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ORIGINAL ARTICLE

Improvement of the Microbial, Chemical, and Sensory Quality of Local Butter with *Carum Copticum* Essential Oil

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KEYWORDS

Natural preservatives;
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ABSTRACT: This study was conducted to improve the microbial, chemical, and sensory quality of local butter offered in Qazvin City, Iran, with the *Carum capticum* essential oil (EO). Firstly, EO extraction was done by distillation method; its compounds were analyzed using GC-Mass. After determining the EO antimicrobial activity by disk diffusion and microdilution method, concentrations of 2 and 4% were selected for butter treatment. The samples were evaluated in terms of microbial (total bacterial count, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and yeast-mold), chemical (pH value and acidity), and sensory (color, flavor, odor, and overall acceptability), at days 1, 3, 5, 7, and 10. According to the results, thymol, p-cymene, and γ-terpinene were the main components of the EO. The minimum inhibitory concentration (MIC) ranged from 0.05 to 0.125 %; the lowest MIC was related to *E. coli* and the highest was related to *Candida albicans*. Microbial evaluation of treated butter showed that the colony counts of examined organisms on days 3, 5, 7, and 10 decreased significantly ($P<0.05$) in the presence of 4% EO. The concentration of 2% EO significantly ($P<0.05$) reduced the organisms' population except on the third day. In addition, the EO reduced the acidity and increased the pH value in different storage days ($P<0.05$). The sensory evaluation also showed more acceptability of EO-containing butter ($P<0.05$). Considering the effects of *C. Copticum* EO in improving butter quality and its abundance as a native plant in Iran, it seems a proper candidate for replacing chemical preservatives and producing new products.

INTRODUCTION

Food-borne diseases are still one of the most challenging issues related to human health [1–6], involving about 30% of the world's population annually; If occurring with multidrug-resistant pathogens, the risk of death increases in the patients [7,8]. Food-borne pathogens can cause these diseases by consuming different types of food and especially dairy products [9]. These products are among the critical food items to support the growth of microorganisms and their transmission to consumers [9,10]. Butter is one of the most widely used dairy products, usually use in people's daily meals (especially for breakfast meals) and cooking [10]. The presence of significant amounts of fat-soluble vitamins and tocopherol in this product has made it a valuable food in

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terms of nutritional. Although the quality of the butter depends on the quality of the milk used, the storage conditions are also not without influence on this matter. Butter is usually contaminated by microorganisms that grow or survive at refrigerated temperatures. Coliforms, *Escherichia coli*, coagulase-positive staphylococci, yeast-mold are among the organisms that cause lipolysis and unfavorable taste in butter by producing enzymes [10,11].

Today, in line with the increasing awareness of consumers about the side effects of chemical antimicrobial compounds, the demand for the application of natural antimicrobial compounds in the food and pharmaceutical industries has increased [12–14]. Essential oils (EOs) and plant extracts are among the natural compounds used in the food industry for various purposes, such as increasing safety, improving taste, or creating new flavors [10,15,16]. *Carum capticum* EO is obtained from an annual herbaceous plant called *Trachyspermum ammi* (or Ajwain) which belongs to the Apiaceae family. According to the available reports, thymol and carvacrol are among the main ingredients of this essential oil, which have antispasmodic, bactericidal, antifungal, antitussive, and antiseptic properties [17,18]. Previously, its antimicrobial and antioxidant properties have been proven *in vitro* conditions and food [9,17]. Although researchers have confirmed the positive effect of this essential oil on the quality of meat products [19], such an evaluation has not been done in the case of butter. Therefore, the current study aims were to evaluate the microbiological and physicochemical quality of traditional butter offered in the retail market from Qazvin and the effect of *C. capticum* EO on these characteristics.

MATERIALS AND METHODS

Organisms and materials

In this study, *E. coli O157: H7* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), and *Candida albicans* (ATCC 10231) taken from the collection of bacteria in the Health Faculty of Qazvin University of Medical Sciences was used in experiments. All microbial culture media belong to Mirmedia, Iran.

Extraction of EO and quantification

First, *Trachyspermum ammi* plant seeds were purchased from Qazvin, Iran, spice market and transferred to the food safety and hygiene laboratory. After drying the plant seed, 100 grams of it were weighed, and EO extraction was conducted by distillation with water for 3 hours using a Clevenger apparatus. Diethyl ether was used as the collector solvent. Finally, the collected EO was dried with anhydrous sodium sulfate and transferred to the freezer in a dark container for further use. Gas Chromatography-Mass Spectrometer (GC/MS) was used to determine essential oil compounds, as described by [11].

In vitro experiments

EO antibacterial assay

C. Copticum EO antimicrobial activity was qualitatively and quantitatively investigated using disk diffusion and microdilution methods on *E. coli*, *P. aeruginosa*, *S. aureus*, and yeast-mold pathogens.

Disk diffusion method

Some empty discs were immersed in pure EO (100 µL) for 15min. Meanwhile, 100µL of the bacteria (10^6 log CFU ml^{-1}) were spread on the plates containing Mueller Hinton agar (MHA) in three replicates. Next, the disc containing the EO was placed in the center of the plates and incubated at 37°C for 24h. Finally, the inhibition zone (mm) was measured with a caliper. Antibiotic discs were used as positive control and empty disc as a negative control [20].

Microdilution method

It was curry out in 96-well microplates, according on Mortazavi et al. descriptions. First, 200µL EO was diluted with 2 ml of DMSO (10 %), and then it was diluted with an appropriate amount of tryptic soya broth (TSB) to create concentrations of 0.05 to 0.5% of EO in microplate wells. Finally, by adding 5 µL adjusted bacteria (10^7 CFU ml^{-1}), the final volume of each microplate was increased to 200 µL. A well without bacteria (containing EO and TSB) and a well without EO (containing bacteria and TSB) were also placed to ensure

sterility and bacterial growth, respectively. After 24-hour incubation at 37°C, the minimum inhibitory concentration (MIC) was obtained by observing the inhibition of bacterial growth in the well with the lowest concentration of EO [21].

Butter experiments

Preparation and microbial count

At first, local butter was purchased from the retail market in Qazvin, Iran, and quickly transferred to the Health and Food Safety Laboratory in the Faculty of Health, Qazvin University of Medical Sciences, by maintaining the cold chain. Then, to enumerate the total bacteria count, *E. coli*, *P. aeruginosa* , *S. aureus*, and yeast-mold, 10 gr of butter sample was weighed and suspended in peptone water (PW), and 100 µl of its different dilutions were cultured on plate count (PC), Eosin methylene blue (EMB), Cetrimide, Baird Parker (BP), and Yeast Extract Glucose Chloramphenicol (YGC) agar plates, respectively. After the required incubation period for each organism, the colonies were counted and recorded [22].

EO antimicrobial assay

Following the MIC determination of EO in TSB, and based on the result of a study that showed MIC of EO in food is about 35-fold MIC in TSB [19], concentrations of 4 % and 2 % of EO were selected for addition in butter. In detail, two butter treatments (with concentrations of 4 % and 2 % of EO) and a control sample (without EO) were prepared and stored at refrigerated temperature after homogenization. Then, on days 1, 3, 5, 7, and 10, various dilutions of the treatment and control groups were cultured on specific culture media of the target microorganisms; after the incubation period, the colonies were counted and the data recorded as log CFU ml^{-1} [19].

Measuring pH and acidity

At the same time as microbial evaluation, the treated and control groups were checked for acidity and pH value on all test days, according to the standard examination methods for dairy products [23].

Sensory evaluation

Sensory features such as color, flavor, odor, and overall acceptance of treatments and the control group were assessed by a trained panel on test days. It was performed with a 9-item questionnaire (1: very undesirable to 9: quite desirable) [12].

Statistical analysis

In the present study, the data were reported as a mean ± SD of three repetitions of each test. Analyses of the significant differences ($P < 0.05$) between means were performed using one-way ANOVA and Duncan's multiple range tests in SPSS ver.24 software [11].

RESULTS AND DISCUSSION

Chemical composition of EO

The analysis of *C. Copticum* EO using GC-Mass identified nine main chemical compounds, which include 95.87% of the total compounds. Table 1 shows their names, percentages, and retention times separately. Based on the results, the main EO compounds include thymol, p-cymene, and γ-terpinene, more than 85% of the identified compounds. So far, many reports have been published about the strong antimicrobial effects of thymol and its precursors (cymene and terpinene); indeed, thymol disrupts the lipid part of the plasma membrane, and it causes leakage of intracellular substances and inhibition of the organism by changing the permeability [24, 25].

Similarly, many researchers reported these three compounds as the main essential oil compounds; Of course, the different percentages of these three main compounds have been reported in studies [18,19, 26–30]. Moreover, in a review study on *C. Capticum* EO, the mentioned compounds were identified as the main compounds in most of the surveyed [18]. Besides, nearly similar to the current study, the main components of EO *C. Copticum* 45, 24, and 23 % of thymol, p-cymene, and γ-terpinene, respectively, were reported [31]. Thymol, γ-terpinene, and p-cymene content in EO Ajwain (*C. Copticum)* compound also reported 50, 23, and 22 %, respectively, with coordination in the current study [24]. In another study, the analysis of the components of *C.*

Copticum taken from 10 different samples identified the same three compounds as the main components in all the analyzed samples, and in 5 of 10 samples, thymol was included in a higher percentage than the other two compounds [32].

According to the statements of other authors, the amount of active components in essential oils can vary depending on which part of the plant the essential oil is made from [33]. Conceivably, the difference in the amount of effective EO compounds may be related to the initial seed of the plant and the climatic conditions [34].

Table 1. Major chemical composition of *C. Copticum* EO.

Compounds	Retention time (min)	Percentage (%)
Tymol	11.88	52.16
p-cymene	6.46	20.78
γ-terpinene	7.06	13.36
β-pinene	5.39	2.60
α-Thujene	4.48	1.45
β –Myrcene	5.56	1.12
cis-sabinene hydrate	7.53	0.87
A-Terpinolene	7.39	0.77

EO antibacterial activity in vitro

The details of the antimicrobial activity of the EO on the examined organisms are given in Table 2, as inhibitory zone and MIC.

Disc diffusion method

Generally, the obtained data confirmed the inhibitory effect of *C. copticum* EO on the examined pathogens (Table 2). Interestingly, the inhibitory zone of EO on *P. aeruginosa* and *C. albicans* was almot similar to or even significantly ($P<0.05$) higher than the effect of antibiotics. It also showed an inhibitory zone of EO against *S. aureus* and *E. coli*, although it was less than the inhibitory zone caused by antibiotics. In this regard, some researchers have confirmed the inhibitory effect of *C. Capticum* EO on different types of bacteria by disc diffusion method [20]. In additional, various studies have published reports of greater sensitivity of gram-positive bacteria than gram-negative bacteria to EOs, attributed to the thick cell wall in gram-negative bacteria [20,33]. However, in this study, the opposite results of their claims were observed, and *E. coli* was relatively more sensitive than *S. aureus*. Furthermore, Goudarzi et al. also showed EO antibacterial activities against *S. typhimurium*, *S. aureus* and *E. coli*, by disc diffusion method, but in the same study *P. aeruginosa* colonies grew on plates containing EO discs without any inhibitory zone [25]. In addition, researchers have published information on the antimicrobial effects of the extract of *C. Copticum* on a group of Gram-negative and Gram-positive bacteria; the highest and lowest inhibition diameters were related to *S. aureus* and *Acinetobacter baumannii*, respectively [35]. Interestingly, in that same study, the highest anti-biofilm effect (about 98%) of the methanolic extract on *A. baumannii* had been reported [35]; They also recorded the diameter of the non-growth zone of *E. coli* in the presence of the extract as 12 mm, which is consistent with the results of the present study (10 mm); however, the diameter of the non-growth zone recorded for *S. aureus* (20 mm) and *P. aeruginosa* (18 mm) was greater than in the present study (0.8 mm and 13 mm, respectively) [35].

Generally, differences in the rate of pathogen inhibition have been observed in different study, however, the researchers agree on the antimicrobial effect of EO *C. Copticum*. The observed differences in antimicrobial activities can be caused by the difference in the amount/ kind of effective compounds of the EO, bacterial strain, or test conditions.

Microdilution method

According to the results of the microdilution method, MIC ranged from 0.05 to 0.125% against the examined

pathogens (Table 2). Different studies have reported similar or different concentrations (higher or lower) than MIC observed in this study; this difference can be related to the difference in the bacterial strain and the percentage/type of the effective substance of the EO. For example, Mehdizadeh et al. reported the MIC of the EO against *E. coli O157:H7* as 10 mg ml^{-1} (1%), which was significantly more than observed MIC in this study [9]; Notably, the amount of thymol in their EO compounds (18%) was significantly lower than that of the present study (55%). The MIC caused by the ethanolic extract of this plant was also reported as 0.3125 and 0.625 % on *S. aureus* and *E. coli*, respectively [35]. It may be related to the difference in the thymol content of the extract compared to the thymol content of the EO in the present study.

Table 2. Antibacterial activity of *C. Copticum* EO *in vitro.*

Organisms	Inhibition zone (mm)			MIC of EO (%)
	EO	TE*	GE**	
E. coli O157:H7	10± 0.08[a]	24± 0.06[b]	17± 0.08[c]	0.05± 0.010
S. aureus	8± 0.06[a]	30± 0.07[b]	22± 0.08[c]	0.083± 0.020
P. aeruginosa	13± 0.03[a]	0±0.00[b]	12± 0.05[ac]	0.125±0.015
Candida albicans	19± 0.04[a]	18± 0.03[a]	11± 0.09[b]	0.1± 0.011

[a, b] Means± SD within a row with no common superscript differ (P<0.05)
*Tetracycline; **Gentamycin

Information has been published that the MIC of *Ajwain* (*C. Copticum*) EO changes depending on the *E. coli* strain. In detail, MIC of EO against *E. coli* LMG 8223 was 500 µg ml^{-1} (0.05 %), which was the same as the result of the present study (0.05 %), but a higher concentration (1000 µg ml^{-1} = 0.1 %) of the EO was needed to inhibit *E. coli* LMG 15862; Moreover, *P. aeruginosa* was inhibited with concentrations higher than 0.1% (>1000 µg ml^{-1}) of EO, similar to the MIC observed in the present study for this pathogen (0.125 %). However, different strains of *S. aureus* were inhibited with an equal MIC of the EO (500 µg ml^{-1}= 0.05 %), which was lower than the MIC observed in the present study (0.078 %) [31]; Considering that the main components of EO compounds are the same in both studies, these differences can be attributed to the difference in the characteristics of bacterial strain. In addition, as expected, the same MIC (0.05 %) was observed where the same *E. coli* strain was used in this study [19, 36].

Antimicrobial activity of C. Copticum EO in butter

Figures 1 to 5 show the antibacterial activities of different concentrations of EO on target microorganisms in butter at different storage times. Generally, for all target microorganisms on days 3, 5, 7, and 10, the 4 % EO concentration showed a higher inhibitory effect (*P<0.05*) than the control group (without EO). On the third day, there was no significant inhibitory difference between the lower concentration of the EO (2 %) and the control group for any of the microorganisms, however, with the increase in the storage period, the inhibition rate of the pathogens increased significantly (*P<0.05*) compared to the control group. Interestingly, on days 7 and 10 for *P. aeruginosa*, day 7 for yeast-mold, and days 5 and 7 for the total count of bacteria, no significant difference was observed in the reduction of bacterial population by two concentrations of 2 and 4 % EO; both treatments showed a significant decrease (*P<0.05*) of mention organisms compared to the control group. On the other hand, *P. aeruginosa* in the presence of EO 4% and 2% at 5 and 7 days, respectively, was entirely killed. Basically, by looking at the Figures, it can be seen that the reduction of the organism occurred depending on the EO dose, which was similar to the previous studies. For instance, bacterial growth in the culture medium and minced meat containing *C. Copticum* EO showed a dose-dependent decrease [19]. Moreover, the addition of *Ziziphora clinopodioides* EO to local butter showed a significant decrease in the population of the total count of bacteria, *E. coli*, and yeast-mold count at a duration storage time, similar to the present study was dependent on EO concentration [11]. Adding cinnamon extract to butter reduced yeast-mold count to zero at the beginning

of the storage period up to 10 weeks; more precisely, it reduced the yeast-mold count by 1.33 log units more than the control group, which in the present study was 1.1 log units in the high concentration of EO *C. Copticum*. This difference can largely depend on the initial quality of the butter sample because, in the present study, more than 7 logs CFU ml^{-1} of yeast-mold were recorded on the day of the test, while this value was 33.3 logs CFU ml^{-1} for the study in question [10]. However, the total count of bacteria in butter containing cinnamon extract decreased (1.34 log CFU ml^{-1}) almost similarly to the present study in 2% of EO (1.19 log CFU ml^{-1} reduction) [10]. Others showed that coliform bacteria and yeast-mold counts were significantly reduced in butter containing 2% EO Black Cumin (*Nigella sativa L.*) compared to the control group [37]. Likewise, in another study, the microbial quality of butter-containing green tea (*Camellia sinensis* L.) extract increased, and the total bacterial and yeast-mold counts were significantly reduced [38]. Based on previous studies, Gram-positive bacteria are more sensitive to essential oils compared to Gram-negatives [20, 33]; however, in the present study, the sensitivity of *E. coli* (even in the evaluation of microbial activity in vitro) to different concentrations of EO was significantly higher than that of *S. aureus* [33]. In this regard, Mahmoudi et al. also reported the high sensitivity of *E. coli* to *Z. clinopodioides* EO [11]. Beside, in another study, one of the strains of *E. coli* was more sensitive to Ajwain essential oil compared to *S. aureus* [28].

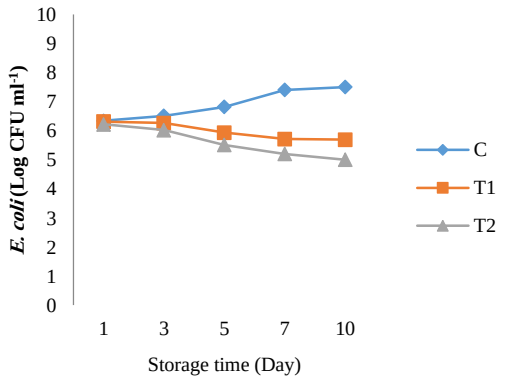

Figure 1. Effect of different treatments (T1: EO 2% and T2: EO 4%) on *E. coli* population (CFU ml^{-1}) in butter at different storage times.

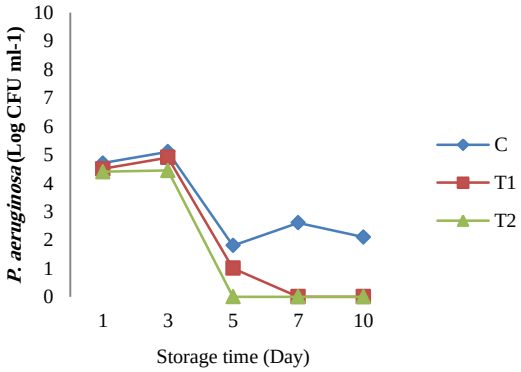

Figure 2. Effect of different treatments (T1: EO 2% and T2: EO 4%) on *P. aeruginosa* population (CFU ml^{-1}) in butter at different storage times.

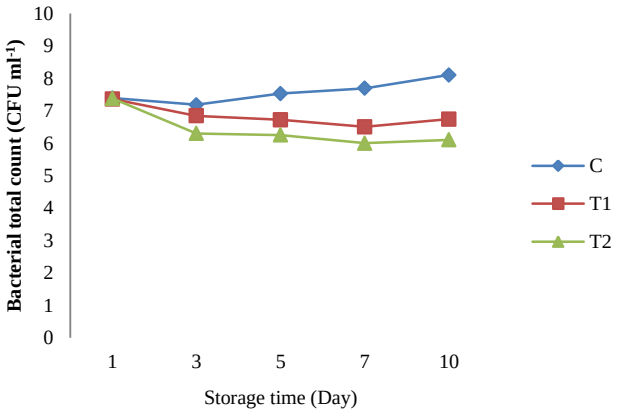

Figure 3. Effect of different treatments (T1: EO 2% and T2: EO 4%) on bacterial total count (CFU ml^{-1}) in butter at different storage times.

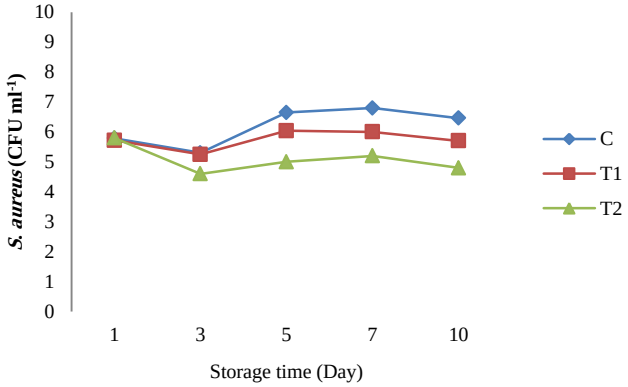

Figure 4. Effect of different treatments (T1: EO 2% and T2: EO 4%) on *S. aureus* population (CFU ml^{-1}) in butter at different storage times.

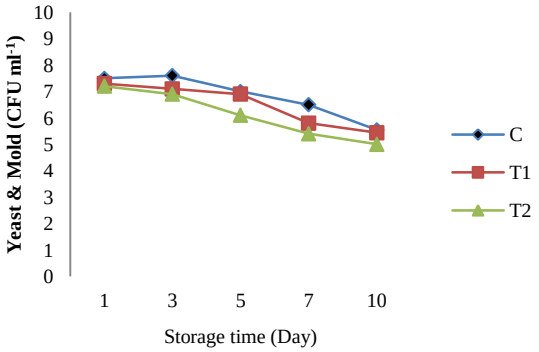

Figure 5. Effect of different treatments (T1: EO 2% and T2: EO 4%) on yeast-mold total count (CFU ml^{-1}) in butter at different storage times.

Acidity and pH value

The results of pH value and acidity are shown in Table 3. In general, a decrease in pH can be seen in all cases of control and treatments during the storage period, but in butter containing different concentrations of essential oil, a higher pH than the control sample was observed, which was significant ($P<0.05$) on all days of storage, except on the first day. However, there was no significant difference in the pH control between the two concentrations of the EO on the other days. In line with the present study, adding cinnamon extract to butter moderated the decreasing trend of pH compared to the control group. They showed that with prolonged storage of butter, its hydrolytic fat breaks down and turns into free fatty acids, and the pH of the product decreases.

Indeed, adding cinnamon to the butter content by controlling the fat decomposition affects the pH reduction process and prevents a high decrease compared to the control group [10].

Regarding acidity, although the acidity value increases in all treatment and control groups with the increase in storage time, in the presence of different concentrations of essential oil, the acidity value was significantly lower than in the control group, except on the first day. No significant difference was observed between the two EO concentrations (Table 3). Similarly, other studies have also reported a decrease in the acidity of butter the presence of different EO [11,37, and 39]. In addition, the results of a decrease in acidity and an increase in pH in yogurt containing oil and flavonoid extracted from orange peel have been published [40].

Table 3. The result of pH and acidity value during storage time treated butter.

Variant	Storage time (day)	Butter		
		Control	T1 (EO 2%)	T2 (EO 4%)
pH	1	5.11±0.01[a]	5.10±0.01[a]	5.10±0.00[a]
	3	4.85±0.00[a]	5.08±0.02[b]	5.06±0.02[b]
	5	4.57±0.01[a]	5.00±0.03[b]	4.90±0.01[b]
	7	4.21±0.02[a]	4.71±0.00[b]	4.70±0.00[b]
	10	4.06±0.01[a]	4.20±0.01[b]	4.12±0.01[b]
Acidity (%lactic acid)	1	0.59±0.01[a]	0.56±0.02[a]	0.57±0.02[a]
	3	0.65±0.01[a]	0.59±0.00[b]	0.60±0.00[b]
	5	0.70±0.02[a]	0.63±0.01[b]	0.66±0.01[b]
	7	0.81±0.00[a]	0.70±0.03[b]	0.73±0.02[b]
	10	0.93±0.01[a]	0.79±0.02[b]	0.81±0.03[b]

[a, b] Means± SD within a row with no common superscript differ (P<0.05)

Sensory evaluation

The results indicated a higher acceptance of the treated samples in all the investigated factors compared to the control group (Figure 6). More specifically, although the sample containing the lower concentration of EO had more acceptance in terms of odor, taste, and general acceptability than the sample containing the higher concentration of essential oil, their difference was not significant; the acceptance rate of both concentrations was even the same on the last day. So far, many studies have been done on the sensory evaluation of essential oils added to food, and the analysis of the results has shown an improvement in the sensory quality of the products. For example, adding the *Z. clinopodioides* EO to butter in the study of Mahmoudi et al., *Teucrium polium* EO to probiotic yogurt in the same author's other study, and *Bunium persicum* Boiss. EO in Gouda cheese in Taherkhani et al.'s study had produced favorable sensory effects compared to control samples [11,41–43]. In addition, in another study, butter containing 3% cinnamon extract was the most acceptable compared to the control group [10].

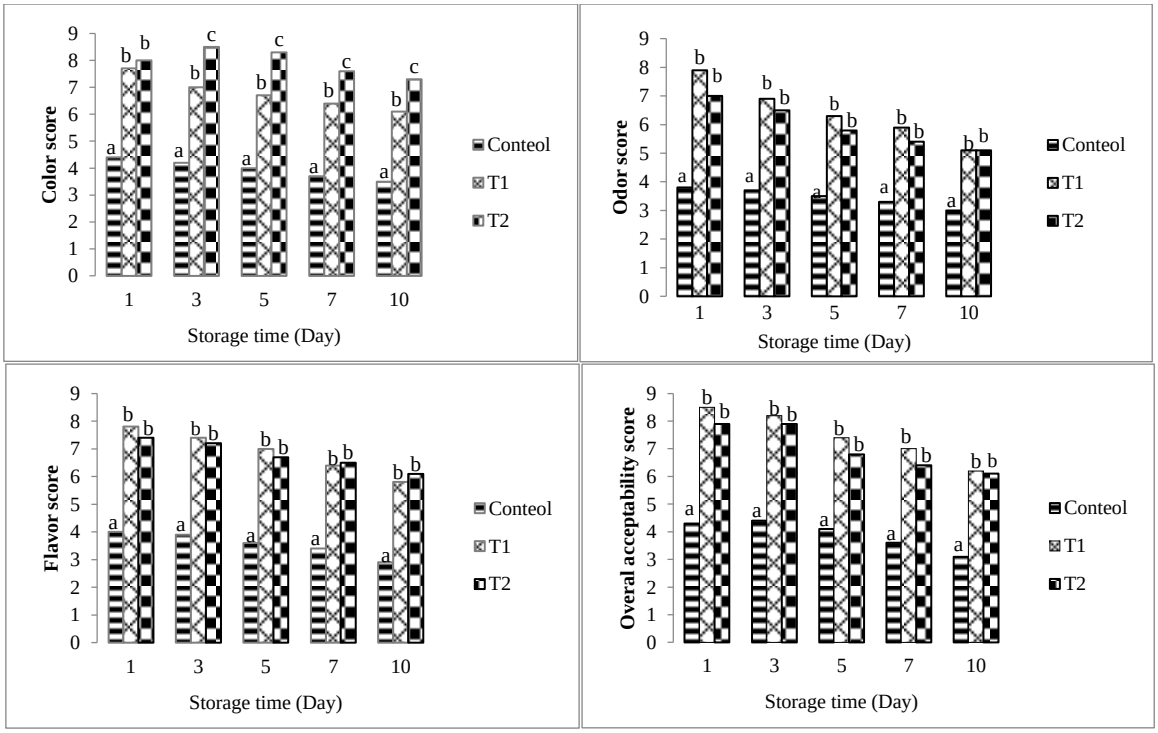

Figure 6. Sensory scores of different treatments of butter stored at 4◦C. Bars with different letters in each storage day indicate significant statistical differences (P < 0.05) among treatments. T1 (EO 2%), T2 (EO 4%).

CONCLUSIONS

In the current study, by adding different concentrations of *C. Copticum* EO to traditional butter, its microbial, chemical properties, and organoleptic properties increased significantly, which is confirmed by the findings of other studies. Indeed, due to the presence of a high amount of thymol and its precursors in the composition of *C. copticum*, it seems that this EO is one of the compounds with a high potential to improve the food's microbiological and chemical properties during the storage period. However, it is suggested to carry out more studies on the effect of the EO on genotypic characteristics of food pathogens, both in vitro and in food model systems.

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Conflict of interests

There are none to declare.

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