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INHIBITORY POTENTIAL OF *SALVIA SCLAREA* AND *OCIMUM BASILICUM* AGAINST CHEMICAL AND MICROBIAL SPOILAGE IN CHEESE

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ABSTRACT

The inhibitory activities of salvia and basil essential oils (EOs) against chemical and microbial spoilage in Iranian white cheese were investigated. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration obtained for salvia against *Listeria monocytogenes* were 0.015 and 0.02%, and for basil were 0.05 and 0.06%, respectively. The activities of EOs against *Aspergillus flavus* were assayed yielding MIC and minimum fungicidal concentration for salvia of 0.5 and 0.65%, and for basil of 0.6 and 0.8%, respectively. At 1%, salvia inhibited fungal growth in the cheese throughout the storage period and reduced bacterial growth of up to 6 log/colony-forming unit/g. By comparison, basil showed weaker antimicrobial effects, as well as lower peroxide and thiobarbituric acid reactive substance values at both 4 and 26C in cheese. As regard to oxidative stability, the activities at different concentrations of EOs were as follows: 1% basil, >0.75% basil, >0.5% basil, >1% salvia, >0.75% salvia and >0.5% salvia.

PRACTICAL APPLICATIONS

White cheese is normally considered to be a stable product with a reasonable shelf life. However, during storage for extended periods at ambient temperature, shelf life may be shortened considerably due to lipid oxidation or microbial spoilage. Chemical preservatives are used in white cheese to inhibit chemical deterioration and growth of microorganisms. Because of the risk of harmful effects of synthetic preservatives on consumer health, both the food industry and the authorities are paying increasing attention to medicinal and aromatic plants as sources of natural preservatives for use in food products.

INTRODUCTION

Fungi are significant spoilage microorganisms of foodstuffs during storage, rendering them unfit for human consumption by reducing their nutritive value and sometimes by producing mycotoxins (Kumar *et al.* 2007). Mold growth on cheese is a common problem for cheese manufacturers during ripening and curing, as well as for the retailer and consumer during refrigerated storage. Species of *Penicillium* and *Aspergillus* are common contaminants of cheese (Bullerman and Olivigni 1974).

Listeria monocytogenes has gained increasing attention as a pathogen of public health importance owing to large numbers of foodborne outbreaks of listeriosis. Ingested by mouth, *Listeria* is among the most virulent of foodborne pathogens with up to 20% of clinical infections resulting to death (Bennion *et al.* 2008). Various types of foods, mostly dairy products such as cheese, have been associated with these outbreaks (Farber and Peterkin 1991), and there is considerable interest in stopping this upward trend. In order to retard or prevent oxidative deterioration and extend the shelf life of many food products, the addition of

antioxidants is necessary. Few studies, however, have been conducted regarding the prevention of lipid oxidation in cheese using plant extracts.

As a result of the negative consumer perception of chemical preservatives, attention is shifting toward natural alternatives. A technique that has been used since ancient times to prevent fungal growth on foods such as cheese involves physically rubbing the product with certain herbs or spices or their oils (Vazquez *et al.* 2001). This is now understood to be due to the antimicrobial properties. Essential oils (EOs) are naturally occurring terpenic mixtures isolated from various parts of plants by steam distillation or other methods. As these compounds are "eco-friendly" and harmless to humans, they are attracting the attention of both industry and academic researchers to medicinal and aromatic plants and the antimicrobial properties they and their oils display against food spoilage (Paranagama *et al.* 2003; Velluti *et al.* 2003; Singh *et al.* 2006).

Ocimum basilicum and *Salvia sclarea* are plants belonging to the Lamiaceae family, collectively called basil and salvia, respectively. They are widely cultivated and extensively used in food, perfumery, cosmetics, pesticides, medicine and traditional rituals because of their natural aromas and flavors and other properties (Zheljazkov *et al.* 2008; Atas *et al.* 2011). Basil and salvia have been shown to have antioxidant properties as well as antimicrobial activities against bacteria and fungi (Chaumont *et al.* 2001; Tepe *et al.* 2005; Bozin *et al.* 2007; Kelen and Tepe 2008).

In this study, we have evaluated the effects of *Salv. sclarea* and *O. basilicum* EOs on the growth of both *L. monocytogenes* PTCC 1298 and *Aspergillus flavus* ATCC 15546, spore production by the latter microorganism and also oxidative stability in Iranian white cheese.

MATERIALS AND METHODS

Eos

Commercially available EOs from basil and salvia were used in this study (Pranarôm International, Ghislenghen, Belgium). EOs were analyzed by gas chromatography mass spectrometry (GC-MS) at the Department of Chemistry, University of Mazandaran, Iran. Both EOs were diluted in 50% ethanol according to the manufacturer's instructions and stored at 4C before use.

Antimicrobial Activity

Bacterial Suspension. *L. monocytogenes* PTCC 1298 was obtained from the Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. The lyophilized culture was grown twice in tubes containing 10 mL of BHI (Brain-heart infusion) broth (Merck KGaA, Darmstadt, Germany) at 35C for 18 h followed by streaking on BHI agar (Merck KGaA) slants and incubation under the same conditions. Cultures were stored at 4C and subcultured monthly. *Listeria* broth culture was adjusted to an optical density (absorbance) of 0.01 at 600 nm in a 13×100 mm sterile cuvette using a spectrophotometer (BSA 3000 Chemistry Analyzer, SFRI, Saint Jean d'Illac, France). This adjustment yielded a cell concentration of 5×10^7 colony-forming unit (cfu)/mL. The number of cells in suspension was estimated by duplicate plating from 10-fold serial dilutions on BHI agar and colony counting after 24 h of incubation at 35C (Basti *et al.* 2007).

Determination of Minimum Inhibitory and Minimum Bactericidal Concentrations. A broth microdilution assay (NCCLS 2000) was employed to determine the lowest concentration (minimum inhibitory concentration, MIC) in which visible growth of the bacterium is inhibited. The assay was carried out with BHI broth culture medium. To obtain and maintain the oil/water emulsion in broth substrate during the experiment, the method of Mann and Markham (1998) was used with some modifications. Dilutions (0.005–1%) of EOs were set up using a 96-well microtiter plate. The final bacterial inoculation in each microwell was 10^5/cfu/mL. The plate was then incubated at 35C for 24 h. Bacterial growth was determined by measuring absorbance at 600 nm. In order to determine the minimum bactericidal concentration (MBC; the lowest concentration that reduces the bacterial population 99.9% after incubation at 35–37C for 24 h), 100 μL of those microtiter wells with no visible growth in the MIC determination assay was spread on BHI agar and incubated at 35C for 24 h. The concentration of EO present in those wells that yielded plates with no visible colonies was considered to be the MBC (Azizkhani *et al.* 2013).

Preparation of Conidial Suspension. The strain used in this study was *A. flavus* ATCC 15546 obtained from the Department of Mycology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. The strain was cultured on potato dextrose agar (PDA; Merck KGaA) slopes for 10–14 days at 26 ± 1C. Conidia were harvested by adding 10 mL of 0.05% Tween 80 solution (Merck KGaA) to the culture and gently scraping the mycelia with a sterile inoculating loop to free the spores. The conidial concentration was determined using a Neubauer chamber (Merck S.A., Madrid, Spain), and the suspension was diluted with 0.05% Tween 80 to give a final concentration of 10^6/conidia/mL (Nguefack *et al.* 2004; Gandomi *et al.* 2009).

Determination of Minimum Inhibitory and Minimum Fungicidal Concentrations. The effect of EOs on radial growth of mycelium was assayed using an agar

dilution method. EO concentrations across the range of 0.05–1% were prepared by adding appropriate volumes of EOs to sterilized molten (40–45C) PDA medium just prior to pouring into sterilize Petri plates. A 5 mm in diameter Whatman no. 1 filter paper disc (Merck KGaA, Darmstadt, Germany) was placed at the center of each plate and inoculated with 10 μL of spore suspension (10^4 spores). Plates were incubated for 10 days at 26 ± 1C in darkness, and the average of two perpendicular diameters of the colony was calculated daily. After the incubation period, discs showing no growth were transferred to PDA plates without EO and incubated as previously described to determine the fungistatic or fungicidal effects. The lowest concentration that inhibited the growth of the fungus (the fungus revived on untreated PDA) was considered to be the MIC, whereas the lowest concentration of EO that killed the fungus (no growth observed on fresh medium) was taken to be minimum fungicidal concentration (MFC). All treatments were carried out in triplicate. The antifungal effect is expressed as the percent inhibition of radial growth by the following formula (Gandomi *et al.* 2009):

$$MIC = [(\mathrm{Dc} - \mathrm{Ds})/\mathrm{Dc}] \times 100$$

where Dc is the diameter of the colony in the control sample and Ds is the diameter of the colony in the treated sample.

Evaluation of Conidial Production. PDA plates containing different concentrations of EOs were inoculated with 0.1 mL of spore suspension (10^5 spores) that was spread evenly on the agar surface and incubated for 7 days at 26 ± 1C. The fungal spores produced in each dish were collected and transferred to an Erlenmeyer flask in 50 mL of 0.05% Tween 80 solution and shaken strongly. The conidial concentration was estimated using a Neubauer, and the number of spores per cm^2 of plate was duly calculated (Tzortzakis and Economakis 2007). The percent inhibition of spore production was computed by the following equation (Gandomi *et al.* 2009):

$$[(\mathrm{Nc} - \mathrm{Ns})/\mathrm{Nc}] \times 100$$

where Nc is the number of spores in the control sample and Ns is the number of spores in the treated sample.

Sample Preparation and Evaluation of Fungal and Bacterial Growth in Cheese. Iranian ultrafiltered white cheese was produced in a commercial factory with different concentrations of EOs (0.5, 0.75 and 1%). EO-treated samples containing *L. monocytogenes* (10^4/cfu/g) were incubated at 4C for 21 days and examined for *L. monocytogenes* counts from the beginning of the experiment (day 0) and subsequently on days 1, 2, 3 and then every 48 h until day 21. The counts were taken on polymyxin-acriflavin-LiCl-ceftazidime-aesculin-mannitol agar plates by surface plating appropriate dilutions of the samples aseptically in triplicate.

Other cheese samples containing EOs alone were aseptically cut into pieces (ca. 20 g) and trimmed to fit into 10 cm in diameter plates. The cheese slices were placed in the center of the plates, exposed to germicidal UV light for 30 min and then surface inoculated with 10 μL of spore suspension (10^4 spores). Plates were partially sealed with adhesive tape and incubated at 26C for 21 days. The means of two perpendicular diameters of the fungal colony were calculated every 24 h (Vazquez *et al.* 2001).

Antioxidant Activity

Peroxide Values. The extraction of lipid was based on the method described by Mortensen *et al.* (2002). About 10 g of each cheese sample was transferred to a 500 mL plastic centrifuge tube and 200 mL of chloroform : methanol (7:3) was added. The sample was homogenized using a homogenizer (Heidolph, Diax 600, Kelheim, Germany), 50 mL of 1 mM $CaCl_2$ was added to the suspension that was shaken for 10 s. The mixture was then centrifuged at 1,400 g at 20C for 30 min and the supernatant was transferred to a 500 mL separation funnel to collect the chloroform (lower) layer. The upper layer and the solids were combined and mixed with 150 mL of chloroform. The mixture was homogenized and then centrifuged as before. This procedure was repeated once more. The three separated chloroform layers were combined and poured into a 1,000 mL conical flask, and the chloroform was evaporated at 60C using a Buchi vacuum Rotavapor R-215 (Büchi Labortechnik AG, Flawil, Switzerland). The remaining oil was then flushed with nitrogen and frozen for peroxide value (PV) analysis. Lipid samples (0.02 g) were weighed into a 25 mL volumetric flask and 15 mL of chloroform methanol (7:3) was added. To each sample were added 0.2 mL of 1% ferrous chloride and 0.2 mL of 4 M ammonium thiocyanate, and the final volume was made up to 25 mL using chloroform : methanol (7:3). Samples were mixed and kept under dimmed light for 5 min for absorbance determination at 505 nm. The result was expressed as milliequivalents of oxygen per kilogram of lipid. A sample blank and a reagent blank were also measured. All the measurements were carried out in triplicate and were carried out under dimmed light. A standard curve was determined under the same conditions using ammonium ferric sulfate (AR grade) as the standard.

Thiobarbituric Acid Reactive Substances. Thiobarbituric acid reactive substances (TBARS) were measured using a method described by Kristensen and Skibsted (1999). The thiobarbituric acid (TBA) reagent was prepared

immediately before use by mixing equal volumes of freshly prepared 0.025 M TBA (brought into solution by neutralizing with NaOH) and 2 M H_3PO_4/2 M citric acid. The combination of citric acid and phosphoric acid was used as both acidulant and metal chelator. To 6 g of accurately weighed cheese was added 18 mL of TBA reagent, and the resulting mixture was homogenized using a homogenizer (Heidolph, Diax 600) for 2 min until the mixture was homogeneous. An aliquot (6 mL) of the suspension was transferred to a Pyrex tube to which 3.5 mL of chloroform was added, followed by gentle mixing for 5 min. The mixture was centrifuged at 6,000× g for 15 min at room temperature. The aqueous layer was transferred to another test tube that was placed in a water bath at 100C for 10 min, followed by cooling on ice. The orange-red cyclohexanone supernatant was decanted and its absorbance was measured spectrophotometrically at 532 nm (BSA 3000 Chemistry Analyzer, SFRI, Saint Jean d'Illac, France). The results were expressed as absorbance units at 532 nm/g of cheese.

Sensory Analyses

Eight trained panelists performed sensory analyses. Sensory evaluation was carried out according to sensory assessment scheme of Paulus *et al.* (1979). The panelists scored for color, odor, flavor, overall acceptability and texture using a 9-point hedonic scale (1, dislike extremely, to 9, like extremely).

Statistical Analysis

All data were reported as the mean ± standard deviation of independent replicates. Data were subject to analysis of variance (ANOVA; $P < 0.05$) and the means were separated by Duncan's multiple range test (ANOVA procedure). Pearson's correlation coefficients were calculated. Results were processed by computer programs Excel (Microsoft, Washington, USA) and STATISTICA version 5.1.3 (StatSoft, Tulsa, OK, USA).

RESULTS

GC-MS analysis resulted in the identification of 34 components for *Salv. sclarea* EO and 23 components for that of *O. basilicum*, representing more than 90% v/v of each oil's content. The main components of *Salv. sclarea* EO include linalyl acetate (52.8%), linalool (18.1%), α-terpineol (5.0%) and α-pinene (4.5%), whereas those of *O. basilicum* EO were linalool (58.6%), α-cadinol (10.0%), α-bergamotene (7.6%) and γ-cadinene (4.9%). The MICs for the EOs of *Salv. sclarea* and *O. basilicum* against *L. monocytogenes* were 0.015 and 0.05%, and their MBCs against this bacterium were 0.02 and 0.06%, respectively.

The effects of different concentrations of salvia and basil EOs on *A. flavus* radial growth and spore production were studied by inoculating filter paper discs with fungal spores and placing them at the center of agar medium plates containing the EO. The results obtained are presented in Table 1. Both EOs showed significant inhibition of both fungal growth and spore production ($P < 0.05$) at all the concentrations tested. Growth of the fungus was completely prevented at concentrations over 0.5% for salvia and 0.6% for basil. To differentiate between fungistatic and fungicidal (MFC) effects, those discs showing no growth on EO plates were transferred to plates without EO for further incubation. Discs taken from plates containing salvia EO at 0.65% and basil EO at 0.8% failed to show evidence of growth after transfer to non-EO plates, thus indicating the fungicidal activity of these oil concentrations. At salvia EO concentrations of 0.3, 0.35, 0.4 and 0.45% radial growth was reduced by 26.2, 51.9, 77.3 and 94.8%, respectively; spore production was 65.7% inhibited at 0.3% EO and completely inhibited at the higher concentrations. These observations indicate dose-dependent effects. At basil EO concentrations of 0.45, 0.50 and 0.55% radial growth decreased by 10.2, 32.7 and 67.4%, respectively, and spore production was totally inhibited at 0.5% EO (Table 1).

L. monocytogenes colony counts in cheese over the course of the 21 day storage period are represented graphically in Fig. 1. Cheese samples treated with salvia EO showed lower *L. monocytogenes* counts than those containing basil EO. The addition of salvia EO at concentrations of 0.5 and 0.75% caused significant reduction in the number of *Listeria* cfu (Fig. 1A); the treated samples showing decreases of nearly 1.5 and 2.5/log/cfu/g, respectively, compared with the control samples after 7 days of storage at 4C. Nevertheless, 3 days after inoculation the growth rate of *Listeria* in the treated samples was comparable with that of the control. The addition of 1% salvia EO reduced the initial *Listeria* population from 4.92 to 1.66 log cfu g^1 after 21 days of storage at 4C, while the corresponding population in the control cheese rose from 4.82 to 8.96/log/cfu/g. Treatment with 1% salvia EO revealed a significant effect against *Listeria* after the fifth day of storage ($P < 0.05$).

Significant differences were also observed in the activity against *Listeria* between the three concentrations of basil EO and the control (Fig. 1B), but the maximum difference was recorded after 7 days ($P < 0.05$). Increasing the concentration of basil EO to 1% enhanced its inhibitory effect against *L. monocytogenes*. On the last day of storage the difference between samples treated with 1% basil EO and the control was more than 3 log cfu g^1 ($P < 0.05$). The mean counts of *L. monocytogenes* in cheese samples treated with salvia and basil EOs were 1.66 and 5.91/log/cfu/g, respectively, after 21 days. A difference of 4.25/log/cfu/g between

TABLE 1. EFFECTS OF *SALVIA SCLAREA* AND *OCIMUM BASILICUM* EOs ON RADIAL GROWTH AND SPORE PRODUCTION BY *ASPERGILLUS FLAVUS* ON PDA

	Growth		Spore production	
EO concentration (%)	Colony diameter (mm)	Inhibition (%)	Spore count ($\times 10^7$ spores/cm^2)	Inhibition (%)
Salv. sclarea				
0	102 ± 1.5	0*	1.9 ± 0.2	0*
0.3	75.2 ± 1.9	26.2a	0.65 ± 0.03	65.7a
0.35	49 ± 0.8	51.9b	0	100b
0.40	23.1 ± 2.6	77.3c	0	100b
0.45	5.3 ± 0.7	94.8d	0	100b
0.50†	NG	100e	0	100b
0.55	NG	100e	0	100b
0.60	NG	100e	0	100b
0.65‡	NG	100e	0	100b
O. basilicum				
0	102 ± 1.5	0*	1.9 ± 0.2	0*
0.45	91.5 ± 2.2	10.2a	0.97 ± 0.05	48.9a
0.50	68.6 ± 1.3	32.7b	0	100b
0.55	33.2 ± 1.5	67.4c	0	100b
0.60†	NG	100d	0	100b
0.65	NG	100d	0	100b
0.70	NG	100d	0	100b
0.75	NG	100d	0	100b
0.80‡	NG	100d	0	100b

Figures in each column followed by different letters are significant at the 5% level.
* Means ± standard error of three separate experiments carried out in three replicates.
† Minimum inhibitory concentration.
‡ Minimum fungicidal concentration.
EOs, essential oils; NG, no growth; PDA, potato dextrose agar.

the two EOs showed that salvia EO had a notably stronger activity against *Listeria* ($P < 0.001$).

At all concentrations tested, the EOs had inhibitory effects against radial fungal growth on cheese (Fig. 2). In the samples treated with 0.5 and 0.75% basil EO, radial growth started from days 8 and 12, respectively, while no visual growth was recorded until day 19 at 1%. Although none of the concentrations of basil EO used completely inhibited the growth of *A. flavus* on cheese, at 1% EO the inhibition attained was 96.2% compared with the control ($P < 0.05$) at the end of the storage period (day 21). Greater reduction in colony diameter was generally observed in samples treated with salvia EO. At 0.5 and 0.75%, growth was inhibited until days 11 and 14. No fungal growth was observed in 1% salvia EO during the whole period of storage.

Lipid oxidation was determined by analysis of lipid peroxides (Fig. 3). The PVs of all samples treated with basil EO increased slightly during the first 21 days of storage at 26C (accelerated condition). These values were lower than those of samples treated with salvia EO at all storage times. Values for samples stored at 4C were also determined; there were no significant differences in the PV results between salvia- and basil-treated samples over 66 days of storage (data not shown).

Secondary lipid oxidation products were quantified using the TBARS method. The development of TBARS in the cheese samples stored at 26C is shown in Fig. 4. The TBARS of samples containing salvia EO increased noticeably during the first 9 days of storage at 26C and then increased gradually up to 39 days. A similar trend in TBARS was observed for samples treated with basil EO, but the values at each storage time were lower than those for salvia EO. No change in TBARS was observed in both samples stored at 4C for 39 days (results not shown).

Odor, flavor, texture and overall acceptability score of cheese samples were considerably affected by the EO treatment during the storage period ($P < 0.05$). The EO of basil at 0.75% and EO of salvia at 0.5% showed the highest acceptability score, compared with other concentrations and control, during storage period ($P < 0.05$). Totally, odor, flavor and overall acceptability scores of samples treated with basil EO were higher than ones treated with salvia and also control ($P < 0.05$).

DISCUSSION

Natural products and naturally derived compounds from plants may have applications in controlling pathogens and oxidative changes in food (Valero and Salmeron 2003), and in this regard plant EOs are potentially useful sources of antimicrobial compounds (Bagamboula *et al.* 2004). Indeed, the anti-*Listeria* activity of EOs in culture media

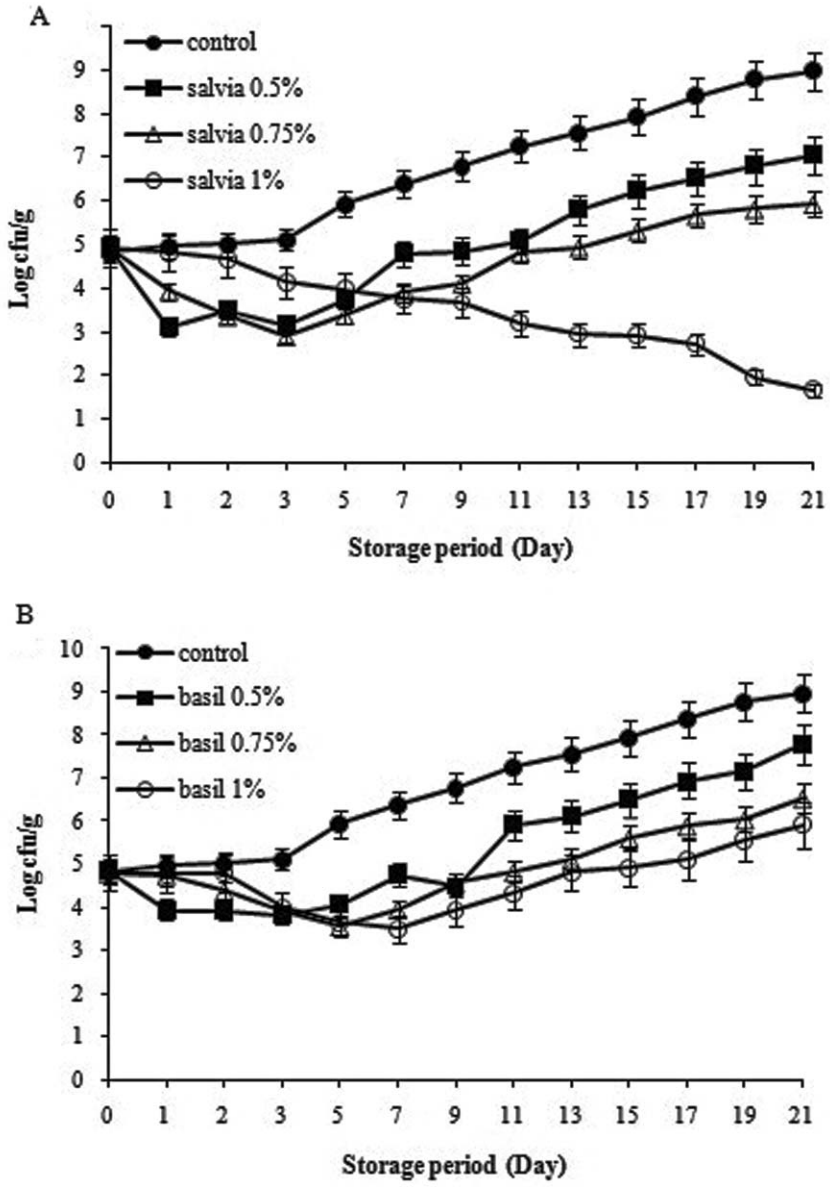

FIG. 1. EFFECTS OF (A) *SALVIA SCLAREA* AND (B) *OCIMUM BASILICUM* ESSENTIAL OILS ON THE GROWTH OF MONOCYTOGENES IN IRANIAN WHITE CHEESE

and food samples has been reported in several earlier studies (Sandasi *et al*. 2008; Awaisheh 2013; Abdollahzadeh *et al*. 2014). The present work reveals the existence of antimicrobial and antioxidant activities in salvia and basil EOs when assayed on both culture media and cheese as a food substrate.

The antimicrobial activities of salvia and basil have been documented more *in vitro* and less *in situ* against a number of food spoilage and pathogenic microorganisms, including (among others) *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Bacillus cereus*, *Proteus vulgaris*, *Escherichia coli*, *Vibrio* spp. and *Salmonella typhimurium* as well as several yeasts and molds, with MIC or MBC ranging between 0.0015 and 0.5% for salvia (Dzamic *et al*. 2008; Arora *et al*. 2013) and 0.003 and 3% for basil (Opalchenova and Obreshkova 2003; Viyoch *et al*. 2006). In the current report, *L. monocytogenes* was neither eliminated nor completely inhibited by basil EO, but salvia EO was able to inhibit its proliferation in cheese. The effect was even more pronounced with 1% salvia oil compared with 0.5 or 0.75%. Basil EO at a concentration of 1% caused a 7 day delay in the growth of *L. monocytogenes*. A growth delay of this type is particularly useful in terms of food safety for the short-term storage of products but not on prolonged storage as *Listeria* may reach high levels in foods over longer periods. In an earlier study Vrinda Menon and

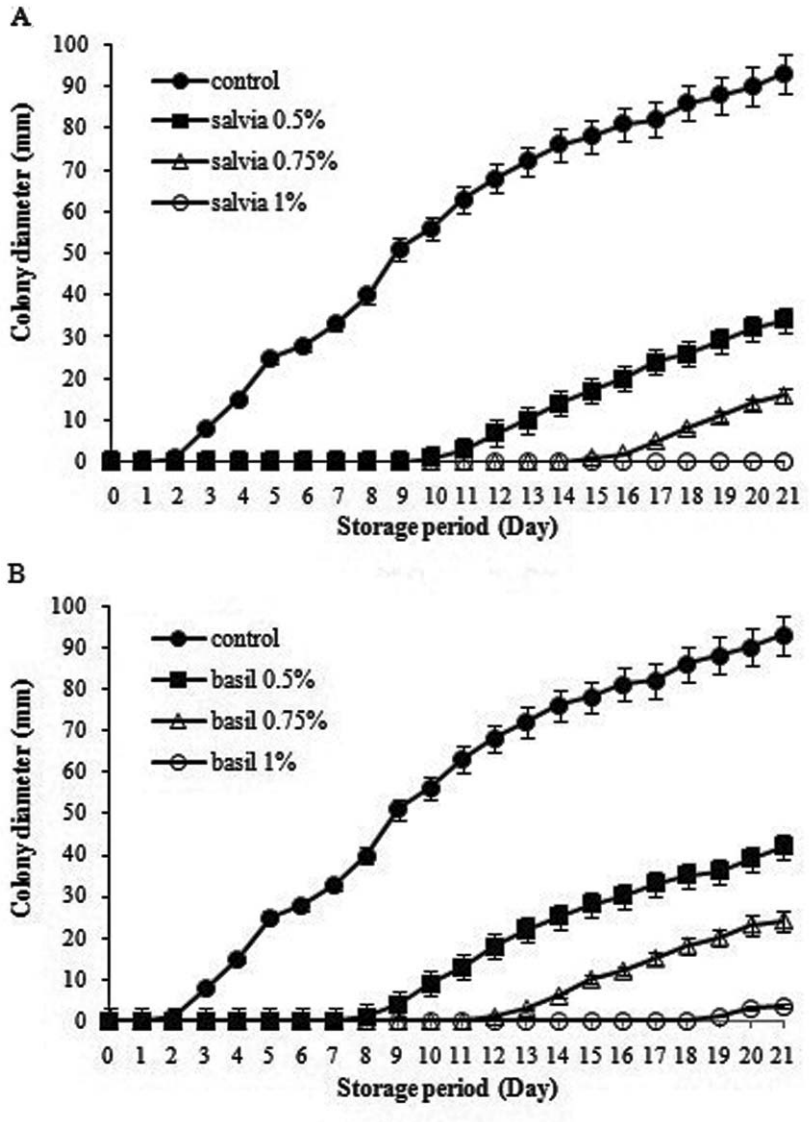

FIG. 2. EFFECTS OF (A) *SALVIA SCLAREA* AND (B) *OCIMUM BASILICUM* ESSENTIAL OILS ON THE GROWTH OF *ASPERGILLUS FLAVUS* IN IRANIAN WHITE CHEESE

Grag (2001) showed that 1% clove EO inhibited this microorganism in cheese (almost the same effect as salvia in the present study).

Molds are major determinants of spoilage in cheese (Cheong *et al.* 2014). In the present study, no growth of *A. flavus* was observed in the presence of 1% of salvia EO, and colony diameter attained less than 5 mm by the 21st day of cold storage in cheese samples treated with 1% basil EO. The inhibitory effects of EOs against sporulation indifferent fungi have been reported previously (Kuate *et al.* 2006; Pawar and Thaker 2006; Gandomi *et al.* 2009). Tantaoui-Elaraki *et al.* (1993) studied the effect of three Moroccan EOs on fungal asexual reproduction and reported that the partial inhibition of spore production could be attributed to mycelia destruction or the inhibition of fungal growth. Mahanta *et al.* (2007) suggested that the impact of *Cymbopogon citratus* L. EO on sporulation may reflect the effects of volatile compounds from the oil on the surface of developing mycelia, and thus the "platform" to support spore production involved in the switch from vegetative to reproductive development. In another study, growth, spore production and aflatoxin synthesis by *A. flavus* ATCC 15546 on Iranian ultrafiltered white cheese were all inhibited by *Zataria multiflora* Boiss. EO (Gandomi *et al.* 2009). In the control group as well as at EO concentrations of 0.005, 0.01 and 0.02% fungal growth started from day 1. However, no visual growth was recorded until day 2 at 0.04 and 0.06%, and until day 4 at 0.1% EO. None of those EO concentrations examined completely inhibited the growth of

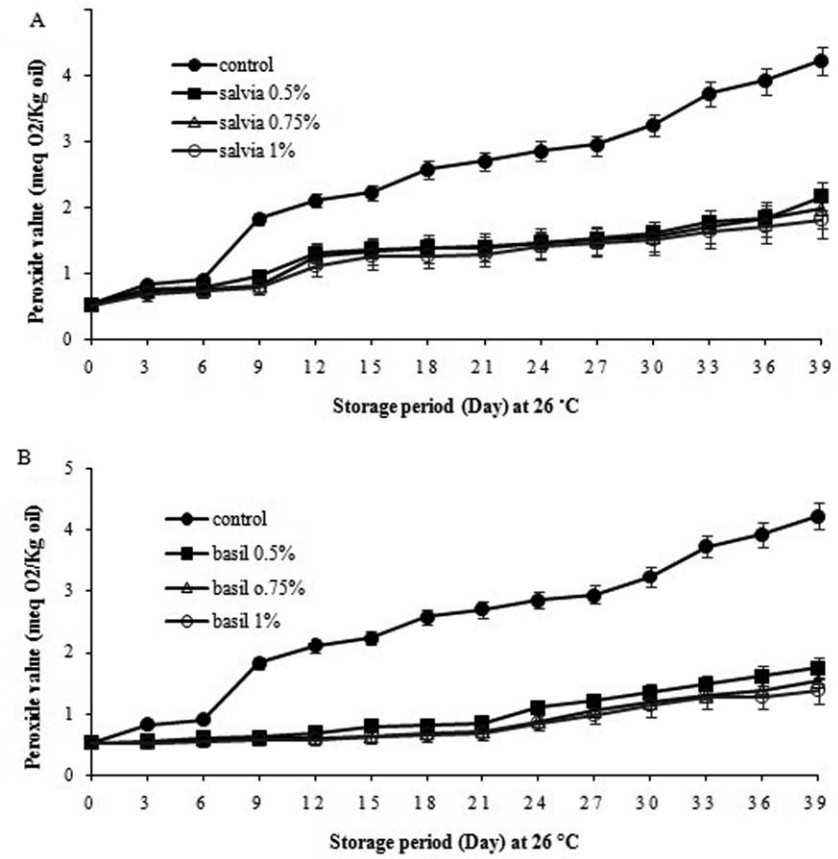

FIG. 3. EFFECTS OF (A) *SALVIA SCLAREA* AND (B) *OCIMUM BASILICUM* ESSENTIAL OILS ON THE PEROXIDE VALUES IN IRANIAN WHITE CHEESE AT 26C

A. flavus on cheese, as at 0.1% EO the inhibition was 75.4% compared with the control. However, decreased colony diameter was generally observed as the EO concentration rose. The results of the present study are in agreement with these findings.

Primary lipid oxidation products are peroxides but they provide little information about the nature of the oxidative processes during storage of cheese. Lipid peroxides are cleaved to yield secondary lipid oxidation products (Kristensen *et al.* 2000). Asensio *et al.* (2015) demonstrated that different oregano EOs added to organic cottage cheese significantly inhibited lipid oxidation over the course of 30 days under thermal storage (40C) and decreased the deterioration process of quality parameters thereby prolonging the shelf life of the product. Similar effects were observed for the addition of various concentrations of oregano and rosemary EOs to flavored cheese prepared with a cream cheese base (Olmedo *et al.* 2013). In a study by Zhang *et al.* (2013), sausages containing sage (*Salvia officinalis*) showed a significant delay in the increase in TBARS values and protein carbonyl formation during refrigerated storage. The sage used in that study had no negative effects on the sensory properties of the sausages. Estevez *et al.* (2006) evaluated the antioxidant effects of sage and rosemary and one synthetic antioxidant (BHT) on refrigerated stored porcine liver pâté (4C/90 days). Both EOs exhibited similar antioxidant properties to those of BHT, thus illustrating their suitability as alternatives to synthetic antioxidants. The main components of EOs in present study are terpene compounds. These compounds can act as direct antioxidants through free radical scavenging mechanisms and/or as indirect antioxidants by enhancing the antioxidant status (enzymatic and non-enzymatic) (Gonzalez-Burgos and Gomez-Serranillos 2012).

Evidently, there is a link between the antifungal action of terpene compounds and the abnormal cells, including plasma membrane and mitochondrial dysfunction. The flow cytometry data and the impairment in the biosynthesis of ergosterol in a study confirmed the EO-induced lesion of the plasma membrane structure as the antifungal target of oil. Afterward, the lesion of the plasma membrane may be associated with ion loss, reduction of membrane potential, collapse of the proton pump and consumption of the ATP pool (Tian *et al.* 2012).

The mechanisms by which EOs bring about their antibacterial effect is incompletely understood, but there are a

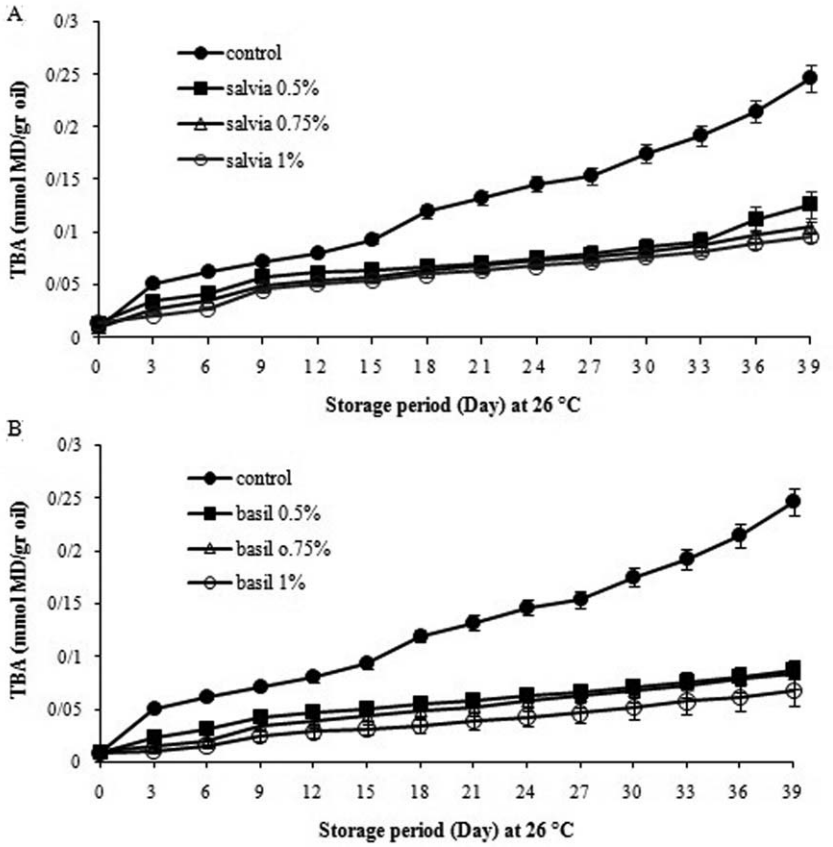

FIG. 4. EFFECTS OF (A) *SALVIA SCLAREA* AND (B) *OCIMUM BASILICUM* ESSENTIAL OILS ON THIOBARBITURIC ACID REACTIVE SUBSTANCES OF IRANIAN WHITE CHEESE AT 26C

number of proposed mechanisms (Holley and Patel 2005). Certainly, there are morphological changes that are apparent. The major thickening and disruption of the cell wall, together with increased roughness and lack of cytoplasm, has recently been reported in *L. monocytogenes* on treatment with thyme EOs (Rasooli *et al.* 2006). Terpenes, one of the most extensive and varied structural compounds occurring in nature, display a wide range of biological and pharmacological activities.

Sensory evaluation is a key factor in judging about foodstuff quality. Cheese samples with different concentrations of EOs were evaluated and compared with the control sample to ascertain consumer acceptability for it. Significant differences were detected among samples containing EOs and the control sample in odor, color and texture, but the samples containing 0.75 and 1% of salvia EO were significantly impaired in both odor and taste as compared with the other samples. With regard to the overall acceptability, the cheese sample containing 0.75% of basil EO was the highest acceptable sample. Generally, it is well known that in complex systems such as cheese, several ingredients interact with each other and affect the sensory properties (Heenan *et al.* 2010).

CONCLUSION

The results of the present study demonstrate the effectiveness of basil and salvia EOs on microbial growth inhibition, the retardation of lipid oxidation and the extension of shelf life of Iranian white cheese during both refrigerated (4C) and ambient temperature (26C) storage. The EO of salvia showed the greatest effect on limiting microbial growth while the EO of basil strongly inhibited lipid oxidation. Both EOs could thus have potential for commercial use in improving the preservation of these products without the need for propionates or other synthetic additives. Further research could examine the utility of the combined application of basil and salvia EOs in different dairy products such as different types of cheese, as well as the use of different quantities/ratios for optimization of their antimicrobial and antioxidative effects.

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