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Research Article
Antioxidant, Rheological and Sensorial Properties of Ultra-filtrated Soft Cheese Supplemented with Basil Essential Oil

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Abstract

Background and Objective: There is a risk to use the artificial antioxidant materials in food sector. So, the current study was designed to figure out the properties of Ultra-filtrated (UF) soft cheese supplemented with natural antioxidant material as basil essential oil *(Ocimum basilicum* var. Genovese). **Materials and Methods:** The extracted basil essential oil (B-EO) was investigated for its main constituents by Gas Chromatography-Mass Spectrometry (GC-MS) as well as its antioxidant activity. The UF-soft cheese samples were traditionally prepared from buffalo's milk retentate. Three treatments were achieved; the first was served as control while the second and the third samples were supplemented by B-EO at ratios of 0.005 (T_1) and 0.010 (T_2) μL/100 mL, respectively. The obtained data were statistically analyzed using SAS analysis method. **Results:** The results revealed that the main compound of B-EO was identified as linalool with 63.23%, while, the second was 1,8-cineol which reached to 11.69%. It was obvious, from obtained data, that B-EO had high antioxidant activity. Data also indicated that fortification of B-EO increased the antioxidant activity of the fortified cheese samples (T_1 and T_2) compared with control. Supplementation with B-EO had decreased the hardness, gumminess and chewiness of treated cheese samples than control one. On another side, judging degrees displayed that there were noticeable differences in flavor scores between treated and control samples. The treated samples with low level of B-EO (T_1) were more favorable than those with high level (T_2). **Conclusion:** It could be concluded that basil essential oil increased the antioxidant properties of UF-soft cheese samples and improved their rheological and sensorial characters, where the samples seemed to have refreshing and acceptable taste.

Key words: UF-soft cheese, basil essential oil, antioxidants, textural profile, natural materials, sensory evaluation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antioxidant materials either synthetic or natural can be poignant in scavenging of free radical formation with the eventual repression of some disorders[1]. There are risks about the use of synthetic or artificial antioxidants for their instability and activity as promoters of carcinogenesis[2]. Recently, Plants can be used as antioxidants source to keep health and preserve food[2-4]. Many herb spices show highly antioxidant activity[5,6]. *Ocimum basilicum* (Basil) is a member of the *Lamiaceae* family. It is known as aromatic and medicinal plant. In view of its several therapeutic effects and its importance, basil deserves scientific attention. It contains vitamins and phenolic compounds[7,8] which acting as powerful antioxidants and free radical scavengers. On the other side; basil essential oil showed many different biological activities i.e., antimicrobial, antioxidant[9,10], antifungal[11] and insecticidal antioxidant[12,13]. The oil is a lightly yellowish liquid and had smelled characteristic. The yield of basil essential oil from different plant parts varies between 0.15-1.59% and it depends on some factors and locality. For the composition of B-EO, more than 200 compounds were identified and different chemo-types have been classified[14,15]. Lawrence[16] mentioned that basil contains essential oils based primarily on monoterpene derivatives such as linalool. While Ozcan and Chalchat[17] recorded that the main active compounds of basil oil are linalool, methyl chavicol, eugenol, estragole, thymol and p-cymene.

Conan[18] reported that the essential oil of basil cultivated in Egypt contained 48% linalool, 3.04% methyl chavicol and 5.9% eugenol, while Omer *et al.*[19] found that linalool is the most prominent component in Genovese basil grown in Egypt.

From other scope, dairy industries have great social and economic relevance, especially that concerning cheese production which occupying the sixth position in world production. Ultra-filtrated soft cheese is produced by Ultra-filtration (UF) of pasteurized milk with five times' higher concentration and addition of mesophilic lactic starter bacteria and rennet[20].

Today, foods are intended not only to satisfy hunger and to provide necessary nutrients but also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers[21,22]. Demand for dairy products with essential oils has increased in recent years. There are very few searches; concerning using basil essential oil in dairy field. Subsequently, the hopeful goal of this study was producing an acceptable product by adding basil essential oil (B-EO). Thus, assaying the influence of it's adding in UF-soft cheese as natural, flavoring and antioxidant agent and evaluates the rheological and sensory properties of the resultant UF- soft cheese.

MATERIALS AND METHODS

Basil essential oil

- **Basil source and its cultivation:** The plant material which used for extracted essential oil is the leaves of basil (*Ocimum basilicum* var. Genovese). Plants had grown in the experimental station of National Research Centre in Nobaria, Beheira Governorate, Egypt. Seeds of basil were obtained from SEKEM Company and were sown in seed bed. When seedlings height reached 10 cm (after 45 days), they were transplanted in the permanent field in rows at spacing of 60 cm between rows and the distance of 30 cm between hills. The growing plants had received the normal agricultural treatments (irrigation, fertilization and weed control) usually applied for the basil fields. Two harvests were carried out during the two successive seasons of study. The 1st cut was carried out after 2 months of transplanting (full bloom stage, which is the optimal commercial stage for oil production. The 2nd cut was done after two months from the 1st cut at the beginning of bloom stage. The harvested plants were air dried in shades, then powdered and kept tell essential oil extraction
- **Basil essential oil extraction:** The essential oil of air dried leaves was extracted by hydro distillation for 3 h according to Egyptian Pharmacopeia (MoH)[23]. The resulted essential oil was dehydrated over anhydrous sodium sulfate and a part of it was kept at deep freezer till GC-MS analyses, while the main part was used in UF-soft cheese preparation
- **GC-MS analysis of extracted B-EO:** The procedure of GC-MS was applied using gas chromatography-mass spectrometry instrument with the following specifications

Instrument of TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer) was used. The GC-MS system was equipped with a TG-WAX MS column (30 m×0.25 mm i.d., 0.25 μm film thickness). Analysies were carried out using helium as carrier gas at a flow rate of 1.0 mL min^{-1} and a split ratio of 1:10. The injector and detector were held at 210°C. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always

injected. Qualitative and quantitative analysies of the main compounds of the volatile oil was carried out using mass spectra (Wiley spectral library collection and NIST library). The identification of these compounds was confirmed by the published data by Adams[24].

- **Free radical scavenging activity measurements of extracted B-EO:** The antioxidant activity of essential oil was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical DPPH[25]. The amount of sample necessary to decrease the absorbance of DPPH (IC_{50}) by 50% was calculated graphically. The inhibition percentage of the DPPH radical was calculated according to the equation:

$$I(\%) = \frac{A_B - A_S}{A_B} \times 100$$

where, I is the DPPH inhibition (%), A_B is the Absorbance of control sample (t = 0 h), A_S is the Absorbance of a tested sample at the end of the reaction (t = 30 min)

UF-soft cheese preparation:

- **UF-soft cheese materials:** Full fat buffalo milk retentate (TS 33.67%, Fat 12%) was obtained from Animal Production Research Institute, Ministry of Agriculture, Giza, Egypt

Calf rennet powder (HA-LA) and starter cultures *(Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*) were purchased from Chr. Hansen's Lab., A/S Cophenhagen, Denmark.

- **Preliminary experimental trail:** Preliminary trail was conducted by using 5 different concentrations of B-EO (0.001, 0.005, 0.010, 0.015 and 0.020 µL/100 mL) to estimate the suitable ratios which will be applied and gave the most acceptable sensory properties. Two ratios had been chosen in UF-soft cheese manufacture according to organoleptic evaluation. They were 0.005 and 0.010 µL/100 mL
- **UF-soft cheese processing:** The UF-soft cheese was manufactured according to the method described by Renner and Abd El-Salam[26]. Buffalo's milk retentate was heated at 75°C, cooled to 38°C, then inoculated with 2% starter culture and held for 30 min. Salt (3%) and calf rennet were added to the retentate, then divided into three equal portions. The first one served as control (C). The second and the third parts fortified with 0.005 and 0.010 µL/100 mL B-EO as first and second experimental treatments (T_1 and T_2), respectively. All resultant milk retentate samples dispensed into plastic bags (500 mL) and held at 38°C until a uniform coagulum was formed. All cheese samples were stored at 6±2°C and analyzed when fresh, then after 15, 30 and 60 days of cold storage
- **Antioxidant activity of UF-soft cheese samples:** The DPPH (2,2-diphenyl-1picrylhydrazil) free radical scavenging activity of cheese samples was also performed according to the method of Locatelli *et al.*[27] as mentioned by Zaky *et al.*[28]. About 400 µL of cheese sample or ethanol (blank) was added to 3600 µL of a 100 µm DPPH solution in ethanol and well mixed. After 20 min, absorbance at 517 nm was measured. The DPPH radical scavenging activity (%) was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ is absorbance of blank and A_{sample} is absorbance of sample.

- **Rheological properties estimation of UF-soft cheese samples:** Texture profile analysis was performed on cheese samples using the double compression test (Multi test 1d Memesin, Food Technology Corporation, Slinfold, W. Sussex, UK)

Experiments were carried out by a compression test that generated a plot of force (N) versus time (s). A 25 mm diameter perplex conical-shaped probe was used to perform the analysis at five different points on the sample surface. In the 1st stage, the sample was compressed by 80% of their original depth at a speed of 2 cm min^{-1} during the pretest, compression and the relaxation of the sample. From the force–time curve, the following parameters were determined according to the definition given by the International Dairy Federation (IDF)[29].

Hardness (N)	=	Maximum force of the 1st compression
Cohesiveness	=	Area under the 2nd compression/area under the 1st compression (A_2/A_1)
Adhesiveness (N.s)	=	Negative area in the curve
Springiness (mm)	=	Length of 2nd compression/length of 1st compression (L_2/L_1)
Gumminess (N)	=	Hardness × cohesiveness
Chewiness (mm)	=	Gumminess × springiness

Sensorial evaluation of UF-soft cheese samples: The sensory properties of the resultant UF-soft cheese samples were evaluated periodically by 20 regular-score panelists when fresh and after 15, 30 and 60 days of cold storage for flavor (50 points), body and texture (40 points) and appearance (10 points).

Statistical analysis: Statistical analysis of experimental data was performed by using SAS Statistical Analysis Software Version 9.1.3[30]. Probability equal ($p \leq 0.05$) was significant. Each assay was carried out in triplicate.

RESULTS

Basil essential oil analysis

Chemical constituents of B-EO: The main constituents of the essential oil of basil were studied qualitatively and quantitatively with GC-MS. The identified compounds reached thirty three which accounted for 99.01% (Table 1). The main one was identified as linalool with 63.23%. The second was 1,8-cineol and reached to 11.69%, followed by a-cis-ocimene and α-copaene with 4.63 and 3.86%, respectively. The total monoterpenoids were the principal compounds since they reached to 87.84% while the sesquiterpenoid compounds were 11.16%. The total oxygen containing compounds were the dominant and formed 87.22%, while the hydrocarbon compounds were 11.73%.

Essential oil composition depends upon internal, environmental and agricultural practices as well as genetics and ecological conditions[31,32]. These results were agreed with the findings of Karawya *et al.*[33] and Omer *et al.*[19], who found that linalool was the most prominent component in Genovese basil grown in Egypt. On the other side, Lawrence[34] established four essential-oil-chemo-types (methyl chavicol, linalool, methyl eugenol and methyl cinnamate) and also numerous subtypes of oils extracted from *Ocimum basilicum*. Generally, *Ocimum* species have a clear variation in their composition. Pandey *et al.*[35] had explained that the action of volatile oils is the result of the combined effect of both their active and inactive compounds. The inactive compounds might affect resorption, rate of reactions and bioavailability of the active compounds. Biological activity of an essential oil is related to its chemical composition. The relation between composition and bioactivity of the essence from the aromatic plants may be attributable both to their major components and the minor ones present in the oil.

Antioxidant activity of basil essential oil: The inhibition percentage of the DPPH radical and IC_{50} of different concentration of both basil essential oil and ascorbic acid are shown in Table 2. Increasing the concentration of the essential oil from 0.5-5 mg mL^{-1} gradually increased the inhibition percent from 23.58-81.59%, respectively. The IC_{50} value of the essential oil was 1.78 mg mL^{-1} while it was 0.31 mg ascorbic acid mL^{-1}. As can be seen from the results summarized in Table 1.

Table 1: Main constituents of basil essential oil and their relative percentages as separated and identified by GC-MS

Compound	RT (min)	Relative (%)	Compound	RT (min)	Relative (%)
α-pinene	5.38	0.31	a-cis-Ocimene	25.78	4.63
Sabinene	6.51	0.24	α-humulene	26.80	0.52
β-pinene	6.70	0.72	germacrene-D	27.91	1.26
Myrcene	6.97	0.43	Cis-β-farnesene	28.01	0.70
Limonene	8.41	0.25	Germacrene-B	28.52	0.26
1,8-cineol	8.58	11.69	Farnesol	28.75	0.71
Trans linalool oxide	10.02	0.44	Neryl acetate	29.06	0.34
Linalool oxide	10.69	0.28	cedra-8-ene	29.34	1.56
Linalool	11.32	63.23	Globulol	31.73	0.31
Cis-epoxy-ocimene	12.92	0.18	(z.z)-α-pharmene	32.06	0.47
Camphor	13.47	0.56	Trans-α-bergamotene	33.6	0.42
Borneol-l	14.50	0.57	α-copaene	34.77	3.86
Terpineol	15.55	1.21	(+)-lavandulol	35.27	0.26
Estragole	15.68	0.69	Total identified constituents	-	99.01
N-octyl acetate	16.06	0.17	Total non-identified constituents	-	0.99
Linalyl acetate	19.39	1.61	Total oxygenated constituents	-	87.22
Limonene oxide	21.86	0.10	Total non-oxygenated constituents	-	11.73
Eugenol	22.68	0.56	Total monoterpenoids	-	87.84
β-elemene	23.87	0.92	Total sesquiterpenoids	-	11.16
Trans caryphellene	25.22	0.12			

RT: Retention time

Table 2: Antioxidant activity of basil essential oil

	Inhibition (%)								
	C1		C2		C3		C4		
Samples	Mean	STDV	Mean	STDV	Mean	STDV	Mean	STDV	IC_{50}*
Essential oil	23.58	±0.25	39.07	±0.28	67.42	±0.16	81.59	±0.17	1.78
Ascorbic acid	80.28	±1.18	81.53	±0.98	82.32	±0.65	83.43	±0.39	0.31

*Concentration (mg mL^{-1}) for 50% inhibition, C1: 0.5 mg mL^{-1}, C2: 1 mg mL^{-1}, C3: 3 mg mL^{-1}, C4: 5 mg mL^{-1}

DISCUSSION

The DPPH scavenging ability of B-EO can be attributed to the presence of some components that have antioxidant activity, for example: 1,8 cineol, α-pinene, β-pinene[36], camphor, α-thujone and B-thujone[37]. Several activities of the natural products are related to the antioxidant capacity of this product i.e., the bacterial growth inhibitory activity of the *Cakile. maritima* extracts generally correlated with their antioxidant capacities as mentioned by Omer *et al.*[38]. Oxidative stress is associated with many human diseases including atherosclerosis, cancer, chronic inflammation and Alzheimer's disease[39]. Individuals with elevated dietary intakes of non-enzymatic antioxidants such as vitamins A, C and E are less likely to suffer from some diseases including cancer and chronic inflammation[40]. Furthermore, several studies have demonstrated bacterial growth inhibitory and anti-inflammatory activities for several culinary plants with high antioxidant capacities and have linked the bioactivities to their free radical scavenging activities[41-45]. These results had been pointed out that the essential oil could serve as a good and natural antioxidant agent not only in food and cosmetics production, but also in prevention and treatment of various human diseases.

Cheese analysis

Antioxidant activity of UF-soft cheese samples: Antioxidant activity of UF-soft cheese samples are elucidated in Fig. 1. Data revealed that as increment of B-EO ratio as radical scavenging activity increasing. All samples supplemented with B-EO were significantly ($p \leq 0.05$) higher in the scavenging activity than control samples either fresh or during storage period. However, non-significant ($p \geq 0.05$) decrease was noticed after 30 days of storage period.

High antioxidant activity of cheese samples could be attributed to the existence of linalool compound in basil oil which had high ratio (63.23%). Same results were recorded by Hussain *et al.*[9], who backed the antioxidant activity of B-EO to its major component (linalool). Bassole and Juliani[46] also, stated that higher antioxidant activity of B-EO was attributed to two components (linalool and eugenol) contents and a synergistic effect was noticed. The antioxidant activity was due to the existence of hydroxyl group (-OH) of phenols. Furthermore, Ramadan *et al.*[47] produced ice cream supplemented with three essential oils (thyme, basil and marjoram), they recorded that there were a directly correlation between concentration of essential oil and antioxidant activity.

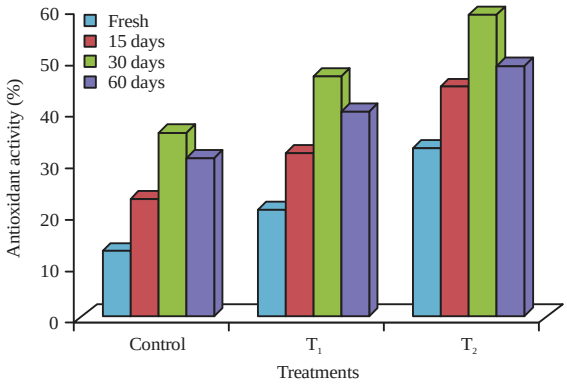

Fig. 1: Antioxidant activity (%) of UF-soft cheese samples supplemented with basil essential oil during storage. T_1 = 0.005 and T_2 = 0.010 (µL/100 mL)

Texture characteristics of UF-soft cheese samples: Rheological characterization of cheese is paramount as a means of defining body and texture properties and for checking how these parameters are influenced by processing techniques and storage conditions[48]. These characteristics were presented in Table 3.

The data which presented in Table 3 revealed that the addition of basil-essential oil significantly ($p \leq 0.05$) decreased the hardness of treated cheese samples; T_1 decreased from 15.80-13.45 N and T_2 from 15.40-12.30 N after 60 days of storage. Non-significant decrease was observed in hardness of control samples where they ranged from 15.70-14.78 N after 60 days of storage.

It could be observed in the same table that gumminess and chewiness had the same trend of hardness. A significant difference ($p \leq 0.05$) was observed in fresh control samples (9.906 N, 11.907 N/m) compared to fresh treated samples

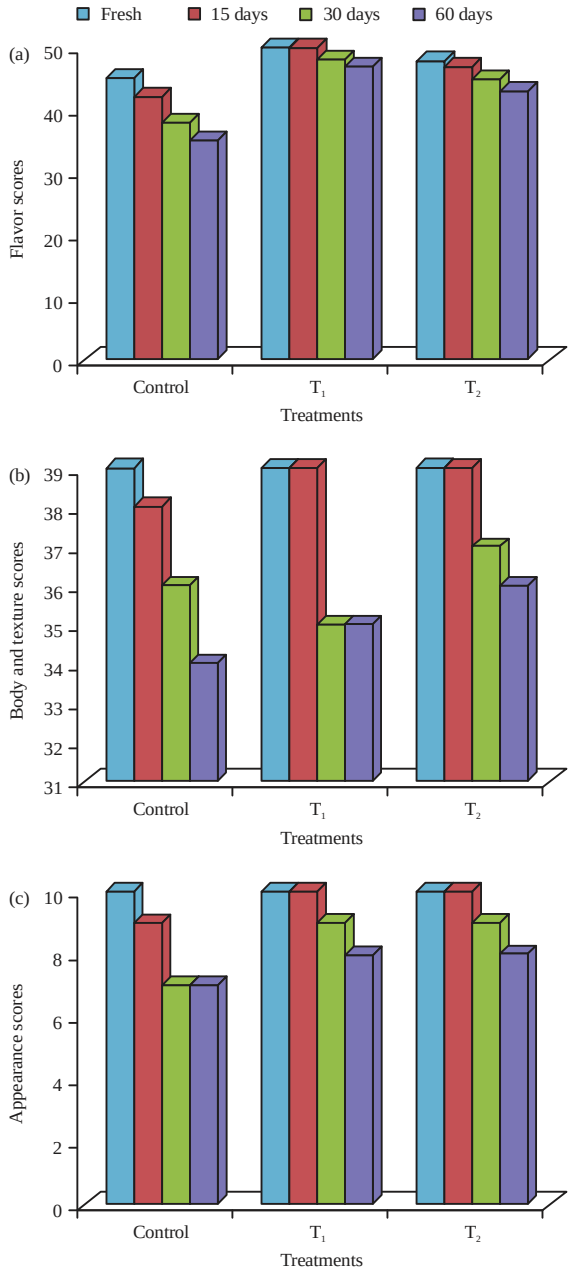

Fig. 2(a-c): Sensory evaluation of UF-soft cheese samples supplemented with basil essential oil during storage period
T_1 = 0.005 and T_2 = 0.010 µL/100 mL

(10.08, 6.995 N) for T_1 and (9.440, 6.834 N m^{-1}) for T_2. As long as storage period increased gumminess and chewiness decreased in all samples.

The hardness values which decreased with advanced storage in all cheese samples may be due to the cheese proteolysis. Fredrick *et al.*[49] recorded a correlation between hardness and proteolysis, thus, the softening of the cheese samples is likely a result of proteolysis.

Table 3: Texture profile analysis of UF-soft cheese supplemented with basil essential oil during storage (5±2°C)

Samples	Hardness (N)*	Cohesiveness (B/A area)*	Springiness (Mm)*	Gumminess (N)*	Chewiness (N m^{-1})*
Fresh					
C	15.70^{A}	0.631^{EF}	1.202^{A}	9.906^{C}	11.907^{A}
T_1	15.80^{A}	0.638^{DEF}	0.694^{A}	10.08^{C}	6.995^{E}
T_2	15.40^{A}	0.613^{F}	0.724^{A}	9.440^{D}	6.834^{E}
15 days					
C	15.50^{A}	0.821^{A}	0.854^{A}	12.725^{A}	10.867^{B}
T_1	14.60^{B}	0.689^{C}	0.791^{A}	10.059^{CD}	7.956^{CD}
T_2	13.80^{AB}	0.666^{CDE}	0.729^{A}	9.191^{E}	6.700^{EF}
30 days					
C	15.00^{A}	0.669^{C}	0.751^{A}	10.035^{C}	7.536^{D}
T_1	13.80^{AB}	0.774^{B}	0.778^{A}	10.681^{B}	8.309^{C}
T_2	12.50^{AB}	0.654^{CD}	0.774^{A}	8.175^{F}	6.327^{F}
60 days					
C	14.78^{A}	0.657^{C}	0.742^{A}	10.099^{C}	7.509^{D}
T_1	13.45^{AB}	0.762^{B}	0.763^{A}	10.668^{B}	8.320^{C}
T_2	12.30^{AB}	0.633^{CD}	0.760^{A}	8.159^{F}	6.310^{F}

Data expressed as mean of 3 replicates. Means with the same letter are not significantly different ($p \leq 0.05$), C: Control T_1 = 0.005 and T_2 = 0.010 µL/100 mL, *International Dairy Federation (IDF[29])

These data were agreed also with Al-Otaibi and Wilbey[50], who showed that hardness of UF-white cheese was decreased during ripening. These results were also confirmed by Mohamed *et al.*[51], who revealed that using Caraway and Dill essential oil in manufacture of labneh enhanced the starter activity and so increase its protyletic and lypoletic activities. A same trend was observed when using essential oil in production of soft cheese by Foda *et al.*[52] and Farbod *et al.*[53]. The hardness of cheese was also found to be affected by the composition and pH of cheese. Positive correlation was found between hardness and total solids, TP, PH and S.N/TN and negative correlation was found with Fat/DM and lactose[54,55].

Sensorial properties: Sensory evaluation is considered one of the most important parameters especially when using flavoring agent like basil oil in dairy products. Basil essential oil significantly ($p \leq 0.05$) enhanced the flavor of treated samples (T_1 and T_2) as compared to control sample either fresh or during cold storage as shown in Fig. 2. Addition of B-EO to UF-soft cheese samples gave pleasant smell and refreshing odor. It also could be noticed that samples with lower concentration of B-EO (T_1) were more acceptable than that with higher concentration (T_2).

Concerning body and texture degrees, data showed that there were clear significant differences ($p \leq 0.05$) between control and treated samples. It was found that fortified samples with B-EO were softener than control sample during storage period. While there were no observable differences ($p \geq 0.05$) in sample appearance when fresh till the end of storage period.

According to existence of flavored components in basil oil that had been used led to improve the cheese flavor. In addition, enhance body and texture of produced cheese. Consequently, these results indicated that using B-EO in the preparing of UF-soft cheese as natural flavoring agent had got great effectiveness. A same trend was observed by Zaky *et al.*[28], in free-salt labneh samples fortified with dill and caraway essential oil and Foda *et al.*[52] in soft cheese fortified with spearmint essential oil.

CONCLUSION

It could be concluded that acceptable soft cheese fortified with basil essential oil (B-EO) was conducted by using 0.005 µL/100 mL. The treated samples had an antioxidant effect more than control one. The product had soft body and acceptable texture with refreshing and favorable taste. Thus, the goal of this search was achieved by adding a natural flavoring agent like basil-EO which have notable antioxidant activity in addition of its previous properties.

SIGNIFICANCE STATEMENTS

This study can give a flasher on using basil essential oil as a natural antioxidant agent to produce healthy soft cheese. Natural antioxidant materials should be used also in dairy sector to prepare safe and healthy product.

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