Effect of Some Essential Oils on the Quality of UF-Soft Cheese During Storage

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ABSTRACT

The present study aimed to evaluate the chemical composition, antioxidant activity, antimicrobial properties and the minimum inhibitory concentrations (MICs) of three essential oils extracted from cumin, rosemary and thyme and their mixture and their effect on physicochemical, microbial, rheological and sensorial attributes of ultrafiltrated (UF)-soft cheese. UF-soft cheese was prepared from UF milk retentate with adding 0.1% of these essential oils. The results revealed that the different essential oils had remarkable antimicrobial effect on the growth of *Escherichia coli* (*E. coli*), *Salmonella typhimurium* (*S. typhimurium*), *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Bacillus cereus* (*B. cereus*), and *Aspergillus niger* (*A. niger*). Among essential oils, thyme oil had the highest antioxidant activity and antimicrobial effect. Addition of essential oils appeared to affect cheese pH and total volatile fatty acid content during storage period, while total solids and fat contents were slightly affected. Addition of essential oils to retentate resulted in an increment in antioxidant activity and decreased the total bacterial count compared with the control cheese. Sensory evaluation revealed that UF-soft cheese containing essential oils remained acceptable even at the end of storage period. Adding cumin essential oil to UF-soft cheese gained the highest scores for the sensorial attributes. The results concluded that a concentration of 0.1% of essential oils extracted from cumin, rosemary or thyme or their mixture can be used to extend the shelf life of UF-soft cheese for up to 28 days. These essential oils could be successfully used as natural and safe additives in production of UF- soft cheese.

Key words: UF-Soft cheese, cumin, rosemary, thyme essential oils, antioxidant activity and antimicrobial properties.

INTRODUCTION

Soft cheese is the most popular cheese consumed in Egypt. It is made by different procedures, i.e. traditional methods and ultrafiltration (UF), and is stored at low temperature with or without brine. UF technology has many advantages in cheese making such as increasing cheese yield and nutritive values, decreasing the production cost and solving the environmental problems related to whey disposal (Mehaia, 2006). On the other hand, UF-soft cheese is characterized by weak flavour, which is attributed to the concentration of proteinase and peptidase inhibitors by UF procedures (El-Soda, 1997).

Spices and herbs, commonly known as aromatic plants, are an important group of agricultural commodities and being used by many civilizations all over the world to add flavour, taste, and nutritional values and to improve shelf life of food. These plants can also heal various physical, mental and emotional problems and to restore human health (Bhat *et al*., 2014). However, each spice or herb is characterized by a peculiar qualitative and quantitative composition for its essential oil and all of these oils contain compounds with established and well known biological activity (Stefanini *et al*., 2006).

Essential oils are complex mixtures of different chemical compounds and most of them have been recognized as safe extracts (GRAS). These oils have remarkable antioxidant and antimicrobial activities and have the potential to be used in food industry as a biopreservative to prevent spoilage and to extend the shelf life of products (Burt, 2004). The antimicrobial effect of essential oils has been attributed to the presence of many phenolic components and polypeptides (Ismail *et al*., 2006). El-Nawawy *et al*. (1998) concluded that essential oil extracts can be used as food flavouring agents and biopreservatives. Additionally, essential oil extracts may have potential medical application as they possessed an important antioxidant activity which may play a role in reducing the risk of some chronic diseases.

Cumin (*Cuminum cyminum* L.) is an annual herb from *Apiaceae* family. The spice, green cumin is cultivated in Iran, Egypt, Turkey, North Africa, and Asia. Cumin seeds possess an aromatic odour and have a spicy and bitter taste and largely used in the Egyptian kitchen and is locally known as "Kammoun" (Hajlaoui *et al*., 2010). Green cumin is an important medicinal and aromatic plant that has medicinal properties, including antimicrobial and antioxidant activities (Gachkar *et al*., 2007, Einafshar *et al*., 2012). Many phytochemical studies have been conducted to investigate the chemical composition of the essential oils of cumin seeds. They stated that the major components of cumin are aldehydes.

Rosemary (*Rosmarinus officinalis* L.) is a spice and medicinal herb which widely used in the world and accepted as one of the spices with the highest antioxidant activity (Peng *et al*., 2005). Rosemary essential oil extract is also used as an antibacterial and antifungal (Kabouche *et al*., 2005). The main compounds responsible for the antimicrobial activity are α-pinene, bornyl acetate, camphor and 1, 8-cineole (Pintore *et al*., 2002).

Thyme (*Thymus vulgaris* L.) is an aromatic plant belonging to the *Labiatae* family. Thyme is used in the food and aroma industries and is widely used as a culinary ingredient and it serves as a preservative for foods especially because of its antioxidant effect (Zarzuelo & Crespo, 2002). Thyme is an excellent source of essential oils and natural antibiotic properties as a consequence of the presence of thymol which constitutes around 50 % of the components in its essential oil extract. Carvacrol is also of importance in this respect (Anonymous, 2009). Thyme oil is among the world's top ten essential oils regarding to its use as a food additive (Stahl-Biskup & Saez, 2002).

The present study aimed to identify major constituents found in essential oils extracted from cumin, rosemary, thyme and a mixture of them at ratio (1:1:1). Also evaluate the antioxidant and antimicrobial activities of the extracted oils. The effect of the extracted essential oils of previous plants and their mixture on physicochemical, microbial, rheological properties and sensorial attributes of UF-soft cheese was investigated.

MATERIALS AND METHODS

Materials

Cumin (*Cuminum cyminum* L.), rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.) were obtained from commercial market in Alexandria, Egypt. Buffalo's milk retentate used in the manufacture of UF-soft cheese was obtained from Dairy Industry Units, Animal Production Research Institute, Ministry of Agriculture, Giza, Egypt. The retentate contained 63.06 % moisture, 17.06 % protein, 14.00 % fat, 4.06% ash, 1.82 % lactose and 0.18% titratable acidity. Animal rennet was obtained from Chr-Hansen's Laboratories, (Copenhagen, Denmark). Rennet Powder was diluted with distilled water to a standard rennet solution before use. Commercial edible grade table salt (sodium chloride) produced by El-Nasr company, Alexandria, Egypt was obtained from the local market. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Butylated Hydroxy Toluene (BHT) were obtained from Sigma-Aldrich, Germany. All chemicals, and reagents used in the present study were of analytical grade.

Microorganisms

The tested microorganisms were Gram-negative (*Escherichia coli* ATCC 6933, *Salmonella typhimurium* ATCC 14028), Gram-positive (*Staphylococcus aureus* ATCC 20231, *Bacillus subtilis* DSMI 08, *Bacillus cereus* ATCC 33018) and *Aspergillus niger*. All microorganisms were obtained from the Egyptian Microbial Culture Collection Faculty of Agriculture, Ain Shams University, Cairo, Egypt. All bacteria were enumerated on nutrient agar medium at 37°C, while potato dextrose agar medium was used to enumerate the fungus at 25°C.

Methods

Extraction of the essential oils

Distillation using a Clevenger-type apparatus has been used for essential oils extraction. About 100g (cumin, thyme and rosemary) were set in Clevenger apparatus with enough distilled water. Distillation was carried out for 3 hr as described by the British Pharmacopoeia (1963). The obtained essential oils were stored at -20°C till used.

Manufacture of UF-soft cheese

UF-soft cheese was made according to the method described by Renner &Abd El-Salam (1991). Buffalo's milk retentate was divided into five equal portions; each portion was salted to a concentration of 2.5 %, well mixed and pasteurized at 65ºC for 30 min and immediately cooled to 37ºC. Frist portion was served without essential oil as (control) sample. One ml essential oil/kg retentate of cumin, rosemary, thyme or their mixture were

added to the other four portions at 37ºC to prepare UF-soft cheese and well mixed with a blender with high speed, then renneted at 37ºC and stirred for 1min, dispensed into plastic containers and kept at 37ºC±2ºC until a proper coagulum was formed after about one hr and then transferred to refrigerator at 4ºC±1ºC for 4 weeks. Cheese samples were taken from fresh and after 7, 14, 21 and 28 days of refrigerated storage for chemical composition, rheological, microbiological and sensory evaluation.

Gas chromatography-mass spectroscopy (GC-MS) analysis

GC/MS analysis was performed separately with a Hewlett Packard model 5890. Gas chromatograph equipped with 5 series Mass selective detector 8644 (HP) with flame ionization detector (FID) on a fused silica 132 capillary column DB-5 (25 m in length, 0.32 mm i.d., and 0.5 mm film thickness). The oven temperature was maintained at 60°C for 2 min after injection and then programmed at 4°C min^{-1} to 270°C. The split injector temperature was 270°C and MS conditions were kept at 280°C and 42ev. The percentage of major constituents of cumin, rosemary, thyme and the mixture were estimated by measuring the peak area of the different compounds of the chromatogram according to Gunther & Joseph (1978).

Determination of the antioxidant activity

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of purple coloured methanol solution of DPPH. The effect of oils on DPPH radical was estimated according to Kose *et al*. (2010). One ml of various concentrations (200, 300, 400 and 500 ppm) of the essential oils and BHT in methanol was added to a 4 ml of DPPH radical solution in methanol (0.004%). The mixture was shaken vigorously and allowed standing for 30 min. The absorbance of the resulting solution was measured at 517nm with a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$$I\% = 100 \times (A_{Control} - A_{Sample}) / A_{Control}$$

Where, $A_{Control}$ is the absorbance of the control reaction (containing all reagents without the test sample), and A_{Sample} is the absorbance of the tested sample. BHT was used for comparison.

The antioxidant activity of UF-soft cheese was determined by DPPH as described by Brand Williams *et al*. (1995) and expressed as percentage inhibition of the DPPH radical.

Antimicrobial activity

Agar well diffusion method

The agar-well diffusion method was conducted for the determination of antimicrobial activity of essential oil extracts as described by Schillinger & Lucke (1989). Semi solid (0.7% agar w/v) nutrient medium or potato dextrose medium was used to perform test against indicator bacterial strains and fungus, respectively. The semi-solid media were molten, cooled down to 48°C, inoculated with 0.1 ml of fresh overnight culture of the indicator strains (approximately 10^5 cfu/ml) and poured into a Petri disk. Wells of 6 mm in diameter were cut into the agar and filled with 100 µL of the extract of essential oil. After holding the plates at room temperature for 2 hr to allow diffusion of the extract into the agar, the plates were incubated at 37°C for 24 hr. Then, they were examined for inhibition of the bacterial lawn and the diameters of the inhibition zones were measured. All tests were performed in triplicate.

Minimum inhibitory concentration (MIC) test

Determination of MIC of the essential oil against the test bacterial strains was determined as described by National Committee for Clinical Laboratory Standards (NCCLS 2002). All microorganisms were prepared for 24 hr and the suspensions were adjusted to 10^8 cfu/ml. Essential oil solutions at concentrations 1, 0.5, 0.25, 0.125 and 0.063% were prepared by diluting the concentrated essential oil in broth containing 0.5% tween 80. Inoculated plates were incubated at 37ºC for 18 hr. The MICs were determined by agar well diffusion method as the lowest concentration of extracts inhibiting visible growth of each organism on the agar plate.

Physicochemical analysis of UF-soft cheese

Cheeses were analyzed in duplicate for moisture, total protein, fat, ash and titratable acidity using the methods of the AOAC (2007). Total volatile fatty acids (TVFA) were determined according to Kosikowski (1978). Cheese pH was determined using glass electrode pH meter (Persica model pH 900, Switzerland).

Rheological analyses of UF-soft cheese

Texture properties were measured at 20°C using a texture analyzer (TA1000, Lab Pro (FTC TMS-Pro, USA) and connected to a computer pro-

grammed with Texture Pro™ texture analysis software (program, DEV TPA with holding time of 2 seconds between cycles. A flat rod probes (49.95 mm in diameter) to uniaxially compresse the "cheese samples with the following parameters conduction to 30% of their original height. Each sample was subjected to two subsequent cycles (bites) of compression-decompression. The data were collected on computer and the texture profile parameters were calculated from DEV TPA texture analyzer and computer interface. Calculation described by Bourne (1978) was used to obtain the following texture profile parameters (hardness, cohesiveness, springiness, gumminess and chewiness)

Microbiological analyses

The total bacterial count of cheese was determined according to Marshal (1992). Coliform bacteria, moulds and yeasts were enumerated according to IDF (1985_a, 1985_b), respectively. Samples were analyzed at zero, 7, 14, 21 and 28 days. The results were recorded as log number of colony forming units per g ($\log_{10}$ cfu/g).

Sensory evaluation

Sensory evaluation of cheese was evaluated by nine-point hedonic scale described by Pajohi *et al*. (2011). Sensory evaluation was performed by ten trained panellists belong to staff members of Dairy Research Department and Food Technology Research Institute, Egypt. Cheeses were evaluated for their appearance and colour, body and texture, odour, taste and overall acceptability. Prior to be subjected to panellists, cheese samples were cut into cubes (1.5 x 1.5 x 1.5 cm) and covered with plastic wrap to prevent dehydration. Cubes were coded with three-digit random numbers and held for at least 1 hr at 20°C to equilibrate. Each panellists was given three cubes of each sample. Water and non-salted crackers were provided to clean their palates between tasting.

The maximum acceptable concentration of the studied essential oils in UF- soft cheese samples was determined through sensory evaluation tests. For each of the examined essential oils (cumin, rosemary, thyme and their mixture), different UF-soft cheese samples were made by adding different concentrations (0.0, 0.05, 0.1, 0.2 and 0.4%) for each essential oil.

Statistical analysis

The obtained data were analyzed statistically by two-way analysis of variance using (ANOVA) followed by t test (LSD) with $P \leq 0.05$ being considered statistically significant using SAS program software program (SAS Institute 2004).

RESULTS AND DISCUSSION

The yield of extracted essential oils were 2.4, 2.1 and 1.77% of the gross composition of cumin, rosemary and thyme, respectively. These values are in agreement with those previously reported for cumin (2.3-5%) and rosemary (1-2.5%) extracts (Azeez, 2008, Walsh, 2000). For thyme, the European Pharmacopoeia demands a minimum of 1.2% oil content for thyme herb (Rey & Sáez, 2002).

GC/MS analyses of extracted essential oils

Cumin essential oil

Table (1) shows the main constituents identified in the cumin essential oil extract by the GC-MS. The GC-MS analysis resulted in the identification of 15 constituents in cumin extract. Six major components accounted more than 97% of the composition of cumin extract. Those components were identified as *p*-menth-2-en-1-ol (35.86%), cumin aldehyde (34.97%), *β*-pinene (8.98%), *α*-phellandrene (8.59%), *p*-Cymene (7.79%), and *γ*-Terpinene (1.22%). Meanwhile, El-Ghorab *et al.* (2010) mentioned that the major components in cumin volatile oil were cuminal, *γ* terpinene and pinocarveol which represented antioxidant activity. The authors also reported that cumin essential oil was better at reducing Fe^{3+} ions than dried or fresh cumin. In general, cumin aldehyde, *γ*-terpinene, *p*-cymene and *β*-pinene were considered to be the major constituents of volatile oils of green cumin (Lis-Balchin *et al*., 1998).

Rosemary essential oil

GC-MS analysis resulted in the identification of 14 major constituents in rosemary oils (Table 1). The identified constituents were Limonene (53.58%), *α*-Pinene (17.17%), Camphor (10.58%), Camphene (4.02%), *p*-Cymene (3.09%), Bornyl acetate (2.77%), Eugenol (1.77%), 1,8-Cineole (1.31%), *β*-Pinene (1.21%) and Borneol (1.04%). Similarly to the results obtained in the present study, Pintore *et al*. (2002) reported that the main components of rosemary oil extract were *α*-pinene, bornyl acetate, camphor and 1, 8-cineole. In addition, 3 minor components, Sabinene, *β*-Myrcene and *α*-Terpineol, were identified in the oil extract at

Table 1: Volatile components identified in essential oils extracted from cumin, rosemary, thyme and their mixture at a ratio of 1:1:1

No	Compound	Essential oil %			
		Cumin	Rosemary	Thyme	Mixture
1	*α*-Pinene	0.20	17.17	1.00	2.04
2	*β*-Pinene	8.98	1.21	1.43	0.69
3	*p*-Cymene	7.79	3.09	1.21	1.18
4	*γ*-Terpinene	1.22	1.54	2.80	3.40
5	Myrcene	0.46	0.44	4.53	3.30
6	Limonene	-	53.58	0.39	6.30
7	Camphene	-	4.02	0.39	18.62
8	*α*-Terpineol	0.37	0.66	-	0.89
9	*p*-menth-2-en-1-ol	35.86	-	-	13.47
10	Thymol	-	-	71.17	30.05
11	Cumin aldehyde	34.97	-	-	12.67
12	*α*-Terpinene	-	-	9.93	4.15
13	Camphor	-	10.58	-	0.86
14	*α*-phellandrene	8.59	-	0.29	0.46
15	Carvacrol	0.16	-	-	0.66
16	Sabinene	-	0.82	1.79	0.43
17	Linalool	-	-	1.79	0.83
18	o-Cymene	0.41	-	-	-
19	*γ*-Terpinene-7-al	0.35	-	-	-
20	Citranellol	0.32	-	-	-
21	*α*-Terpinene-7-al	0.27	-	-	-
22	*α*-Thujene	0.05	-	-	-
23	1,8-Cineole	-	1.31	-	-
24	Borneol	-	1.04	-	-
25	Bornyl acetate	-	2.77	-	-
26	Eugenol	-	1.77	-	-
27	*β*-Ocimene	-	-	1.06	-
28	Geraniol	-	-	0.54	-
29	*γ*-terpinolene	-	-	1.68	-
30	Geraniol	-	-	0.54	-

(-) = Not Found

levels of 0.44-0.82% of the entire composition of rosemary essential oil.

Thyme essential oil

Thyme essential oil extract had high amounts of thymol (71.17%), *α*-terpinene (9.930%), myrcene (4.53%), *γ*-terpinene (2.80%), linalool (1.79%), sabinene (1.79%) and *γ*-terpinolene (1.68%) (Table1). Also, other minor compounds (*α*-phellandrene, *β*-pinene, *α*-pinene and camphene) were detected at low concentrations and varied from 0.29 to 1.43%. Farag *et al*. (1989) reported that thyme essential oil contains 43% thymol and 36% p-cymene.

Mixture essential oils

GC-MS analysis resulted in the identification of 17 constituents in essential oils extract of mixed herbs (Table 1). The main constituents were thymol (30.05%), camphene (18.62%), *p*-menth-2-en-1-ol (13.47%), cumin aldehyde (12.67%), limonene (6.30%), *α*-terpinene (4.15%), *γ*-terpinene (3.40%), myrcene (3.30%) and *α*-pinene (2.04%). Also, other minor compounds including *β*-pinene, Carvacrol, Camphor, *α*-Terpineol *p*-cymene and linalool were detected at low concentrations ranged from between 0.43 to 1.18%.

Antioxidant activity of essential oils

Fig. (1) shows the antioxidant activity of different concentrations (100, 200, 300, 400 and 500 ppm) of essential oil extracts. The antioxidant activity of oil extracts was expressed as a relative percentage to antioxidant activity of BHT. Generally, the antioxidant activity of any essential oil extract increased as its concentration increased.

Thyme essential oil extract showed the highest antioxidant activity particularly at high concentrations (300 to 400 ppm). Previous studies attributed the increased scavenging activity of thyme extract to the presence of thymol which has potential ability to scavenge free radicals and to inhibit lipid oxidation. Thymol and other phenolic compounds, present in thyme extract, have been shown to have potential antioxidant activity by inhibiting lipid peroxidation by acting as chain-breaking peroxyl-radical scavengers. In addition, phenols directly scavenge reactive oxygen species, including hydroxyl radicals, peroxynitrite

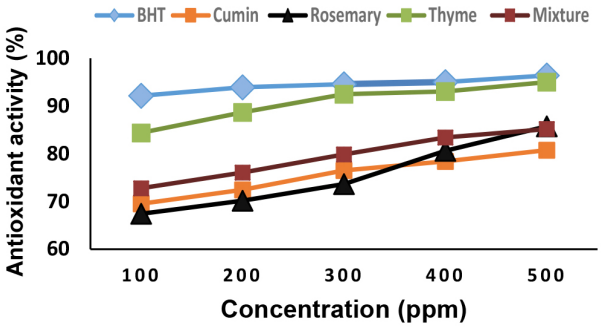

Fig. 1: Antioxidant activity of essential oils

and hypochlorous acid (Wang *et al*., 2008, Miguel, 2010). The potential antioxidant activity of thyme extract reported in the present study support its application as natural resource to improve quality and extend shelf-life of food and dairy products. Early in 2004, Kulisic et al. (2004) reported that thyme essential oil could be used as potential resource of natural antioxidants for food industry and it would be important to examine its application in some final food products.

Cumin essential oil extract at concentrations of 100, 200, 300, 400 and 500 ppm showed antioxidant activity equivalent to 69.53, 72.47, 76.54, 78.43 and 80.76%, respectively as compared to those determined for the same concentrations of BHT. Similar antioxidant activity has been reported for cumin essential oils extract in previous study (Singh *et al*., 2005, Fakoor & Rasooli, 2008). Hajlaoui *et al*. (2010) reported that cumin essential oils had interesting results in term of its ability to neutralize free radicals and prevent unsaturated fatty acid oxidation.

The antioxidant activity of rosemary essential oil was positively correlated with its concentration. The extracted oils at concentrations of 100, 200, 300, 400 and 500 ppm showed antioxidant activity equivalent to 67.42, 70.15, 73.64, 80.64 and 85.75%, respectively as compared to the same concentrations of BHT. The results obtained in the present study are in agreement with those reported by Wang *et al*. (2008) who attributed the antioxidant activity of rosemary oil extract to the presence of antioxidant compounds such as camphor and borneol. In addition, Yosr *et al*. (2013) reported that the essential oil extracted from Tunisian rosemary was characterized by high amount of camphor (14.5%), 1,8-cineol (35.8%) and α pinene (10.6%) which improved its antioxidant activity compared to other types cultivated elsewhere.

The antioxidant activity of the mixture essential oils ranged from 72.78 to 85.16% depending on the tested concentrations. These activities were lower than that reported for thyme extract but slightly higher than cumin and rosemary extracts.

Antimicrobial effects of essential oils

The antimicrobial activity of different essential oils against *B. subtilis, B. cereus, S. typhimurium, Staph. aureus, E. coli* and *A. niger* is shown in Fig. (2). In general, thyme essential oil had the highest antimicrobial activity against tested microorganisms, while rosemary extract showed the least activity. Similarly, Celikel & Kavas (2008) found that thyme essential oil extract had superior antibacterial activity than sage, myrtle, laurel and orange oils.

In the dose response study, the inhibition zone produced by any of the tested essential oil extracts increased with increasing concentration of tested essential oil. Thyme essential oil produced the highest inhibitory zones (9–30 mm diameter) with potential activity against *B. subtilis, E. coli* and *S. aureus*. This is in accordance to the results obtained by Celikel & Kavas (2008). On the other hand, cumin essential oil extract produced inhibitory zones with average diameters ranged from 7 to 24 mm. The susceptible microorganisms against cumin extract were *B. subtilis* and *E. coli.* Rosemary essential oil produced the lowest inhibitory effect with inhibition zone ranged from 7-13 mm in diameter. It appeared to be effective at inhibiting *S. typhimurium* and *E. coli*. A previous study has reported that rosemary essential oil displayed antimicrobial activity against 13 bacterial strains and 6 fungi (Bozin *et al*., 2007). The mixture essential oil had inhibitory effects against tested microorganisms and produced inhibition zone ranged from 7-22 mm in diameter. The most sensitive strains to mixture essential oils were *B. subtilus, B. cereus* and *E. coli*.

All the tested extracts exhibited a moderate inhibitory activity against *A. niger* (mold commonly associated with food spoilage). Essential oils extracted from thyme, cumin and the mixture showed potential antifungal activity against *A. niger*. Thyme essential oil had the highest antifungal activity followed by cumin and the mixture essential oil, respectively, while rosemary essential oils had the least inhibitory effect against *A. niger.* These results are in agreement with those reported by Viuda-Martos *et al.* (2008). In general, essential oils rich in phenolic compounds, aldehydes and alcohols had remarkable inhibitory effects against yeasts and moulds (Sacchetti *et al*. 2005).

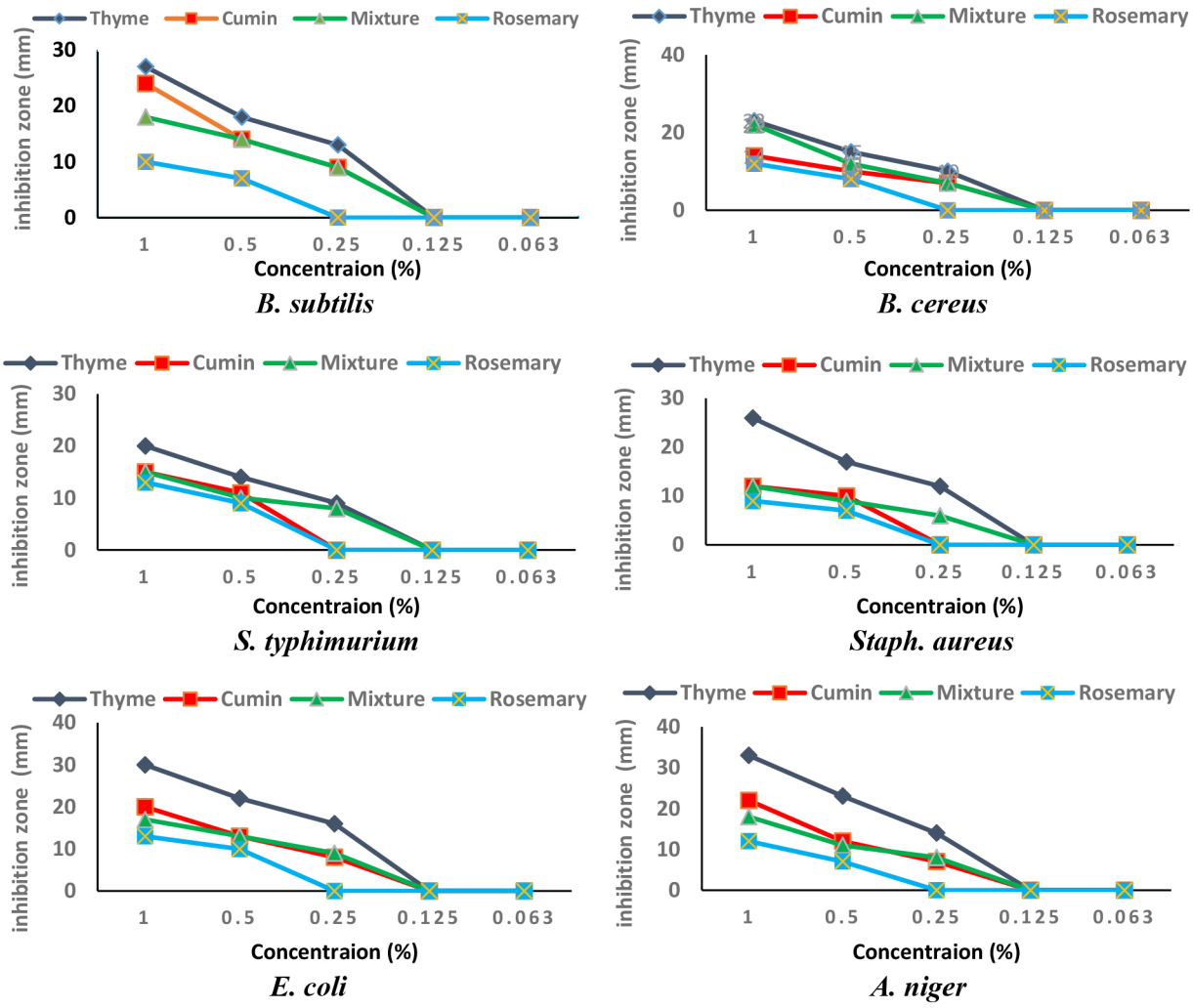

Fig. 2: Antimicrobial effects of essential oils

Minimum inhibitory concentration (MICs)

The minimum inhibitory concentrations (MICs) of essential oils (cumin, rosemary, thyme and the mixture) ranged from 0.063 to 1% and the results are illustrated in Fig. (2). The results revealed that MIC for thyme, cumin and the mixture essential oils was 0.25%, while MIC for rosemary essential oil was 0.5%.

The differences found between the results obtained in the present study and other authors' results can be attributed to the way in which the essential oil is extracted (Moreira *et al*. 2005).

Physicochemical properties of UF-soft cheese

The changes in physicochemical properties during cold storage of UF-soft cheese containing essential oils are shown in Table (2).

It was obvious that the moisture content of UF-soft cheese samples containing the different essential oils were more or less the same as the control sample throughout the storage period. The results also showed that within the same essential oil there were no significant differences in the moisture content during storage period. The results in Table (2) showed that the addition of essential oil significantly ($P \leq 0.05$) increased ash content of UF-soft cheese. Also, storage period appeared to have a significant effect ($P \leq 0.05$) on ash content of the UF-soft cheese. Keke *et al.* (2009) reported an increase in ash content of cheeses treated with *Sorghum vulgaris* and *Pimenta racemosa*.

Cheese with added essential oil extracts showed significantly ($P \leq 0.05$) higher fat content compared with the control cheese. However, no significant differences were found among cheese samples with added oil extracts. Also refrigerated storage could significantly ($P \leq 0.05$) affect fat content. This was in accordance to the results reported by Tornambé *et al.* (2008).

Table 2: Effect of essential oils on physicochemical properties during refrigerated storage of UF-soft cheese

Components	Storage (days)	Control	Cheese with Cumin	Cheese with Rosemary	Cheese with Thyme	Cheese with Mixture	Mean**
Moisture content (%)	Fresh	64.34±0.08	64.84±0.15	64.92±1.09	64.88±0.27	64.90±0.79	64.67^{A}
	7	64.30±0.13	64.77±0.10	64.87±0.87	64.82±0.03	64.81±0.74	64.62^{A}
	14	64.22±1.22	64.22±1.22	64.72±0.26	64.50±0.37	64.64±0.57	64.46^{A}
	21	64.17±0.40	64.52±0.25	64.69±0.15	64.41±0.54	64.22±0.15	64.33^{A}
	28	63.67±0.05	64.24±0.89	64.24±0.97	64.28±0.39	63.93±1.6	64.07^{A}
Mean**		64.14^{a}	64.26^{a}	64.69^{a}	64.57^{a}	64.69^{a}	LSD=0.64
Ash %	Fresh	3.91±0.02	4.13±0.12	3.95±0.03	3.93±0.37	3.99±0.07	3.98^{B}
	7	3.94±0.00	4.14±0.06	4.04±0.01	3.96±0.22	4.05±0.05	4.03^{AB}
	14	3.95±0.14	4.14±0.02	4.06±0.01	4.00±0.10	4.07±0.02	4.05^{AB}
	21	3.96±0.07	4.16±0.07	4.05±0.08	4.10±0.10	4.10±0.02	4.08^{AB}
	28	3.99±0.04	4.22±0.01	4.16±0.04	4.20±0.04	4.21±0.00	4.15^{A}
Mean**		3.95^{c}	4.16^{a}	4.05^{b}	4.04^{b}	4.09^{b}	LSD=0.098
Fat %	Fresh	14.00±0.07	14.12±0.03	14.17±0.03	14.18±0.00	14.15±0.03	14.12^{D}
	7	14.07±0.03	14.18±0.03	14.19±0.06	14.22±0.03	14.20±0.03	14.17^{C}
	14	14.14±0.03	14.19±0.04	14.20±0.01	14.23±0.01	14.22±0.03	14.19^{C}
	21	14. 20±0.05	14. 29±0.04	14.32±0.03	14.28±0.03	14.29±0.04	14.27^{B}
	28	14.23±0.05	14.35±0.06	14.37±0.03	14.38±0.04	14.40±0.01	14.34^{A}
Mean**		14.13^{b}	14.23^{a}	14.25^{a}	14.25^{a}	14.26^{a}	LSD=0.033
Protein %	Fresh	17.18±0.86	17.09±0.71	16.91±0.91	17.01±0.10	16.94±0.09	17.03 ^{A}
	7	17.18±0.77	17.17±0.23	16.94±0.20	17.01±0.16	16.96±0.06	17.05 ^{A}
	14	17.27±0.46	17.28±0.07	16.96±0.81	17.14±0.39	17.06±0.67	17.14 ^{A}
	21	17.41±0.25	17.36±0.06	17.00±0.29	17.18±0.41	17.28±0.19	17.25 ^{A}
	28	17.09±0.08	17.46±0.19	17.23±0.93	17.27±0.28	17.34±0.05	17.28 ^{A}
Mean**		17.23 ^{a}	17.27 ^{a}	17.01 ^{a}	17.23 ^{a}	17.12 ^{a}	LSD=0.44
Total volatile fatty acids (TVFA*)	Fresh	2.60±0.14	2.80±0.03	2.65±0.01	2.60±0.03	2.65±0.03	2.66^{E}
	7	4.20±0.04	3.00±0.04	3.60±0.03	2.87±0.03	3.47±0.07	3.42^{D}
	14	4.63±0.035	3.60±0.07	3.78±0.04	3.25±0.43	3.70±0.03	3.79^{C}
	21	5.62±0.09	3.65±0.04	3.90±0.03	3.80±0.06	3.75±0.01	4.14^{B}
	28	8.82±0.04	3.80±0.03	4.10±0.03	3.90±0.01	3.80±0.07	4.88^{A}
Mean**		5.17^{a}	3.37^{d}	3.60^{b}	3.28^{e}	3.47^{c}	LSD=0.047
pH	Fresh	6.7±0.00	6.7±0.00	6.7±0.00	6.7±0.00	6.7±0	6.70 ^{A}
	7	6.6±0.00	6..6±0.00	6.6±0.00	6.6±0.00	6.6±0.00	6.60 ^{B}
	14	6.5±0.07	6.45±0.07	6.6±0.00	6.6±0.00	6.45±0.07	6.51 ^{C}
	21	6.35±0.07	6.45±0.07	6.5±0.07	6.6±0.00	6.45±0.07	6.46 ^{D}
	28	6.35±0.07	6.45±0.07	6.4±0.00	6.5±0.00	6.45±0.07	6.43 ^{D}
Mean treatment		6.49^{c}	6.53 ^{bc}	6.55 ^{b}	6.60 ^{a}	6.53 ^{bc}	LSD= 0.0412
Acidity %	Fresh	0.196±0.00	0.191±0.00	0.189±0.00	0.191±0.00	0.189±0.00	0.191 ^{E}
	7	0.203±0.00	0.194±0.00	0.1950.00	0.195±0.00	0.199±0.00	0.197 ^{D}
	14	0.203±0.00	0.198±0.00	0.196±0.00	0.196±0.00	0.201±0.00	0.199 ^{C}
	21	0.215±0.00	0.205±0.00	0.205±0.00	0.198±0.00	0.207±0.00	0.206 ^{B}
	28	0.228±0.00	0.205±0.00	0.209±0.00	0.201±0.00	0.207±0.00	0.210 ^{A}
Mean**		0.210 ^{a}	0.199 ^{c}	0.199 ^{c}	0.196 ^{d}	0.201 ^{b}	LSD= 0.0016

* TVFA values expressed as NaOH ml/10g

Means with different superscripts in a raw or column are significantly different at P≤0.05 level.

For protein content, no significant differences were detected among cheese samples, including the control cheese, indicating that the inclusion of essential oil extracts in UF-soft cheese did not affect their protein content. However, there were slight changes in protein contents during refrigerated storage. These changes coincided with changes in cheese moisture content (Ahmed & Abdel–Razig, 1998). The results obtained in the present study are in agreement with those obtained by Mutlag & Hassan (2008), who reported significant increase in protein content during storage period of labneh containing essential oil compared to the untreated control labneh. At the end of storage period, UF-soft cheese contain-

ing cumin and mixture oil had the highest protein content (17.46 and 17.34%, respectively) followed by the UF-soft cheese containing both the rosemary (17.23%) and thyme essential oils (17.27%). However, the lowest protein content was found in the control UF-soft cheese (17.09%).

The total volatile fatty acids (TVFA) contents were significantly ($P \leq 0.05$) affected by the inclusion of essential oil extracts in UF-soft cheese. All cheese samples showed the same trend in which TVFA increased significantly ($P \leq 0.05$) throughout the storage period. In fresh cheese, TVFA contents of cheese containing essential oil were insignificantly differed from that determined in the control cheese. However, the rate of increment in TVFA content varied considerably among treatments during storage. Control cheese had the highest values of TVFA throughout storage period compared with cheese containing essential oil. Similar results were reported by Ahmed & Abdel–Razig (1998). The highest mean value of TVFA were recorded in the control cheese followed by the sample containing rosemary essential oil, whereas the lowest values were recorded in cheese sample containing thyme essential oil. These results are in agreement with those reported by Ragab (2000), who attributed the formation of low amounts of TVFA in cheese containing essential oils to the inhibitory effect of these oils to moulds and lipolytic bacteria. The TVFA contents of untreated UF-soft cheese sample and those containing the essential oil of cumin, rosemary, thyme and the mixture at the end of storage were 8.82, 3.80, 4.10, 3.90 and 3.80 ml of 0.1 N NaoH/10 g cheese, respectively.

All cheese samples showed slight decrease in pH value and an increase in titrtable acidity values during refrigerated storage. This could be attributed to the breakdown of lactose into lactic acid during storage (Hassan & Amjad, 2010). Acidity development was slower in cheese samples containing essential oils compared to the control cheese. Similar results were reported by Hussein (2004) and Shan *et al.* (2011).

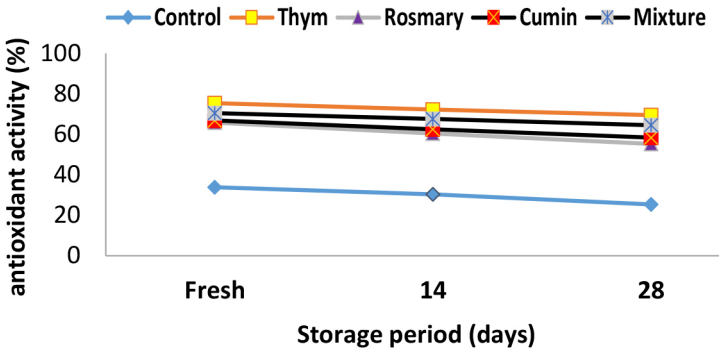

Fig. 3: Antioxidant activity of UF-soft cheese containing different essential oils during refrigerated storage

Antioxidant properties in UF-soft cheese

Changes in antioxidant activity during refrigerated storage of UF-cheese samples containing essential oils are shown in Fig. (3). In general, the inclusion of essential oils increased the antioxidant activity in the resultant UF-soft cheese compared with the control cheese. The antioxidant activities, relative to BHT, of the fresh cheese were 33.85, 66.83, 65.73, 77.34 and 70.42%, for the control cheese and cheese samples containing cumin, rosemary, thyme and the mixture essential oils respectively. Throughout refrigerated storage, all cheese samples showed the same trend in which the antioxidant activity decreased gradually as storage period progressed. After 28 days of storage, the antioxidant activity dropped to 25.34, 58.34, 55.22, 69.24 and 64.45%, in the control cheese and cheese samples containing cumin, rosemary, thyme and the mixture essential oils respectively. In general, UF- soft cheese containing these essential oils had higher antioxidant activity than the control cheese in the fresh state and during storage periods. Olmedo *et al.* (2013) found that rosemary essential oil demonstrated a protective effect against lipid oxidation and fermentation in flavoured cheese prepared with cream cheese base.

Microbiological analysis

The total viable count (TVC) of all cheeses decreased in the presence of essential oils compared with the control cheese (Table 3) which may be attributed to the antimicrobial effect of essential oils. Similar finding was reported by Hussein (2004). On the other hand, all cheese samples showed the same trend during refrigerated storage in which TVC increased gradually as storage period progressed. After 28 days storage, the TVC reached 8.42 log cfu/g of the control cheese and varied between 3.26 to 5.28 log cfu/g for cheese samples containing essential oils.

Yeasts and moulds were not detected in cheese samples containing essential oils throughout storage period (Table 3). This may indicate the effectiveness of added es-

Table 3: Microbiological analysis of UF-soft cheese containing essential oils during refrigerated storage

Treatments	(Storage period (days				
	Fresh	7	14	21	28
Total bacterial count (log cfu /g)					
Control	6.27	6.88	7.22	8.10	8.42
Cumin	3.10	3.56	3.90	4.33	5.0
Rosemary	3.23	3.88	4.44	4.72	5.28
Thyme	2.0	2.30	2.65	2.88	3.26
mixture	2.78	2.90	3.10	3.40	3.90
Yeasts & moulds counts (log cfu /g)					
Control	ND	ND	2	3	5
Cumin	ND	ND	ND	ND	ND
Rosemary	ND	ND	ND	ND	ND
Thyme	ND	ND	ND	ND	ND
mixture	ND	ND	ND	ND	ND

cfu = colony forming unit ND= Not Detected.

sential oils at inhibiting growth of yeasts and moulds. On the other hand, yeasts and moulds were detected in the control cheese samples after 14 days of storage. The results obtained in the present study are in agreement with those reported by Mutlag & Hassan (2008), who found that essential oils, particularly from thyme, had potential antifungal activity.

Coliform group was not detected in any of the experimental cheese samples. This may also confirm the antimicrobial activity of essential oils tested in the present study. Among constituents, phenolic compounds were considered to be the effective antimicrobial substances present in the essential oil extract (Burt, 2004).

The rheological properties of UF soft cheese

The changes in textural attributes during refrigerated storage of UF-soft cheese containing essential oils are shown in Fig. (4).

Cheese samples containing essential oils showed lower values for hardness, cohesiveness,

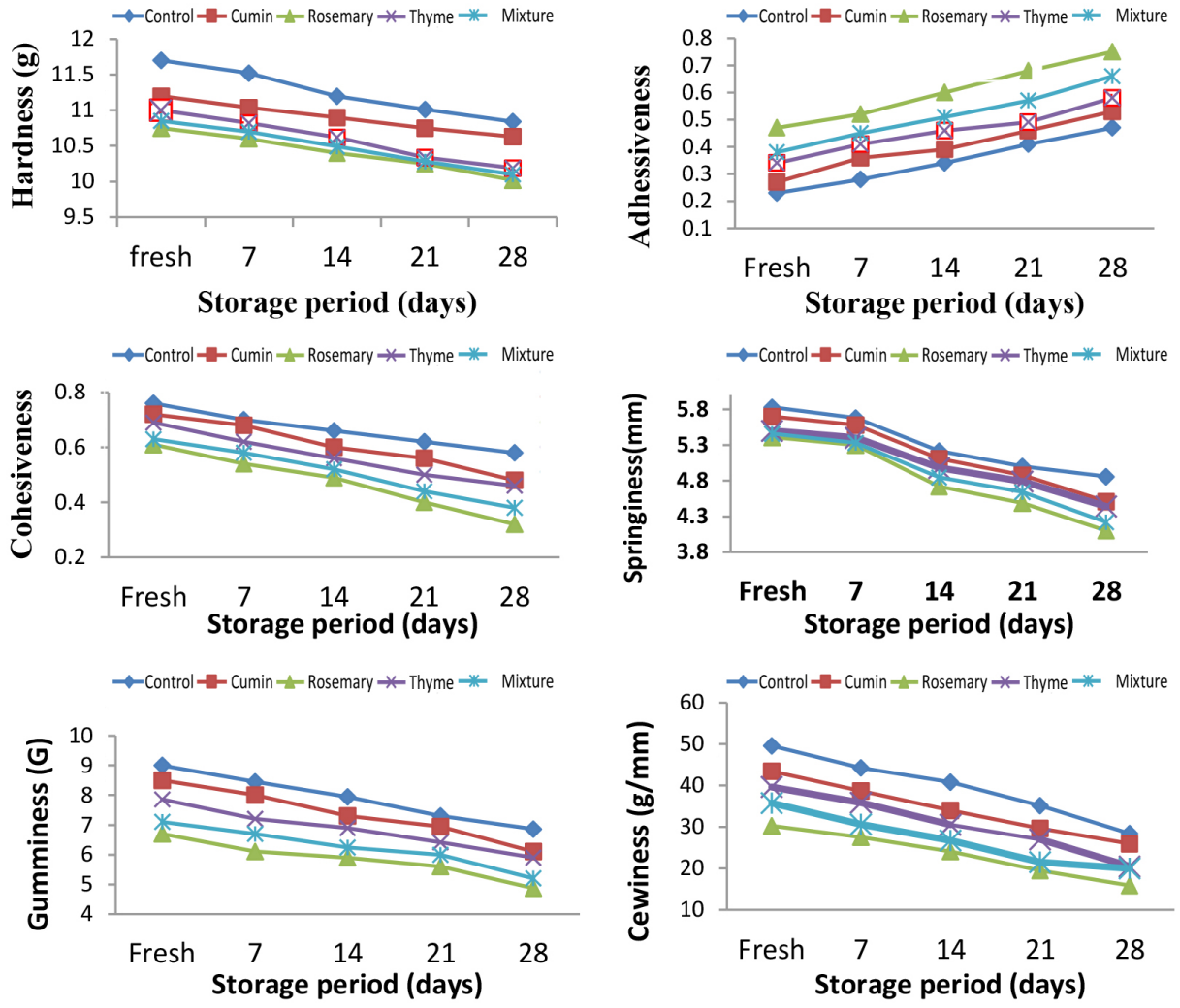

Fig. 4: Changes of rheological parameters of UF-soft cheese containing essential oils during refrigerated storage

springiness, gumminess and chewiness but higher adhesiveness values compared with the control cheese. Among cheese samples containing essential oils, cheese containing cumin essential oils had the highest values for hardness, cohesiveness, springiness and chewiness. While, cheese sample containing rosemary essential oil had the lowest values for the same textural attributes. The difference in textural behaviour among cheese sample may be attributed to many factors including rate of protein degradation, moisture content, fat distribution and pH (Cavalier *et al*., 1991). The early hydrolysis of $\acute{\alpha}_{s1}$-casein at the Phe_{23}- Phe_{24} peptide bond by residual chymosin would result in a marked weakening of para-casein matrix and decrease in fracture stress and hardness (Romeih, 2006). Also, Olson & Johnson (1990) indicated that relative amounts of water, protein, and fat were the dominant factors affecting cheese texture.

During storage, all cheese samples showed the same trend of results for hardness, cohesiveness, springiness, gumminess, and chewiness decreased while adhesiveness increased. This might be due to progress in proteolysis and casein breakdown (Akhgar *et al*., 2016). Proteolysis has been proved to reduce the structural integrity of the protein matrix and cheese become less hard and cohesive (Romeih *et al*., 2002).

Sensory evaluation

The results of maximum acceptable concentration of essential oils in UF- soft cheese samples indicated that the maximum acceptable concentrations of different essential oils were 0.1%, which used in the manufacture of UF-soft cheese samples.

The results in Table (4) indicated that all the scores for the different sensory attributes increased with increasing the storage period of UF-soft cheese

Table 4: Sensory properties of UF-soft cheese containing different essential oils during refrigerated storage

Treatments	Storage period (days)					mean
	Fresh	7	14	21	28	
			Appearance & Colour			
Control	8.50±0.41	8.4±0.32	8.4±0.32	8.2±0.26	6.2±0.78	7.94 ^{B}
Cumin	8.60±0.39	8.60±0.39	8.60±0.45	8.50±0.47	8.45±0.44	8.55 ^{A}
Rosemary	8.60±0.39	8.60±0.39	8.50±0.47	8.45±0.43	8.45±0.37	8.52 ^{A}
Thyme	8.60±0.39	8.60±0.39	8.50±0.47	8.50±0.47	8.45±0.37	8.53 ^{A}
mixture	8.60±0.32	8.55±0.37	8.50±0.47	8.5±0.47	8.5±0.41	8.53 ^{A}
Mean	8.58 ^{a}	8.55 ^{a}	8.50 ^{a}	8.43 ^{a}	8.01 ^{b}	LSD=0.17
			Body & Texture			
Control	8.40±0.46	8.00±0.62	7.10±0.74	6.95±0.98	6.8±0.75	7.45 ^{B}
Cumin	8.50±0.47	8.55±0.37	8.55±0.37	8.45±0.28	8.45±0.36	8.50 ^{A}
Rosemary	8.50±0.40	8.55±0.37	8.50±0.41	8.45±0.37	8.45±0.44	8.49 ^{A}
Thyme	8.50±0.47	8.55±0.37	8.55±0.37	8.40±0.32	8.30±0.35	8.46 ^{A}
mixture	8.50±0.41	8.55±0.41	8.55±0.37	8.45±0.37	8.45±0.37	8.50 ^{A}
Mean	8.48 ^{a}	8.44 ^{a}	8.25 ^{b}	8.14 ^{b}	8.09 ^{b}	LSD=0.19
			Odour			
Control	7.70±0.82	7.50±0.71	7.40±0.52	6.90±0.52	5.9±0.74	7.08 ^{C}
Cumin	8.70±0.35	8.60±0.40	8.60±0.39	8.40±0.39	8.25±0.26	8.51 ^{A}
Rosemary	8.15±0.71	8.10±0.66	8.05±0.80	8.05±0.44	8.00±0.62	8.07 ^{B}
Thyme	8.05±0.64	8.05±0.64	8.00±0.67	8.00±0.53	8.95±0.55	8.01 ^{B}
mixture	8.40±0.46	8.35±0.41	8.33±0.33	8.30±0.35	8.25±0.26	8.33 ^{A}
Mean	8.20 ^{a}	8.12 ^{ab}	8.08 ^{ab}	7.93 ^{b}	7.67 ^{c}	LSD=0.22
			Taste			
Control	8.00±0.58	8.20±0.26	7.9±0.39	6.9±0.70	4.2±1.03	7.04 ^{D}
Cumin	8.60±0.40	8.60±0.31	8.70±0.42	8.60±0.40	8.30±0.35	8.56 ^{A}
Rosemary	8.30±0.35	8.3±0.59	8.20±0.75	8.10±0.66	8.10±0.66	8.20 ^{B}
Thyme	8.00±0.58	8.00±1.0	7.90±0.97	7.85±1.13	7.8±0.75	7.91 ^{C}
mixture	8.40±0.39	8.50±0.32	8.40±0.39	8.40±0.39	8.3±0.35	8.38 ^{AB}
Mean	8.26 ^{a}	8.30 ^{a}	8.22 ^{a}	7.97 ^{b}	7.34 ^{c}	LSD=0.24
			Overall Acceptability			
Control	8.15±0.23	8.03±0.30	7.70±0.35	7.24±0.37	5.77±0.44	7.37 ^{C}
Cumin	8.60±0.22	8.60±0.14	8.6±0.19	8.48±0.24	8.34±0.20	8.52 ^{A}
Rosemary	8.36±0.25	8.36±0.31	8.3±0.41	8.20±0.25	8.16±0.24	8.32 ^{B}
Thyme	8.29±0.19	8.30±0.80	8.24±0.26	8.20±0.39	8.16±0.21	8.24 ^{B}
mixture	8.48±0.18	8.46±0.19	8.44±0.26	8.41±0.24	8.37±0.20	8.43 ^{A}
Mean	8.38 ^{a}	8.35 ^{ab}	8.26 ^{b}	8.11 ^{c}	7.77 ^{d}	LSD=0.11

Means with different superscripts in a raw or column are significantly different at $P \leq 0.05$ level.

samples either untreated or treated with the different essential oils. On the other hand, all the scores of the treated cheese samples were higher than that of the control ones. Also it can be noted that there were slight significant differences between all the scores for the different sensory attributes for the treated UF-soft cheese sample comparing with the control cheese.

In general, the highest scores obtained were for the UF-soft cheese sample containing either the cumin essential oil or the mixture between the three studied essential oils. Also it can be concluded that the untreated and treated UF-soft cheese samples with the different essential oils were still accepted by the panellists even at the end of storage period.

CONCLUSION

The results reported in the present study showed that the inclusion of essential oils into UF-soft cheese would be helpful in extending its shelf life. In addition, essential oils appeared to improve sensory attributes of UF cheeses. The present study concluded that essential oils extracted from thyme followed by herbal mixture, cumin and rosemary could be in use as potential resource of natural antioxidants and antimicrobial compounds for food and dairy industry, so that it is interesting to examine its application as natural antioxidant and antimicrobial additive in some final food products.

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تأثير بعض الزيوت العطرية على جودة الجبن الطرى المعامل بالترشيح الفوقى أثناء التخزين

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أجريت هذه الدراسة بهدف تقييم التركيب الكيميائي، والنشاط المضاد للأكسدة، الخصائص المضادة للميكروبات وأقل تركيز مثبط من ثلاثة أنواع من الزيوت العطرية المستخلصة من الكمون والروزماري والزعتر وخليط من هذه الأنواع الثلاثة بنسبة ١: ١: ١ حجم /حجم وتأثيرها على الخصائص الفيزيائية والكيميائية والميكروبية والريولوجية و الحسية للجبن الطري المعامل بالترشيح الفوقي. تم إعداد الجبن الطري المعامل بالترشيح الفوقي من لبن معامل بالترشيح الفوقي ومضاف إليه ٠,١٪ من هذه الزيوت العطرية. حيث تم تخزين الجبن الطرى المعامل بالترشيح الفوقي بالتبريد لمدة ٢٨ يوماً وذلك لتقييم إمكانية تطبيق استخدام الزيوت العطرية المستخلصة لزيادة العمر التخزيني لهذا الجبن. وكشفت النتائج أن الزيوت العطرية المختلفة لها تأثير مضاد ملحوظ للميكروبات على نمو كل من *Escherichia coli* ، *Salmonella typhimurium* ، *Staphylococcus aureus* ، *Bacillus subtilis* ، *Bacillus cereus* ، *Aspergillus niger*. واتضح من النتائج أن زيت الزعتر العطري سجل أعلى نشاط مضاد للأكسدة وللميكروبات. وكذلك أتضح أن إضافة الزيوت العطرية له تأثير على درجة حموضة الجبن و المحتوى الكلي للأحماض الدهنية الطيارة خلال فترة التخزين، بينما كان تأثيره على المواد الصلبة الكلية والمحتوى الدهنى محدوداً. أدى إضافة الزيوت العطرية إلى retentate إلى زيادة النشاط المضاد للأكسدة وانخفض العدد الكلي للبكتريا مقارنة مع الجبن الكونترول. وأظهرت الخواص العضوية الحسية أن الجبن الطري المعامل بالترشيح الفوقي المحتوى على الزيوت العطرية كان الأكثر قبولاً من حيث اللون والقوام والمظهر والرائحة والطعم والتقبل العام خلال فترات التخزين بالتبريد وذلك مقارنة بالجبن غير المعامل. كما أظهرت الدراسة أن الجبن المضاف له زيت الكمون العطري كان أكثر تقبلاً. وخلاصة نتائج هذه الدراسة هي إمكانية استخدام تركيز ٠,١٪ من الزيوت العطرية المستخلصة من الكمون والروزماري و الزعتر أو خليط منهما لإطالة العمر التخزيني للجبن الطري المعامل بالترشيح الفوقي لمدة تصل إلى ٢٨ يوماً. وأن هذه الزيوت العطرية يمكن أن تستخدم بنجاح كإضافات طبيعية مضادة للميكروبات الممرضة في إنتاج الجبن الطري المعامل بالترشيح الفوقي.