The shelf life of traditional labneh is short, even if stored at low temperatures. 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-flavours and undesirable physico-chemical changes that eventually lead to rejection of the product (Muir and Banks, 2000). One of the most accepted ways to extend the shelf life of perishable food products is through the use of bio-preservatives (Burt, 2004; Draughon, 2004).

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 phe-

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nols and polypeptides (Frag et al., 1989; Gould, 1996; Ismail et al., 2006). Most essential oils have been classified as GRAS (general recognize as safe) and the antimicrobial activity of essential oils and spices are considered to result in extending the shelf life of a wide variety of foods (Burt, 2004). Although there have been few reports regarding the antimicrobial activity of spices on the viability of LAB, most of them suggest that LAB are relatively resistant to the inhibitory effect of spices and essential oils. Moreover, the viability of LAB increased at adjusted concentrations of essential oils (Zaika et al., 1983; El-Nawawy et al., 1998; Ismail et al., 2006).

This study aimed to evaluate the effect of three essential oils, that is, thyme (*Thymus vulgare*), marjoram (*Majorana hortensis*) and sage (*Salvia officinalis*), at different concentrations, on the shelf life of labneh and the viability of the starter culture. The effect of these additives on the chemical, microbiological and organoleptic properties of the resultant labneh was also investigated.

MATERIALS AND METHODS

Materials

Fresh cow's milk, used in the manufacture of labneh, was obtained from the local market in El Hasa, KSA. The bacterial strains *Lactobacillus delbrueckii* ssp. *bulgaricus* 920630 and *Streptococcus thermophilus* HM1, used as starter cultures in the production of labneh, were obtained from the BAFEL Laboratory, Kiel, Germany. The essential oils used in this study, i.e. thyme, marjoram and sage, were obtained from Mebaco Arabic Co., stored at 8°C ± 1 and appropriate dilutions were prepared on the day of their use.

Manufacture of labneh

Labneh was manufactured according to Robinson and Tamime (1994). Fresh cow's milk (3% fat) was heated at 90°C for 20 min, cooled to 45°C and then inoculated with 2% of the yoghurt starter culture (*S. thermophilus* + *L. bulgaricus*). The milk was agitated, dispensed in glass containers and incubated at 40°C for 3 h until it was completely coagulated. The resultant coagulant was mixed thoroughly with 0.5% NaCl and 0.001% Tween- 80 after which the essential oils (thyme, marjoram and sage) were added at concentrations of 0.2, 0.5 and 1.0 ppm, respectively. The mixtures were then put into cheese cloth bags, which were hung in the refrigerator room at 5 ± 1°C for 18 h, to allow drainage of the whey. The fresh labneh from each essential oils-type were poured into small plastic containers and stored for 21 days at 5 ± 1°C. Samples were taken for analysis either fresh (day 0) or during the storage period (days 7, 14 and 21).

Chemical analyses

The methodology reported by Ling (1963) was used to determine the total solid content, the fat content and titratable acidity of the different labneh samples. In addition, the soluble nitrogen (SN) and non-protein nitrogen (NPN) contents were determined by the micro-kjeldahl method according to Ling (1963). Moreover, acetaldehyde and total volatile fatty acids (TVFA) in the different labneh samples were determined as described by Kosikowski (1982) and Ostoev et al. (1958), respectively.

Microbiological analyses

A 1-g sample of labneh was diluted in 9 ml of Ringer solution (Oxoid), 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), while *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. Mould and yeast counts were determined according to Harrigan and McConce (1966), while coliform bacteria were enumerated using the method described by the American Public Health Association (1978). Spore-forming bacteria were enumerated by first placing tubes of the appropriate dilutions in a water bath at 80°C for 15 min, after which they were removed and cooled to room temperature. Aerobic spore-forming bacteria were enumerated on Nutrient agar medium (Difco) and the agar plates were incubated at 30°C for 72 h. Anaerobic spore-forming bacteria were enumerated on RCM agar medium (Merck) and the agar plates were incubated in an anaerobic jar with anaerobic kits (Oxoid) at 30°C for 72 h. The colony forming units (cfu) were converted to log₁₀ and the results are reported as the average from three replicates.

Organoleptic properties

Samples of labneh were organoleptically scored for flavour (50 points), body and texture (40 points), and appearance (10 points) according to score card suggested by Keating and Randwhite (1990).

Statistical analysis

All experiments were replicated and sub-sampled at least once. Results were analyzed using the general linear model (GLM) procedure of the SAS System (SAS, 1996). The level of significance was preset at $P \leq 0.05$.

RESULTS AND DISCUSSION

Chemical properties

Table 1 shows the changes during storage in the total solids (TS) content and fat-to-dry matter (F/DM) of labneh made with several types of essential oils. The TS content increased slightly in all treatments as the storage period increased. Fresh sage labneh had the highest TS content (1.0 ppm; 23.74%), followed by marjoram labneh (1.0 ppm; 23.61%). The lowest TS was obtained for thyme labneh (0.2 ppm; 23.11%), which is comparable to that of the untreated control labneh (22.95%). A similar trend was obtained throughout the rest of storage period (7, 14 and 21 days). No significant differences ($P \leq 0.05$) were observed in the TS and F/DM of the different labneh, either when fresh or during the storage period. During storage, both TS and F/DM increased and could be ascribed to moisture loss. Similarly, Ismail *et al.* (2006) also reported that there were no observable differences in TS and F/DM of labneh produced by addition of six different essential oils. The data is also similar those of Tamime (1978a 1978b), Tamime and Robinson (1985)

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

Property	Storage period (days)	Control	Essential oil additions								
			Thyme			Marjoram			Sage		
			0.2	0.5	1.0	0.2	0.5	1.0	0.2	0.5	1.0
Total Solids %	0	22.95i	23.11h	23.19h	23.40f	23.44e	23.50e	23.61c	23.34g	23.68b	23.74a
	7	23.01b	23.19b	23.33b	23.48b	23.51b	23.58b	23.66ab	23.49b	23.72ab	23.86a
	14	23.19b	23.29b	23.41b	23.54b	23.63b	23.64b	23.74b	23.65b	23.79ab	23.90a
	21	23.31i	23.40h	23.47g	23.59f	23.65e	23.70d	23.83c	23.81c	23.88b	23.94a
Acidity %	0	1.50a	1.51f	1.50g	1.49h	1.60a	1.51c	1.50d	1.54d	1.52e	1.50d
	7	1.54a	1.59c	1.52d	1.49e	1.65a	1.61b	1.56b	1.59b	1.58b	1.54c
	14	1.58bc	1.60d	1.55e	1.55e	1.73a	1.67b	1.60d	1.69b	1.6b	1.59d
	21	1.69a	1.80b	1.74f	1.59g	1.87b	1.78f	1.62b	1.89e	1.82d	1.60g
F/DM %	0	32.29d	32.56f	32.49g	32.61e	33.29d	33.4c	34.07b	32.48g	33.60c	35.21a
	7	32.34j	32.62i	32.65h	32.90f	33.84e	33.89d	34.73b	32.96g	33.94c	36.06a
	14	32.40j	33.05i	33.21h	33.99f	34.20e	34.63d	35.27b	33.24g	34.76c	36.58a
	21	32.50j	33.88i	33.97h	34.11g	34.89e	35.19d	36.10b	34.21f	35.59c	36.99a
SN/TN %	0	13.98j	14.96i	14.39h	14.50g	15.35f	15.34e	15.04d	14.82c	14.97b	15.03a
	7	14.05i	15.38h	15.15e	14.58d	15.94c	15.93a	15.06b	15.73g	15.21f	15.12j
	14	14.29f	16.86d	16.01cd	14.5a	16.94a	16.54a	15.10cd	15.96d	15.90d	15.19e
	21	14.51h	16.27ef	16.09ed	14.51ab	17.30a	17.04b	15.11ed	16.37cd	16.31c	15.19g
TVFA	0	8.53i	8.94e	8.77b	8.72f	8.99a	8.61g	8.58h	8.93e	8.64c	8.86d
	7	8.61i	9.24e	9.18a	8.75b	9.54e	9.43c	8.62g	9.54f	9.31d	8.90h
	14	8.74j	9.75f	9.43d	8.76a	10.48c	10.30b	8.59h	10.12g	9.90e	8.93i
	21	9.02h	10.35g	10.12f	9.86d	11.17c	11.02a	8.60j	10.97e	10.69i	8.89i
Acetaldehyde	0	17d	25c	23a	22ab	24ab	25a	18cd	18cd	20c	17d
	7	19g	25cd	24a	23cb	28ab	27cb	22ef	22ef	24ed	20gf
	14	22c	29a	27a	25b	26b	27b	20cd	20cd	22c	19d
	21	14cd	31a	24a	20b	24a	18b	14cd	12d	18b	15c

Acetaldehyde values expressed as ml/100g

Titratable acidity expressed as % of lactic acid

F/DM %: Fat /dry matter

SN/TN: Soluble nitrogen / total nitrogen

*Significantly differed at p 0.05nt

*The letters (a, b, c, etc.) compared for different properties within a row between treatments at (P 0.05).

and Mehaia and El Khadragy (1999), who reported that the TS of labneh ranged between 22 - 26%.

The change in total acidity (TA) is a very important factor, since it affects the shelf life and the acceptability of labneh. Based on the results presented in Table 1, it is evident that acidity values of the treated labneh increased significantly with an increase in the storage period. The highest values were obtained with labneh containing 0.2 ppm of the essential oils when fresh and it increased up to the end of storage (day 21), suggesting that the essential oils had a stimulatory effect on the starter culture and total viable count (Dawood, 2002). These results were in agreement with that obtained by Abbas and Osman (1998), who reported that the TA increased gradually during storage period.

The percentage of soluble nitrogen per total nitrogen (SN/TN %) of 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 (Table 1). The data also showed that labneh containing 0.2 ppm of marjoram had the highest SN/TN% content (15.35 - 17.30%), followed by labneh containing 0.2 ppm of thyme essential oil (14.96 - 16.39%). The lowest SN/TN% was from the untreated control labneh (13.98 - 14.51%) followed by labneh manufactured with 1.0 ppm of either the thyme, marjoram and sage essential oils. Ismail et al. (2006) found the same trend in labneh cheese prepared with essential oils and they attributed it to an increase in LAB activity.

The total volatile fatty acids (TVFA) of labneh made with the respective essential oils are also indicated in Table 1. The highest value of TVFA was recorded for labneh manufactured with 0.2 ppm of marjoram essential oil, whereas the lowest values were recorded with untreated control labneh, followed by labneh manufactured with 1.0 ppm of the essential oils. However, a decrease in TVFA was observed as the concentration of essential oils increased and may be due to the inhibitory effect of these

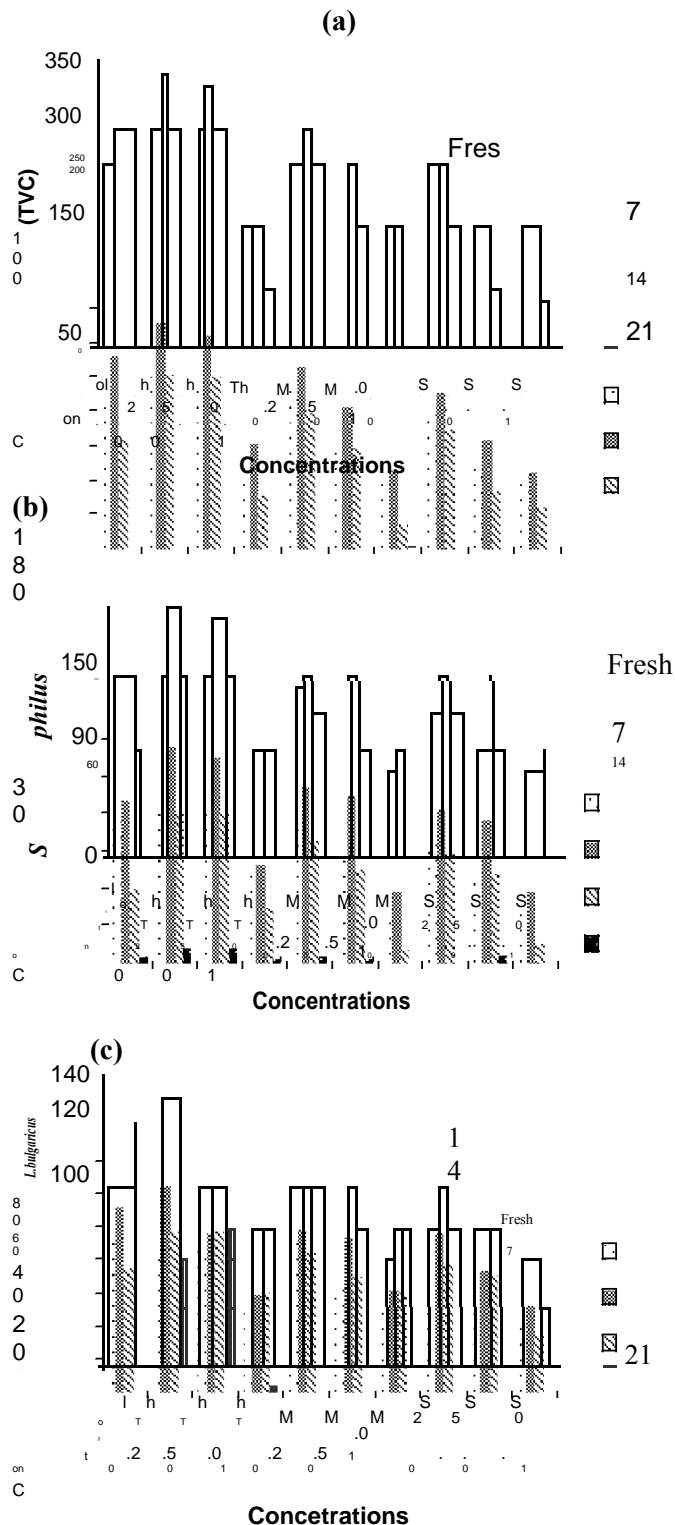


Figure 1. Effect of thyme (Th), marjoram (M) and sage (S) essential oils on the (a) total viable bacterial count (TVC), and on the counts of (b) *S. thermophilus* and (c) *L. bulgaricus* of labneh during storage. The count was calculated as (cfu × 10⁶/g labneh).

essential oils to moulds and lipolytic bacteria, especially at higher concentrations. These results are in agreement with Ragab (2000), who reported that the TVFA contents of labneh were affected by the type of essential oil. Also, it is clear from Table 1 that the acetaldehyde values of all treatments were increased within the first 7 days of the storage period and then decreased up to the end of storage. The highest value was obtained for labneh containing 0.2 ppm of thyme essential oil, while the lowest values were obtained for labneh containing 1.0 ppm of the sage essential oil and for the untreated control labneh. This is presumably due to the ability of the LAB to produce acetaldehyde. Soad et al. (1997) reported that the acetaldehyde content of labneh made with plant oils increased and reached a maximum value after 7 days of storage and then decreased to reach a minimum value after 28 days.

Bacterial counts

Labneh prepared by adding three different essential oils at different concentrations to the food product was subjected to microbiological analyses. Analysis of the results obtained for the total bacterial viable counts (TVC) (Fig. 1a), as well as counts for *S. thermophilus* (Figure 1b) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Figure 1c) indicated that in all cases the respective counts increased gradually up to 7th day of storage and then decreased thereafter. Whereas labneh made with 0.2 ppm of the essential oils had the highest total viable count, those manufactured with 1.0 ppm of the essential oils had the lowest count (Figure 1a). Regarding enumeration of the starter culture bacteria, the results obtained for *S. thermophilus* indicated that the highest count was obtained for labneh manufactured with 0.2 and 0.5 ppm of thyme oil on the 7th day of storage (174 and 166 × 10⁶ cfu/g, respectively), while the lowest counts were obtained for labneh made with different concentrations of sage oil (Figure 1b). In the case of *Lactobacillus delbrueckii* ssp. *bulgaricus*, the highest count was obtained from labneh manufactured with 0.2 ppm of thyme on the 7th day of storage (125 × 10⁶ cfu/g), while the lowest count was observed in labneh made with 1.0 ppm of sage after 21 days of storage (7.3 × 10⁶ cfu/g) (Figure 1c).

The obtained results suggest that the bacterial populations were not inhibited by low concentrations of the different essential oils. However, increases in the oil concentrations lead to decreases in bacterial counts. It has previously been reported that addition of some essential oils to yoghurt and labneh cheese during its manufacture had a stimulatory effect on LAB by enhancing their growth and acid production (Khaleel, 2000; El-Nawawy et al., 1998). Notably, El-Nawawy et al. (1998) reported 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.

Notably, coliform bacteria and spore-forming bacteria were not detected in any of the labneh prepared by addition of the respective essential oils (data not shown). This may not be surprising, as Burt (2004) reported that essential oils contain phenolic compounds that are primarily responsible for their antimicrobial properties. Schelz et al. (2006) also reported that both sage and thyme essential oils had an inhibitory effect against *E. coli*. Whereas sage had a minimum inhibitory concentration (MIC)

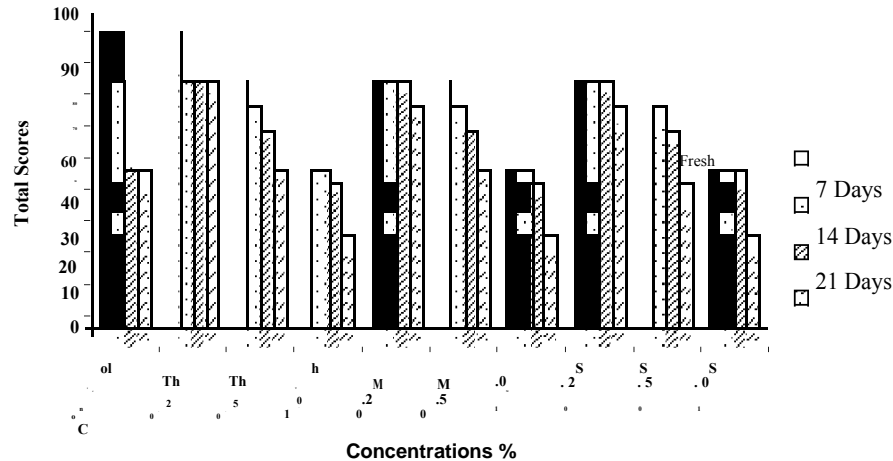


Figure 2. Organoleptic properties of labneh treated with thyme (Th), marjoram (M) and sage (S) essential oils during storage.

of 3.5 - 5 ml⁻¹, thyme had a MIC of 0.45 - 1.25 ml⁻¹. Moreover, Barnes et al. (2002) reported that thujone and camphor present in the volatile oil of sage were inhibitory towards *Bacillus* species.

Yeast and mould counts

Yeast and mould counts are considered indicative of the quality and the shelf life of labneh. In this regard, yeasts and moulds were not detected in labneh containing essential oils throughout the storage period, and yeasts and moulds were detected in the untreated control but only after 14 and 21 days of storage (0.31 and 0.41×10³ cfu/g, respectively) (data not shown). These results are in agreement with those reported by Schelz et al. (2006) and Hassan et al. (2001) whom both reported that essential oil from thyme had antifungal and antimicrobial activities.

Organoleptic properties

The organoleptic properties of the different labneh were also investigated and the results are presented in Figure 2. There were considerable and significant differences ($P \leq 0.05$) in the flavour of these treated samples as compared with the untreated control. The untreated control labneh, when fresh and after 7 days of storage, was preferred compared to the treated labneh. Nevertheless, labneh containing essential oils at 0.2 ppm were the most acceptable after the control. 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.

It can be concluded that 0.2 ppm of thyme, marjoram or sage oils can be used in order to increase the shelf life of labneh for up to 21day at 5 ± 1 C with acceptable flavour

and good appearance without any signs of spoilage organisms.

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