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Inhibitory effect of thyme and cinnamon essential oils against *E. coli* O157:H7 in Tahini

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Abstract

Tahini is a common food product in the Mediterranean area that is used as a main ingredient in variety of ready-to-eat foods. The objective of the current study was to investigate the inhibitory effect of thyme oil (TO) or cinnamon oil (CO) on *E. coli* O157:H7 viability in tahini and diluted tahini at different storage temperatures. Addition of 2.0% CO to tahini reduced *E. coli* O157:H7 numbers by 1.38, 1.79 or 2.20 $\log_{10}$ CFU/mL at 10, 25 or 37 °C, respectively, by 28d. In diluted tahini at 10 °C, no viable cells of *E. coli* O157:H7 by 21d were detected when 1.0% CO was used. However, at 25 and 37 °C, no viable cells were detected by 14d when CO was added at 0.5% level. Addition of 2.0% TO to tahini, resulted in 1.82, 2.01 or 1.65 $\log_{10}$ CFU/mL reduction in *E. coli* O 157:H7 numbers was noted at 37, 25 or 10 °C, respectively, by 28d. In diluted tahini, TO at 0.5% or 1.0% induced complete reduction in the viability of *E. coli* O157:H7 by 28d storage at 37 or 25 °C. At 10 °C, a 3.02 $\log_{10}$ CFU/mL reduction was observed by 28d compared to the initial inoculation level in samples treated with 2.0% TO.

Keywords: tahini; *E. coli* O157: H7; cinnamon oil; thyme oil.

Practical Application: Control the growth of *E. coli* O157:H7 in tahini using thyme oil or cinnamon oil.

1 Introduction

Tahini or sesame paste is a common component of several popular ready-to-eat (RTE) dishes including tahini salad, halva (sweetened tahini), hummus, tarator sauce and mutabbel in Middle Eastern countries including Jordan, and it is also involved in several other ethnic dishes in Greek, North African and Turkish cuisines. The worldwide tahini consumption or its products has raised during the past few years, particularly in the European countries, US and Canada due to its nutritional value and health properties. For example, tahini has moderate amount of carbohydrate (6.4-9.0% wt) and high amount of protein (23-27% wt) as well as lipid (57-65% wt) and vitamins and minerals (Sawaya et al., 1985; Abu-Jdayil et al., 2002). Further, it has been reported that sesame seed products like tahini play a crucial role in preventing cardiovascular diseases and atherosclerosis, improving digestion and metabolic activity and reducing hyperglycemia and cholesterol (Narasimhulu et al., 2015). It is also anticipated that the global consumption of tahini will increase by 12% in the next 5 years. It is expected that the Middle East and the US will be the main consumption regions due to the increasing demand for ethnic Arabic and Mediterranean foods, and seed-based spreads. The US alone was responsible for 78% of the total global tahini consumption in 2015 (Research & Markets, 2017).

Tahini is a RTE product that has a low water activity, a_w, (~ 0.16-0.25) and an extended shelf-life (two years) when stored at ambient temperature. However, its high fat content provides opportunity for microorganisms to survive for long time under different storage environments (Lake et al., 2010). Tahini nowadays has raised a special concern regarding its safety, particularly to both the food regulatory agencies and food industry in view of recent reports of recalls and outbreaks associated with tahini contaminated with pathogenic bacteria. Numerous foodborne recalls and outbreaks linked to consumption of tahini or its products have been documented worldwide in the last two decades, particularly due to their contamination with *Salmonella* serovars (Unicomb et al., 2005; Centers for Disease Control and Prevention, 2012, 2013). Furthermore, *E. coli* O157:H7 has been isolated from tahini or tahini-based products. *E. coli* O157:H7 is also able to survive and grow in commercial and diluted tahini during storage at temperature range of 10 to 37 °C (Al-Nabulsi et al., 2013). Consequently, control of foodborne pathogens in tahini is likely to require novel approaches, including those based on using natural antimicrobials instead of chemical preservatives, which is of popular consumer interest for consumers worldwide, and hence this approach has been utilized in the current study.

Plant-derived antimicrobial substances like essential oils (EOs) have been used for hundreds of years as a mean of inhibiting spoilage and pathogenic microorganisms (Al-Nabulsi et al., 2015). Some edible oils, medicinal herbs and spices are potently effective against different foodborne pathogens such as *E. coli* O157:H7 in

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a variety types of food system (Tajkarimi et al., 2010). However, the inhibitory effects of plant EOs against *E. coli* O157:H7 in tahini have not been investigated. Therefore, the objectives of this study were to: i) screen the antibacterial activity of 14 plant EOs against three *E. coli* O157:H7 strains using disc-diffusion method at 10 and 37 °C; ii) determine the minimum inhibitory (MICs) and minimum bactericidal concentrations (MBCs) of cinnamon and thyme oils (the most potent essential oils) against *E. coli* O157:H7; and iii) investigate the inhibitory activity of cinnamon and thyme oils against *E. coli* O157:H7 cocktail in commercial or diluted tahini which was used as a model for tahini-based products) stored at 10, 25 or 37 °C.

2 Materials and methods

2.1 Preparation of bacterial cultures

Three mutated non-pathogenic (verotoxigenic negative) *E. coli* O157:H7 strains (02:0627, 02:0628, 00:3581), that had been provided by Rafiq Ahmed, National Microbiology Laboratory, Public Health Agency, Canadian Science Centre for Human and Animal Health, Winnipeg, MB. Canada, were used in the present study. The strains were individually kept and maintained in Brain Heart Infusion (BHI, Oxoid Ltd, Basingstoke, UK) broth including 20% (v/v) glycerol at -40 °C. A loopful of each thawed frozen strain was streakedonto the plates surface of Sorbitol MacConkey agar (SMA, Oxoid Ltd, Basingstoke, UK), followed by incubation at 37 °C for 24 h. Thereafter, a colony of each individual strain was transferred to BHI broth which was incubated for 24 h at 37 °C. Three consecutive transfers were performed to revitalize the bacterial culture and a final transfer to BHI broth was conducted before the experiment. The inoculated BHI broth was incubated for 24 h at 37 °C to allow *E. coli* to reach the stationary phase.

2.2 Preparation of cocktail culture

After culturing *E. coli* O157:H7 in BHI broth, the cells were harvested by centrifugation at 4000 *x g* for 15 min. The pellet was obtained after discarding the supernatant. Thereafter, 10 mL of 0.1% sterile peptone water (Oxoid Ltd, Basingstoke, UK) was mixed with the pellets. The *E. coli* O157:H7 count in the resulting bacterial suspension was determined by plating onto SMA and a final bacterial concentration of ~9 $\log_{10}$ CFU/mL was obtained. The suspension was diluted with 0.1% peptone water to the desired concentration and 2.5 mL of each *E. coli* O157:H7 strain was combined to form a cocktail mixture of the three strains.

2.3 Essential oil extracts

The inhibitory effect of 14 EOs of medicinal plants were screened in this study; lavender, thyme, cardamom, rosemary, mint, cinnamon, fir, sage, laurel leaves, ginger, mustard, radish seeds, black cumin seeds and pomegranate seeds. These EOs were purchased from Green Field Oil Factory in Amman, Jordan. Hydro-distillation is usually used in this factory to extract the EOs from their sources with a purity of 99%. The EOs were kept dry in sealed dark glass vials at 4 °C until use.

2.4 Screening of inhibitory effect of plant EOs

The inhibitory effect of the 14 plant EOs against *E. coli* O157:H7 strains was screened using the disc diffusion method (Janakat et al., 2015). Specifically, 20 µL of each plant EO were spread onto sterile discs with diameter of 6 mm (Oxoid Ltd, Basingstoke, UK) which were air dried at room temperature for 10 min under a bio-safety cabinet. The discs were placed onto TSA plates that had been inoculated with 100 µL solution containing ~ 6.0 $\log_{10}$ CFU/mL of each individual *E. coli* O157:H7 strain. Thereafter, the plates were incubated for 24 h at 37 °C or for 7 d at 10 °C. A calibrated ruler was used for measuring the inhibition zones in mm

2.5 Determination of the MICs and MBCs of most potent EOs against E. coli O157:H7 (02:0627)

Of the 14 EOs, the MIC and the MBC of only thyme oil and cinnamon oil were determined using the microdilution method, since these oils showed the strongest inhibitory effect against *E. coli* O157:H7 strains. Briefly, 100 µL of fresh cultures were diluted in Mueller Hinton (MH) broth (Oxoid Ltd, Basingstoke, UK) to give a final concentration of about 5.0 $\log_{10}$ CFU/mL of *E. coli* O157:H7 (02:0627). Cinnamon or thyme oils were individually mixed at a ratio of 1:1 in 0.5% dimethyl sulfoxide (DMSO, HPLC grade). Volumes of 50, 100 or 150 µL of DMSO-essential oil solution were added to 96 well microtiter plates (Greiner Bio-One, CellStar™, Italy). Thereafter, the volume to 250 µL was completed with sterile distilled water. A solution of 0.5% DMSO without cinnamon or thyme oil was mixed with the bacterial culture as a positive control, while DMSO containing cinnamon or thyme oil was used as a negative control. Then, the sealed microplate was incubated for 24 h at 37 °C. Then, 100 µL aliquots from each sample were taken and decimal dilutions were prepared with 0.1% peptone water. Exactly 100 µL from each of the appropriate dilutions was spread onto the surface of TSA (Oxoid) and incubated for 24 h at 37 °C.

2.6 Tahini preparation

Tahini samples were purchased from a local market in Irbid, Jordan. Samples were tested for the presence of *E. coli* O157:H7 prior to its use and confirmed to be free from contamination. Diluted tahini (10% w/v) was prepared by mixing 2.5 g tahini with 22.5 mL sterile distilled water in sterile Duran bottle. A cocktail mixture of *E. coli* O157:H7 was inoculated into tahini and diluted tahini to obtain ~4.5 $\log_{10}$ CFU/mL. Thereafter, 0.0, 0.2, 0.5, 1.0 or 2.0% (v/v) of cinnamon or thyme EOs, which were prepared as formerly described were added to tahini or diluted tahini. Each bottle was agitated using a vortex mixer (Velp, Italy) to distribute the bacterial culture uniformly throughout the samples, and were incubated at 37, 25 or 10 °C for 28 d.

2.7 Microbiological enumeration

Sampling of tahini or diluted tahini was conducted after 0, 1, 3, 7, 14, 21 and 28d at 37, 25 or 10 °C. Samples of 5 mL were taken by sterile syringe from each bottle and transferred into sterile stomacher bags (Seward Ltd., London, UK). The sample was mixed with 45 mL of 0.1% peptone water and pummeled for 2 min using a Stomacher 400 (Seward Ltd,). Then the

homogenate was 10-fold serially diluted in screw-capped test tubes containing 0.1% peptone water and 100 µL of the diluted sample was plated onto the surface of Sorbitol MacConkey agar overlaid with TSA to allow recovery of injured cells (Osaili et al., 2010). The plates were aerobically incubated for 24 h at 37 °C.

2.8 Water activity (a_w) and pH measurement

Water activity of tahini samples was measured at room temperature using an a_w meter (Hygrolab, Rotronic Instr. Corp, Huntington, NY, US). The pH value of regular and diluted tahini was directly measured using a digital pH meter (Eutech model CyberScanpH1100, Singapore).

2.9 Determination of total phenolic content

The total phenolic content of tahini was determined using Folin-Ciocalteu spectrophotometric method as described by Osaili et al. (2017). A standard calibration curve was prepared using a stock solution of gallic acid (50 mg/50 mL). A 100 µL aliquot of the sample containing phenolic compounds was added to 8.40 mL distilled water. Then 0.5 mL Folin-Ciocalteu reagent was added and agitated for 4 min using vortex mixer (Velp, Italy). The mixture was incorporated with 1 mL of 5% Na_2CO_3 solution and mixed similarly. After 1 hour, the absorbance was measured at 725 nm using a spectrophotometer (UV 1800, 50 Hz, UK). The total content of phenolic compounds was calculated as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g). The analysis was conducted in triplicate.

2.10 Statistical analysis

The SPSS software version 19.0 (2009; IBM, Chicago, IL, US) was used to analyse the results which were represented by means ± standard deviation (SD). The effect of time and EO concentrations on bacterial log reduction were determined by one way ANOVA. The significant differences between means of variables was determined by Duncan's post hoc test when p-value < 0.05.

3 Results and discussion

The commercial tahini had an initial pH value of 6.23 and a_w of 0.25, while the initial pH and a_w of diluted tahini were 6.31 and 0.96, respectively. The total phenolic content in tahini was found to be 14.35 ± 0.15 mg/mL.

3.1 Inhibitory effect of plants EOs

The EOs of ginger, sage, radish seeds, black seeds, fir, mustard and pomegranate seeds did not exhibit any inhibitory effect against *E. coli* O157:H7 strains at 10 or 37 °C (data are not shown). While the EOs of laurel leaves, lavender, cardamom, mint and rosemary exhibited moderate inhibitory effect with inhibition zones that ranged between 7.8-15.0 mm at 37 °C (Table 1). The EOs of cinnamon and thyme showed the strongest inhibitory effect with inhibition zones of 24.3-50.8 mm at 37 °C. It was interesting that all the strains behaved similarly in response to all tested EOs at 10 °C with no significant differences being detected among the tested strains.

Comparable results were obtained by Kon & Rai (2012) and Prabuseenivasan et al. (2006) who reported that CO had the strongest inhibitory effect against Gram-positive and Gram-negative bacteria among 21 to 35 plant EOs. Moreover, it has been reported that cinnamon, thyme, bay leaf and garlic have significant inhibitory effects against *E. coli* O157:H7 (Gyawali & Ibrahim, 2014; Dadalioğlu & Evrendilek, 2004). In addition, the difference in the inhibitory effect of EOs may be due to the variation in the composition of bioactive compounds, which is affected by harvesting time the part of the plant which is used, as well as the extraction method (Naghdi Badi et al., 2004; Burt, 2004; Hyldgaard et al., 2012).

Since cinnamon and thyme oils exhibited the strongest *in vitro* inhibitory effect against *E. coli* O157:H7 and since no strain variations were found between the strains, *E. coli* O157:H7 (02:0627) was used to determine the MIC and MBC. The MICs of cinnamon and thyme oils against *E. coli* O157:H7 strain 02:0627 were 0.10% at 37 °C while the MBCs were 0.20% for both oils (data are not shown). These results are compatible with those reported by Oussalah et al. (2007) who indicated that *Thymus vulgaris* (TO) had a significant inhibitory effect against *E. coli* O157:H7 at 0.05% (v/v) and by Hammer et al. (1999) who reported that the MIC of thyme against *E. coli* O157:H7 was 0.12% (v/v). On the other hand, a lower MIC for *Thymus vulgaris* (0.01%) than was found in the present study against *E. coli* O157:H7 was reported by Hossain et al. (2013).

The mechanism responsible for the antimicrobial action of TO and CO is believed due to their ability to disturb the outer membrane (OM) structure of Gram-negative bacteria. As a result, small hydrophilic solutes can cross the OM through profuse

Table 1. Inhibitory effect of plant essential oils (20 µL/disc) against 3 strains of *E. coli* O157:H7 using disc diffusion.

Foodborne pathogen	Plant Essential oils						
	Laurel leaves	Thyme	Lavender	Cardamon	Mint	Rosemary	Cinnamon
a) At 10 °C after incubation for 7 days.							
E. coli O157:H7 02:0628	10.3 ± 0.7^{dB}	32.7 ± 0.5^{bBC}	10.3 ± 0.5^{dB}	8.5 ± 0.5^{eB}	10.5 ± 0.8^{cE}	10.2 ± 0.7^{dB}	63.2 ± 0.7^{a}
E. coli O157:H7 00:3581	12.3 ± 0.9^{dA}	32.2 ± 0.9^{bC}	11.8 ± 0.7^{dA}	12.8 ± 0.7^{cA}	16.7 ± 0.9^{cA}	11.2 ± 0.9^{dA}	62.8 ± 0.7^{aA}
E. coli O157:H7 02:0627	12.0 ± 0.6^{dA}	35.7 ± 0.5^{bA}	10.5 ± 0.5^{dB}	8.8 ± 0.4^{eB}	14.3 ± 0.5^{cB}	9.0 ± 0.8^{dC}	60.0 ± 0.6^{aB}
b) At37 °C after incubation for 24 h							
E. coli O157:H7 02:0628	10.3 ± 0.8^{eA}	26.3 ± 0.5^{bBC}	11.0 ± 0.6^{dAB}	8.9 ± 0.2^{fA}	13.2 ± 0.8^{cB}	10.3 ± 0.5^{deA}	38.7 ± 0.8^{aD}
E. coli O157:H7 00:3581	9.7 ± 0.5^{eB}	26.3 ± 0.5^{bBC}	11.5 ± 0.5^{dA}	8.0 ± 0.6^{fB}	13.5 ± 0.8^{cB}	10.3 ± 0.5^{deA}	34.7 ± 0.7^{aE}
E. coli O157:H7 02:0627	9.0 ± 0.0^{eC}	29.0 ± 4.2^{dB}	10.6 ± 0.5^{dB}	8.3 ± 0.5^{fAB}	13.8 ± 0.4^{cB}	10.2 ± 0.4^{deA}	39.5 ± 0.5^{aC}

Inhibition zone diameter (mm). Values are the mean of 3 experiments (n = 6) ± SD. Values with same capital letters in the same row and with same small letters in the same column are not significantly different ($p \geq 0.05$).

porin proteins. This induces the liberation of OM-associated constituents to the external medium, and in turn these changes decrease the intracellular ATP pool of the bacterial cell, which leads to the loss of cytoplasmic membrane integrity and eventually cell death (Helander et al., 1998; Ultee et al., 1999; Oussalah et al., 2006; Boskovic et al., 2015).

3.2 Survival of E. coli O157:H7 in tahini and diluted tahini

The viability of *E. coli* O157:H7 in tahini was decreased at all tested storage temperatures. The extent of bacterial reduction was 0.57, 1.04 and 1.15 $\log_{10}$ CFU/mL at 10, 25 and 37 °C, respectively, after 28d (Table 2). Our results are in agreement with those reported by Al-Nabulsi et al. (2013) who indicated that the viability of *E. coli* O157:H7 in tahini decreased by 4.53, 2.52 and 2.18 $\log_{10}$ CFU/mL at 37, 21 and 10 °C, respectively, after 28d. The bacterial reduction could be partially related to the presence of phenolic compounds in tahini (14.35 mg/g) as well as to its low a_w (0.25). Beside the low a_w that may inhibit bacterial growth, the high fat percentage of tahini may provide protection to contaminating organisms. In addition, the pH of tahini (~6.8) is not inhibitory by itself to bacteria.

Tahini is usually consumed in a diluted form since water and other food ingredients are added to prepare different types of RTE products. In the present study, tahini was diluted 10-fold which is the most common level of tahini used during preparation of tahini-based products. Progressive *E. coli* O157:H7 growth was noted in diluted tahini at all storage temperatures. The number of *E. coli* O157:H7 cells increased by 2.15, 2.74 and 2.84 $\log_{10}$ CFU/mL after incubation at 10, 25 and 37 °C, respectively, after 28 d (Table 3). Similarly, Al-Nabulsi et al. (2013) reported that *E. coli* O157:H7 grew in 10% diluted tahini. The neutral pH, high a_w (0.96), and nutrients availability are likely the key factors that enabled *E. coli* O157:H7 growth in diluted tahini. Therefore, contamination of tahini with pathogens should be treated as a microbial hazard requiring high attention because of the ability of foodborne pathogens to persist in tahini and sometimes grow, reaching the infectious dose when the a_w is increased during tahini-based products preparation.

3.3 Inhibitory effect of TO and CO on the viability of E. coli O157:H7 in tahini and diluted tahini

The use of plant-based materials including thyme for inactivating *E. coli* O157:H7 in different food products has been reported (Boskovic et al., 2015). It was notable that in the present study the extent of inactivation of *E. coli* O157:H7 in tahini increased with higher EO concentrations and higher storage temperature. The addition of TO to tahini led to a significant reduction in the viability of *E. coli* O157:H7 cells at all tested temperatures. After 28d, TO at concentration of 0.2, 0.5, 1.0 or 2.0% caused a reduction of 1.25, 1.35, 1.67 and 1.82 $\log_{10}$ CFU/mL, respectively at 37 °C (compared to 1.15 $\log_{10}$ CFU/mL in the control). However, the inhibitory effect of TO was reduced at lower temperatures of storage. When 0.2, 0.5, 1.0 or 2.0% TO was added to tahini, a

Table 2. Effect of thyme oil on the viability of *E. coli* O157:H7 ($\log_{10}$ CFU/mL) during storage of tahini at:10 °C (a), 25 °C (b) and 37 °C (c).

Time	Concentration of thyme oil (%)				
(Day)	0.0	0.2	0.5	1.0	2.0
a) At 10 °C					
0	4.45 ± 0.13^{aA}	4.45 ± 0.13^{aA}	4.45 ± 0.13^{aA}	4.45 ± 0.13^{aA}	4.45 ± 0.13^{aA}
1	4.48 ± 0.20^{aA}	4.17 ± 0.05^{ab}	3.90 ± 0.06^{cC}	3.70 ± 0.10^{bCD}	3.55 ± 0.13^{bD}
3	4.53 ± 0.10^{aA}	4.33 ± 0.15^{aA}	4.13 ± 0.05^{bB}	3.85 ± 0.12^{bC}	3.65 ± 0.24^{bC}
7	4.45 ± 0.37^{aA}	4.18 ± 0.35^{abA}	3.73 ± 0.13^{cB}	3.45 ± 0.10^{cB}	2.90 ± 0.14^{cC}
14	3.98 ± 0.25^{bA}	3.81 ± 0.16^{cAB}	3.48 ± 0.26^{dBC}	3.30 ± 0.20^{dC}	2.90 ± 0.16^{cD}
21	3.93 ± 0.13^{bA}	3.66 ± 0.30^{cAB}	3.36 ± 0.30^{deBC}	2.95 ± 0.13^{eC}	2.87 ± 0.67^{cC}
28	3.88 ± 0.15^{bA}	3.90 ± 0.17^{cdA}	3.20 ± 0.14^{eB}	3.00 ± 0.20^{eC}	2.80 ± 0.08^{cC}
b) At 25 °C					
0	4.45 ± 0.13^{aA}	4.45 ± 0.13^{aA}	4.45 ± 0.13^{aA}	4.45 ± 0.13^{aA}	4.45 ± 0.13^{aA}
1	4.05 ± 0.10^{bA}	3.80 ± 0.08^{bB}	3.46 ± 0.19^{bC}	3.20 ± 0.08^{bD}	2.85 ± 0.10^{bE}
3	3.45 ± 0.17^{cA}	3.50 ± 0.25^{cA}	3.14 ± 0.16^{cB}	2.83 ± 0.10^{cC}	2.65 ± 0.06^{bcC}
7	3.38 ± 0.26^{cA}	3.30 ± 0.05^{cdAB}	3.05 ± 0.06^{cdB}	2.78 ± 0.20^{cC}	2.73 ± 0.20^{bcC}
14	3.52 ± 0.20^{cA}	3.10 ± 0.20^{deB}	3.00 ± 0.13^{cdB}	2.38 ± 0.10^{dC}	2.26 ± 0.30^{dC}
28	3.41 ± 0.08^{cA}	3.00 ± 0.20^{eB}	2.90 ± 0.13^{dB}	3.05 ± 0.13^{bB}	2.44 ± 0.20^{cdC}
c) At 37 °C					
0	4.45 ± 0.13^{aA}	4.45 ± 0.13^{aA}	4.45 ± 0.13^{aA}	4.45 ± 0.13^{aA}	4.45 ± 0.13^{aA}
1	3.85 ± 0.06^{bA}	3.65 ± 0.13^{bB}	3.33 ± 0.13^{bC}	3.30 ± 0.08^{bC}	2.56 ± 0.17^{bD}
3	3.03 ± 0.15^{dA}	2.93 ± 0.30^{dA}	2.95 ± 0.50^{bA}	2.28 ± 0.19^{dB}	2.10 ± 0.14^{cdB}
7	3.23 ± 0.10^{cdA}	3.15 ± 0.05^{cdAB}	3.08 ± 0.05^{bB}	2.88 ± 0.10^{cC}	2.58 ± 0.05^{bD}
14	3.33 ± 0.30^{cA}	2.40 ± 0.08^{eB}	2.35 ± 0.26^{cB}	2.09 ± 0.38^{dB}	2.03 ± 0.30^{dB}
21	3.24 ± 0.20^{cdA}	3.51 ± 0.13^{bAB}	2.99 ± 0.20^{bB}	2.29 ± 0.35^{dC}	2.35 ± 0.20^{bcC}
28	3.30 ± 0.10^{cdA}	3.20 ± 0.10^{cA}	3.10 ± 0.14^{bA}	2.78 ± 0.13^{cB}	2.63 ± 0.10^{bB}

Values are the means of 2 experiments (n= 4) ± SD; Values with same capital letters in the same row and with same small letters in the same column are not significantly different ($p \geq 0.05$).

Table 3. Effect of thyme oil on the viability of *E. coli* O157:H7 (log_{10} CFU/mL) during storage of diluted tahini at:10 °C (a), 25 °C (b) and 37 °C (c).

Time	Concentration of thyme oil (%)				
(Day)	0.0	0.2	0.5	1.0	2.0
a) At 10 °C					
0	5.10 ± 0.00^{cA}	5.10 ± 0.00^{bA}	5.10 ± 0.00^{cA}	5.10 ± 0.00^{aA}	5.10 ± 0.00^{aA}
1	5.75 ± 0.85^{bA}	5.30 ± 0.06^{aA}	4.97 ± 0.51^{cAB}	4.38 ± 0.30^{bC}	3.56 ± 0.48^{cC}
3	6.86 ± 0.28^{aA}	6.97 ± 0.08^{aA}	6.90 ± 0.24^{aA}	4.15 ± 0.13^{cB}	3.38 ± 0.30^{cC}
7	7.28 ± 0.15^{aA}	7.06 ± 0.19^{aA}	6.06 ± 0.21^{bB}	4.92 ± 0.17^{bC}	4.33 ± 0.54^{bD}
14	7.15 ± 0.17^{aA}	6.66 ± 0.20^{aB}	5.30 ± 0.46^{bcC}	4.14 ± 0.18^{cD}	3.31 ± 0.19^{cE}
21	7.10 ± 0.23^{aA}	6.94 ± 0.27^{aA}	6.55 ± 0.28^{aA}	3.06 ± 0.96^{dB}	2.88 ± 1.03^{cB}
28	7.25 ± 0.25^{aA}	6.98 ± 0.17^{aA}	6.98 ± 0.19^{aA}	2.26 ± 0.15^{eB}	2.08 ± 0.15^{dB}
b) At 25 °C					
0	5.10 ± 0.00^{cA}	5.10 ± 0.00^{bA}	5.10 ± 0.00^{cA}	5.10 ± 0.00^{aA}	5.10 ± 0.00^{aA}
1	7.20 ± 0.19^{cA}	6.73 ± 0.20^{aB}	6.63 ± 0.24^{aB}	6.18 ± 0.16^{aC}	4.85 ± 0.30^{aD}
3	7.35 ± 0.10^{bcA}	7.09 ± 0.90^{aAB}	6.85 ± 0.13^{aB}	6.24 ± 0.05^{aC}	4.95 ± 0.29^{aD}
7	7.33 ± 0.06^{bcA}	6.88 ± 0.32^{aB}	5.86 ± 0.16^{bC}	5.40 ± 0.36^{bD}	4.40 ± 0.14^{bD}
14	7.42 ± 0.06^{bA}	5.88 ± 0.20^{bB}	5.92 ± 0.25^{bB}	4.28 ±0.51^{cC}	2.18 ± 0.21^{cD}
21	7.48 ± 0.12^{bA}	5.87 ± 0.73^{bB}	4.67 ± 0.13^{dC}	2.62 ± 0.37^{dD}	2.06 ± 0.16^{cD}
28	7.84 ± 0.07^{aA}	4.87 ± 0.49^{cB}	3.94 ± 0.42^{eC}	ND^{eD}*	ND^{dD}
c) At 37 °C					
0	5.10 ± 0.00^{cA}	5.10 ± 0.00^{bA}	5.10 ± 0.00^{cA}	5.10 ± 0.00^{aA}	5.10 ± 0.00^{aA}
1	6.80 ± 0.20^{cdA}	6.90 ± 0.10^{aA}	6.57 ± 0.15^{aB}	6.26 ± 0.35^{aC}	4.89 ± 0.10^{bD}
3	6.70 ± 0.50^{dA}	6.42 ± 0.15^{aA}	6.00 ± 0.20^{bB}	5.83 ± 0.20^{bB}	5.90 ± 0.11^{aB}
7	7.03 ± 0.19^{bcA}	5.14 ± 0.60^{bB}	3.98 ± 0.21^{dC}	3.54 ± 0.31^{dC}	2.90 ± 0.30^{cD}
14	7.18 ± 0.25^{bA}	4.58 ± 0.68^{cB}	3.58 ± 0.12^{eC}	2.54 ± 0.47^{eD}	2.20 ± 0.23^{dD}
21	7.05 ± 0.17^{bcA}	2.67 ± 0.22^{dB}	2.57 ± 0.17^{fB}	2.08 ± 0.15^{fC}	1.93 ± 0.15^{eC}
28	7.94 ± 0.14^{aA}	2.52 ± 0.17^{dB}	ND^{gC}	ND^{gC}	ND^{fC}

*ND: Cells of *E. coli* O157:H7 were not detected (<1 CFU/mL). Values are the means of 2 experiments (n= 4) ± SD. Values with same capital letters in the same row and with same small letters in the same column are not significantly different ($p \geq 0.05$).

1.45, 1.55, 1.40 and 2.01 log_{10} CFU/mL reduction in the number of *E. coli* O157:H7 cells, respectively, was noted compared to 1.04 log_{10} CFU/mL in the control at 25 °C after 28d. At 10 °C, the reduction was 0.55, 1.25, 1.45 and 1.65 log_{10} CFU/mL, respectively, with samples treated with 0.2, 0.5, 1.0 or 2.0% TO compared to 0.57 log_{10} CFU/mL 10 °C in the control samples (Table 2).

In diluted tahini after 28 d storage, TO at 0.5% or 1.0% caused a complete reduction in the numbers of *E. coli* O157:H7 at 37 or 25 °C, respectively. At 10 °C, *E. coli* O157:H7 cells showed more resistance to TO since a 3.02 log_{10} CFU/mL reduction was observed after 28d compared to the initial inoculation level in samples treated with 2.0% TO (Table 3). Burt & Reinders (2003) reported that bacteriostatic action of TO against *E. coli* O157:H7 occurred at 0.12%, while a bactericidal effect was noted at 0.25%. Likewise, Solomakos et al. (2008) investigated the antimicrobial effect of 0.3%, 0.6%, or 0.9% TO against *E. coli* O157:H7 in minced beef and found that 0.6% thyme oil inhibited its growth during storage at 10 °C.

CO reduced the viable numbers of *E. coli* O157:H7 by 1.23, 1.42, 1.72 and 2.20 log_{10} CFU/mL in tahini with 0.2, 0.5, 1.0 or 2.0% CO, respectively, at 37 °C after 28d, compared to 1.2 log_{10} CFU/mL in the control samples. At 25 °C, the bacterial reduction was 0.95, 1.06, 1.44 and 1.79 log_{10} CFU/mL by addition of 0.2, 0.5, 1.0 or 2.0% CO respectively, compared to 0.90 log_{10} CFU/mL in the control after 28 d. At 10 °C, numbers of *E. coli* O157:H7 were reduced by 0.80, 0.95, 1.25 and 1.38 log_{10} CFU/mL by addition of 0.2, 0.5, 1.0 or 2.0% CO to tahini, respectively, compared to 0.47 log_{10} CFU/mL at 10 °C in the control (Table 4). In contrast to its activity in tahini, CO was more inhibitory in diluted tahini. At 10 °C, *E. coli* O157:H7 cells were not detectable when 1.0 or 2.0% was used after 21d. Nonetheless, at 25 and 37 °C, the antibacterial activity of CO was more obvious where *E. coli* O157:H7 cells were not detected after 14d when 0.5% was used (Table 5).

CO showed significant inhibitory effect against *E. coli* O157:H7 in both commercial and diluted tahini. However, its activity was higher than TO in diluted tahini where 0.5% CO prevented *E. coli* O157:H7 detection after 14d at 25 and 37 °C. Olaimat et al. (2019) also indicated that CO had higher antimicrobial activity than TO against *Salmonella enterica* in hummus. Similarly, Ceylan et al. (2004) reported that 0.3% CO reduced numbers of *E. coli* O157:H7 in apple juice by 1.6 and 2.0 log_{10}CFU/mL at 8 and 25 °C, respectively.

In diluted tahini, the addition of TO or CO resulted in more inhibitory activity compared to regular tahini and caused significant

Table 4. Effect of cinnamon oil on the viability of *E. coli* O157:H7 (log_{10} CFU/mL) during storage of tahini at: 10 °C (a), 25 °C (b) and 37 °C (c).

Time	Concentration of cinnamon oil (%)				
(Day)	0.0	0.2	0.5	1.0	2.0
a) At 10 °C					
0	4.70 ± 0.07^{aA}	4.70 ± 0.07^{aA}	4.70 ± 0.07^{aA}	4.70 ± 0.07^{bA}	4.70 ± 0.07^{aA}
1	4.73 ± 0.05^{aA}	4.69 ± 0.06^{aA}	4.54 ± 0.06^{bB}	4.54 ± 0.08^{cB}	4.08 ± 0.09^{bC}
3	4.75 ± 0.04^{aA}	4.77 ± 0.04^{aA}	4.70 ± 0.07^{aA}	4.30 ± 0.12^{cB}	3.80 ± 0.01^{cC}
7	4.48 ± 0.08^{bA}	4.40 ± 0.12^{bA}	4.40 ± 0.09^{cA}	4.16 ± 0.04^{dB}	3.80 ± 0.07^{cC}
14	4.40 ± 0.07^{bA}	4.39 ± 0.06^{bA}	4.22 ± 0.07^{dA}	3.99 ± 0.08^{eB}	3.84 ± 0.10^{cC}
21	4.50 ± 0.07^{bA}	4.30 ± 0.07^{bB}	4.09 ± 0.12^{dC}	3.66 ± 0.06^{eD}	3.45 ± 0.11^{dE}
28	4.23 ± 0.13^{cA}	3.90 ± 0.16^{cB}	3.75 ± 0.05^{cB}	3.45 ± 0.09^{fC}	3.32 ± 0.08^{dC}
b) At 25 °C					
0	4.70 ± 0.07^{aA}	4.70 ± 0.07^{aA}	4.70 ± 0.07^{aA}	4.70 ± 0.07^{bA}	4.70 ± 0.07^{aA}
1	4.71 ± 0.07^{aA}	4.60 ± 0.07^{aA}	4.40 ± 0.07^{bB}	4.35 ± 0.06^{bB}	3.87 ± 0.07^{bC}
3	4.75 ± 0.09^{aA}	4.38 ± 0.04^{bB}	4.13 ± 0.08^{cC}	4.08 ± 0.80^{cC}	3.81 ± 0.02^{bD}
7	4.64 ± 0.04^{aA}	4.30 ± 0.07^{bcB}	4.35 ± 0.05^{bB}	4.03 ± 0.04^{cC}	3.90 ± 0.12^{bC}
14	4.33 ± 0.11^{bA}	4.25 ± 0.06^{cA}	3.93 ± 0.04^{dB}	3.70 ± 0.01^{dC}	3.58 ± 0.08^{cC}
21	3.86 ± 0.06^{dAB}	3.95 ± 0.11^{dA}	3.80 ± 0.07^{eB}	3.38 ± 0.08^{eC}	3.30 ± 0.06^{dC}
28	3.79 ± 0.07^{dA}	3.75 ± 0.05^{eAB}	3.64 ± 0.08^{fB}	3.26 ± 0.06^{fC}	2.91 ± 0.14^{eD}
c) At 37 °C					
0	4.70 ± 0.07^{aA}	4.70 ± 0.07^{aA}	4.70 ± 0.07^{aA}	4.70 ± 0.07^{bA}	4.70 ± 0.07^{aA}
1	4.64 ± 0.05^{aAB}	4.67 ± 0.10^{aA}	4.54 ± 0.04^{bB}	4.11 ± 0.07^{bC}	4.08 ± 0.08^{bC}
3	4.23 ± 0.08^{bA}	4.12 ± 0.12^{bAB}	4.07 ± 0.50^{cBC}	3.95 ± 0.05^{cC}	3.69 ± 0.06^{cD}
7	3.93 ± 0.08^{cA}	3.80 ± 0.12^{cA}	3.63 ± 0.08^{dB}	3.53 ± 0.08^{dB}	3.07 ± 0.09^{dC}
14	3.62 ± 0.04^{dA}	3.58 ± 0.04^{dA}	3.40 ± 0.12^{eB}	3.30 ± 0.07^{eB}	3.05 ± 0.09^{dC}
21	3.59 ± 0.11^{dA}	3.55 ± 0.12^{dA}	3.38 ± 0.08^{eB}	3.16 ± 0.07^{eC}	2.53 ± 0.08^{eD}
28	3.50 ± 0.07^{dA}	3.47 ± 0.04^{dA}	3.28 ± 0.08^{eB}	2.98 ± 0.08^{fC}	2.50 ± 0.10^{eD}

Values are the means of 2 experiments (n= 4) ± SD. Values with same capital letters in the same row and with same small letters in the same column are not significantly different ($p \geq 0.05$).

reductions in *E. coli* O157:H7 numbers which reached below the detection level. This could have been due to lower levels of fat in diluted tahini after being diluted 10-fold with water. Similarly, it was reported that CO at a concentration of 0.5% in full fat cheese reduced numbers of *S.* Enteritidis by 0.3 log_{10} CFU/mL at the first day, compared to 3.1 log_{10} CFU/mL in the low fat cheese (Smith-Palmer et al., 2001). EOs are more soluble in the fat phase compared to the aqueous phase; however, the proliferation of organisms usually occurs in the aqueous phase, which may reduce the effectiveness of TO and CO (Burt, 2004).

EO concentration and temperature are major factors that affect the inhibitory action of TO and CO in undiluted and diluted tahini. The antibacterial activity was significantly increased as the storage temperature increased (37 > 25 > 10 °C). The results in the present study are in agreement with previous findings on the activity of TO and CO against pathogenic bacteria. It was reported that the addition of cinnamaldehyde to apple juice reduced *E. coli* O157:H7 cells to undetectable levels after 5 and 14d at 23 and 4 °C, respectively (Baskaran et al., 2010). Additionally, combination of CO with nisin induced greater inhibitory effect against *E. coli* O157:H7 cells in apple juice at 20 °C compared to 5 °C (Yuste & Fung, 2004).

Considering that the TO and CO mainly damage the cytoplasmic membrane of bacteria, altered permeability of the membrane may affect the passive transport of hydrophobic particles and influence protein-protein interactions (Zhang & Rock, 2008). In addition at lower temperatures, permeability of the bacterial membrane is reduced when higher proportions of saturated fatty acids are contained in the membrane and thus decrease the inhibitory activity of EOs (Al-Nabulsi & Holley, 2006; Mani-Lopez et al., 2012).

Although EOs showed substantial inhibitory effects against foodborne pathogens, the presence of a number of ingredients in foods including fat, proteins, carbohydrates, salt, water, preservatives, antioxidants, and some additives may reduce the inhibitory activity of EOs (Perricone et al., 2015). Moreover, another limitation of using EOs as preservatives is their negative impact on flavor since high concentrations are required to achieve satisfactory antimicrobial activity (Al-Nabulsi et al., 2020; Olaimat et al., 2019). This limitation could be circumvented by using lower concentrations of the EOs along with other barriers (e.g low storage temperature, mild heating of the product, or in combination with organic acids) as part of the hurdle technology. Additionally, further research is needed to study the potential of incorporating EOs such as TO and CO in the packaging material used to pack tahini, and to study the effect of such an active package on the viability of pathogens in tahini.

Table 5. Effect of cinnamon oil on the viability of *E. coli* O157:H7 (log_{10} CFU/mL) during storage of diluted tahini at: 10 °C (a), 25 °C (b), and 37 °C (c).

Time	Concentration of cinnamon oil (%)				
(Day)	0.0	0.2	0.5	1.0	2.0
a) At 10 °C					
0	4.65 ± 0.09^{eA}	4.65 ± 0.09^{aA}	4.65 ± 0.09^{aA}	4.65 ± 0.09^{aA}	4.65 ± 0.09^{aA}
1	6.49 ± 0.12^{dA}	4.89 ± 0.07^{aB}	4.51 ± 0.01^{aC}	4.23 ± 0.08^{bD}	4.15 ± 0.11^{bD}
3	6.17 ± 0.10^{cA}	4.55 ± 0.03^{bB}	4.47 ± 0.11^{aB}	3.99 ± 0.09^{cC}	3.15 ± 0.15^{cD}
7	7.03 ± 0.14^{bA}	4.60 ± 0.19^{abB}	4.03 ± 0.11^{bC}	3.33 ± 0.08^{dD}	2.40 ± 0.29^{dE}
14	7.27 ± 0.04^{aA}	4.14 ± 0.15^{cB}	2.84 ± 0.07^{cC}	1.88 ± 0.13^{eD}	$ND^{eE\star}$
21	7.27 ± 0.11^{aA}	3.81 ± 0.08^{dB}	2.42 ± 0.17^{dC}	ND^{fD}	ND^{eD}
28	7.35 ± 0.04^{aA}	3.58 ± 0.25^{dB}	2.05 ± 0.21^{eC}	ND^{fD}	ND^{eD}
b) At 25 °C					
0	4.65 ± 0.09^{eA}	4.65 ± 0.09^{aA}	4.65 ± 0.09^{aA}	4.65 ± 0.09^{aA}	4.65 ± 0.09^{aA}
1	5.68 ± 0.11^{dB}	6.44 ± 0.07^{aA}	5.23 ± 0.08^{aC}	3.73 ± 0.20^{bD}	3.35 ± 0.09^{bE}
3	6.72 ± 0.09^{cA}	6.00 ± 0.13^{bB}	4.51 ± 0.18^{bC}	3.43 ± 0.04^{cD}	3.28 ± 0.08^{bD}
7	7.36 ± 0.08^{bA}	5.39 ± 0.09^{cB}	3.02 ± 0.10^{cC}	2.88 ± 0.29^{dC}	2.04 ± 0.34^{cE}
14	7.19 ± 0.11^{bA}	5.05 ± 0.11^{dB}	ND^{dC}	ND^{eC}	ND^{dC}
21	7.29 ± 0.11^{abA}	4.69 ± 0.07^{eB}	ND^{dC}	ND^{eC}	ND^{Dc}
28	7.25 ± 0.04^{aA}	3.42 ± 0.08^{efB}	ND^{dC}	ND^{eC}	ND^{dC}
c) At 37 °C					
0	4.65 ± 0.09^{eA}	4.65 ± 0.09^{aA}	4.65 ± 0.09^{aA}	4.65 ± 0.09^{aA}	4.65 ± 0.09^{aA}
1	6.71 ± 0.07^{dA}	6.68 ± 0.12^{aA}	4.36 ± 0.04^{bB}	4.20 ± 0.10^{bC}	3.99 ± 0.05^{bD}
3	6.88 ± 0.08^{dA}	5.06 ± 0.18^{bB}	4.17 ± 0.40^{cC}	3.50 ± 0.20^{cD}	2.25 ± 0.11^{cE}
7	7.19 ± 0.12^{dA}	4.94 ± 0.10^{bcB}	2.75 ± 0.08^{dC}	2.75 ± 0.22^{dC}	ND^{eD}
14	7.39 ± 0.09^{cA}	4.82 ± 0.13^{cdB}	ND^{eC}	ND^{eC}	ND^{eC}
21	7.66 ± 0.15^{bA}	4.75 ± 0.09^{cdB}	ND^{eC}	ND^{eC}	ND^{eC}
28	8.00 ± 0.20^{aA}	3.41 ± 0.09^{eB}	ND^{eC}	ND^{eC}	ND^{eC}

*ND: Cells of *E. coli* O157:H7 were not detected (<1 CFU/mL). Values are the means of 2 experiments (n= 4) ± SD. Values with same capital letters in the same row and with same small letters in the same column are not significantly different ($p \geq 0.05$).

4 Conclusion

The current study reports the results of using 14 different essential oils against *E. coli* O157:H7 in tahini and diluted tahini. TO and CO exhibited the strongest antibacterial activity against *E. coli* O157:H7 with a range of inhibition zones from 33.0 to 50.8 mm. In spite of the fact that tahini did not enhance the growth of *E. coli* O157:H7, the pathogen was able to survive in tahini for up to 28d. Nonetheless, diluted tahini permitted enhanced growth ($p \leq 0.05$) of *E. coli* O157:H7 at all incubation temperatures. The addition of TO or CO at ≤ 2.0% is recommended for use by processors of tahini or tahini-based products to inhibit the growth of *E. coli* O157:H7 when stored over a wide temperature range (10 to 37 °C).

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