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Increase in Activity of Essential Oil Components Carvacrol and Thymol against *Escherichia coli* O157:H7 by Addition of Food Stabilizers

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ABSTRACT

The major components of oregano and thyme essential oils that had previously been shown to inhibit *Escherichia coli* O157:H7 were determined by high-performance liquid chromatography with UV detection and liquid chromatographic tandem mass spectrometry. The MICs and MBCs of carvacrol, thymol, *p*-cymene, and γ-terpinene against a strain of *E. coli* O157:H7 phage type 34 isolated from bovine feces were determined by microdilution assay. The constituents were then tested in checkerboard assays to detect possible interactions. Carvacrol and thymol displayed bacteriostatic and bactericidal properties with MICs of 1.2 mmol/liter and were additive in combination. *p*-Cymene and γ-terpinene displayed no measurable antibacterial activity up to 50 mmol/liter, and neither influenced the activity of carvacrol or thymol. Growth curves in the presence of nonlethal concentrations of carvacrol with the addition of agar (0.05%, wt/vol) or carrageenan (0.125%, wt/vol) as stabilizer were produced by optical density measurement. The stabilizers agar and carrageenan both significantly improved the effectiveness of carvacrol in broth, possibly because of a delay in the separation of the hydrophobic substrate from the aqueous phase of the medium. When carvacrol was dissolved in ethanol before addition to broth, stabilizers were not needed. Carvacrol and thymol, particularly when used in combination with a stabilizer or in an ethanol solution, may be effective in reducing the number or preventing growth of *E. coli* O157:H7 in liquid foods.

Controlling the numbers and growth of *Escherichia coli* O157:H7 remains an important objective for the food industry because this pathogen is found in a wide variety of foodstuffs and causes serious outbreaks of foodborne disease *(2, 27)*. Bovine feces are the chief source of contamination, which means that insufficiently heated ground beef, raw dairy products, and leafy vegetables that have been treated with bovine manure are the foods most often associated with human cases of infection *(5)*. More than 5,000 cases of enterohemorrhagic *E. coli* infection per year are recorded worldwide, with the highest incidence in young children. Cases can be severe and are sometimes fatal. Infection with this pathogen can therefore be classed among the most serious of foodborne infections *(45)*.

Recent studies have revealed that the essential oils (EOs) of oregano and thyme are active against strains of *E. coli (3, 4, 11, 15, 39)*. This activity could lead to opportunities for the use of these EOs or their components in improving the safety of certain foods. Chemical analysis of these oils has identified the major components as carvacrol, thymol, *p*-cymene, and γ-terpinene *(21, 25, 26)*, although the composition of the EO from a particular species of plant can differ among harvesting seasons *(1, 26)* and geographical sources *(6, 18)*. A number of constituents of EO exhibit significant antimicrobial properties when tested separately *(20, 22, 42)*. However, there is some evidence that EOs are more strongly antimicrobial than is accounted for by the additive effect of their major antimicrobial components, which may indicate that minor components also play a role and/or that there may be synergy among components *(22, 23, 32, 33, 40)*.

EOs and their components are hydrophobic, whereas many foods have an aqueous phase. This could restrict their potential application in foods. However, it may be possible to delay separation of EO components from the water phase by the addition of food stabilizers, which would prevent coalescence of hydrophobic droplets by increasing the viscosity of the aqueous medium.

The purpose of this study was to compare the composition of two oregano EOs and two thyme EOs and to determine to what extent their activity against *E. coli* O157:H7 is related to the content of carvacrol, thymol, *p*-cymene, and γ-terpinene. Possible interactions among these constituents were also evaluated. The influence of two permitted food stabilizers, agar and carrageenan, on the activity of carvacrol also was investigated.

MATERIALS AND METHODS

Maintenance and preparation of cultures. Cultures of *E. coli* O157:H7 strain rr98089 phage type 34 isolated by our laboratory from bovine feces were maintained on tryptone soy agar slants (Oxoid, Basingstoke, UK) at 4°C. Inocula were prepared by 16-h culture in Mueller-Hinton broth (MHB; Oxoid) at 37°C. This

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E. coli strain harbors the *eae* and *ehly* genes and shows enterohemolysis on enterohemolysin agar but does not carry genes for Shiga toxin production. The strain was chosen as a surrogate for Shiga toxin–producing *E. coli.* Before use, the optical density (OD) of the suspension was measured using a Pharmacia Ultrospec III spectrophotometer at 620 nm, and the suspension was diluted in MHB to the appropriate concentration.

EOs and components. EOs of oregano (*Origanum vulgare*) were obtained from Jacob Hooy (Limmen, The Netherlands) and C. Melchers GmbH (Bremen, Germany). Two thyme (*Thymus vulgaris*) EOs were obtained from C. Melchers GmbH. Carvacrol (98%) and γ-terpinene (97%) were from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), thymol (98%) was from Merck KGaA, (Darmstadt, Germany), and *p*-cymene (>99.5%) was from Fluka Chemie GmbH (Buchs, Switzerland).

HPLC-UV. All solvents were from J. T. Baker (Deventer, The Netherlands) and were degassed before use. Aliquots of 20 μl of 0.5% EO diluted in 40% (vol/vol) acetonitrile were injected into a high-performance liquid chromatography (HPLC) system consisting of a quaternary gradient HPLC pump (type L-7100, Hitachi, Tokyo, Japan), a UV detector (type 785A, Applied Biosystems, Foster City, Calif.), and an autosampler (type Triathlon, Spark-Holland, Emmen, The Netherlands). The HPLC column was a Phenomenex Luna C18 (150 by 4.6 mm, Phenomenex, Torrance, Calif.). Elution was performed at a flow of 0.8 ml/min using a mixture of water (A) and acetonitrile (B) with the following elution characteristics. For the first 2 min there was an isocratic flow with 40% B, from 2 to 17 min B increased to 60%, and then the column was washed with 90% B for 12 min. After 6 min of equilibration, the next sample was injected. The effluent was monitored at 254 nm (for detection of carvacrol, *p*-cymene, and thymol) or 210 nm (for detection of γ-terpinene). Chromatograms were recorded and processed using a Chromquest software package (version 2.51, Thermoquest, San Jose, Calif.). For calibration, standard solutions of 200 to 1,000 μg/ml pure EO components were injected.

LC-MS-MS. For liquid chromatographic tandem mass spectrometry (LC-MS-MS) analysis, 10-μl aliquots of diluted EO solution were injected into an HPLC system consisting of two HPLC pumps (PE200 series, Applied Biosystems, Foster City, Calif.), an autosampler (PE200 series, Applied Biosystems), and an MDS SCIEX API-365 MS detector (Applied Biosystems) equipped with an APCI interface. The LC-MS-MS was controlled by Analyst software (version 1.1, Applied Biosystems). The HPLC column was a Phenomenex Luna C18 (150 by 4.6 mm, Phenomenex), and elution was performed as described. The APCI of the MS operated at an ionization current of 2 μA and a source temperature of 350°C. The entrance, declustering, and focusing potentials were set at 10, −26, and −90 V, respectively. Tandem MS analysis was performed in negative multireaction monitoring mode. The collision energy was set at −35 V. The following traces were monitored to detect carvacrol and thymol: m/z 149.0 → m/z 133.0, m/z 149.0 → m/z 106.0, m/z 149.0 → m/z 91.0. Ionization and consequently detection of *p*-cymene and γ-terpinene was not possible because of the lack of a hydroxyl group on these molecules.

Determination of the MICs and MBCs. The MICs and MBCs of the EOs and their components were determined using a colorimetric broth microdilution technique as previously described *(4),* with small adaptations. Serial dilutions of the substances to be tested were made up in sterile MHB in sterile 96-well microplates (Greiner Bio-One, Alphen aan den Rijn, The Netherlands). To each well was added 80 μl of inoculum and 20 μl alamarBlue (Biosource International, Inc., Camarillo, Calif.). The inoculum was prepared using a 16-h culture adjusted by reference to the OD at 620 nm and further diluted with MHB to achieve approximately 1.25×10^6 CFU/ml, which produced a total bacterial load of approximately 10^5 CFU per well. A positive control (containing inoculum but no EO) and a negative control (containing EO but no inoculum) were included on each microplate. The contents of the wells were mixed, and the microplates were incubated at 37°C for 24 h. A color change from blue to pink or mauve was indicative of bacterial growth. Aliquots of 5 μl from the wells that remained blue were placed on Mueller-Hinton agar (MHA; Oxoid) and incubated for 24 h at 37°C. Three replicates of each microassay were carried out, and the experiment was carried out twice; averages were calculated for MICs and MBCs. The MIC was the lowest concentration at which bacteria failed to grow in MHB but were cultured when plated onto MHA. The MBC was the lowest concentration at which bacteria failed to grow in MHB and were not culturable after plating onto MHA. These definitions have been established by other workers *(39).*

Checkerboard assay for synergism or antagonism. To detect any synergism or antagonism between the EO components, a checkerboard assay was carried out whereby increasing concentrations of one component were dispensed in the rows and increasing concentrations of the other component were dispensed in the columns of a microplate. Inocula and color indicators were added as for the colorimetric assay. Carvacrol was tested against thymol; and carvacrol and thymol were both tested against *p*-cymene and γ-terpinene. MICs were determined for each component in the presence of the second component, and fractional inhibitory concentrations (FICs) were calculated from these as follows: $FIC_{(A)} = MIC_{(A \text{ in the presence of B})}/MIC_{(A \text{ alone})}$ and $FIC_{(B)} = MIC_{(B \text{ in the presence of A})}/MIC_{(B \text{ alone})}$. The FIC index was obtained by adding the individual FICs. The results were interpreted as synergistic when the FIC index was ≤0.5, as additive when the index was between 0.5 and 1.0, as indifferent when the index was between 1.0 and 2.0, and antagonistic when the index was ≥2.0 *(12).*

Effect of stabilizers on the antimicrobial activity of carvacrol. The effect of carvacrol on the growth of the test strain was verified in the presence and absence of the stabilizers with an automated OD reader (software version 2.28, Bioscreen, Oy Growth Curves AB Ltd., Helsinki, Finland). Stock preparations of 1 mol/liter carvacrol were made up in 96% ethanol or by shaking in sterile distilled water. The appropriate test concentrations of carvacrol were made up in MHB, MHB containing 0.1% bacteriological agar no. 1 (Oxoid), or MHB containing 0.25% carrageenan type II (Sigma) (predominantly iota carrageenan), and 100-μl portions were placed in wells in a sterile Bioscreen microplate with a lid. At these concentrations, the stabilizers increased the viscosity of the medium without forming a firm gel *(7, 8).* Aliquots of 100 μl of bacterial suspension containing approximately 10^6 CFU/ml were added to all wells with mixing, and during the 18-h incubation with continuous shaking the OD measurements were automatically plotted in an Excel file. Each experiment was carried out on two different days with three to five replicates each time. A positive growth control containing no EO component was run on every occasion. The maximum specific growth rate, μ_{max}, and time to reach μ_{max} were calculated automatically by the software.

Determination of final bacterial population after incubation with carvacrol. Viable counts were made by serial 10-fold dilutions in sterile physiological salt solution (0.85% sodium chlo-

TABLE 1. *Percent composition of the four major components of essential oils of oregano and thyme and their MICs and MBCs*

EO	Composition (%, vol/vol)[a]						MIC (%, vol/vol)	MBC (%, vol/vol)	Calculated MIC (%, vol/vol)[b]
	Carvacrol	Thymol	*p*-Cymene	γ-Terpinene	Carvacrol + thymol	Other components			
Oregano 1	14.6	27.7	6.1	3.7	42.3	47.9	0.06	0.08	0.04
Oregano 2	12.5	14.6	8.3	6.4	27.1	58.2	0.14	0.14	0.07
Thyme 1	21.2	22.3	5.2	3.8	43.5	47.5	0.05	0.07	0.04
Thyme 2	—[c]	33.0	6.0	11.5	33.0	49.5	0.08	0.08	0.05

[a] Determined by HPLC-UV and LC-MS-MS.
[b] MIC calculated on the basis of the carvacrol plus thymol content and on MICs of carvacrol and thymol (see Table 2).
[c] Percent composition too low to be determined.

TABLE 2. *MIC, MBC, and fractional inhibitory concentration (FIC) index for the four major components of oregano and thyme essential oils*

	MIC (mmol/liter)	MBC (mmol/liter)	FIC index
Carvacrol	1.2	1.4	1.1
Thymol	1.2	1.2	
p-Cymene	>50	>50	
γ-Terpinene	>50	>50	

ride in distilled water) and plating out of appropriate dilutions in duplicate on MHA. Plates were incubated at 37°C for 24 h.

Statistical analysis. For the experiments using stabilizers, the μ_{max} and time to reach μ_{max} were compared in an analysis of variance using SPSS software (version 10.0, SPSS, Chicago, Ill.).

RESULTS

The composition of the four EOs was determined using HPLC-UV and LC-MS-MS, and the results are presented in Table 1 with the MICs and MBCs of the whole EOs. The oil with the highest MIC and therefore the least antibacterial activity had the lowest percentage of carvacrol plus thymol.

The MICs and MBCs of the four major components of the EOs are shown in Table 2. Carvacrol and thymol were active against *E. coli* O157:H7, but no antibacterial activity was detected for *p*-cymene or γ-terpinene. The MBC for carvacrol was slightly higher than the MIC; for thymol, the MBC was the same as the MIC.

The checkerboard assay revealed that the effects of carvacrol and thymol were additive (FIC = 1.1). Further tests with combinations of the four components revealed no antagonism or synergism (data not shown). Because neither *p*-cymene nor γ-terpinene had antibacterial activity, no MICs or FIC indices could be calculated for them.

The effect of the addition of stabilizers on the activity of carvacrol is represented in Figure 1. When a stock preparation of carvacrol shaken in distilled water was mixed with broth to achieve the desired test concentration (rather than first being dissolved in ethanol as in the MIC determination), little effect was achieved up to and including 6 mmol/liter carvacrol. The use of broth containing stabilizer significantly improved the action of carvacrol so that an extension of the lag phase and a reduction in the final population density were achieved compared with the control. Standard deviations for the mean ODs were large (not presented in Fig. 1 to preserve legibility); therefore, the mean μ_{max} and time to μ_{max} were compared (Table 3). The use of agar or carrageenan as stabilizer resulted in significantly better performance of carvacrol compared with no stabilizer, and agar performed significantly better than carrageenan in the reduction of μ_{max}. The stabilizers were not significantly different from each other with respect to the period of time to reach μ_{max} ($P < 0.05$). The use of 4 or 6 mmol/liter carvacrol produced a significant reduction in μ_{max} in the presence of both stabilizers compared with the controls, but 6 mmol/liter carvacrol was not significantly more ef-

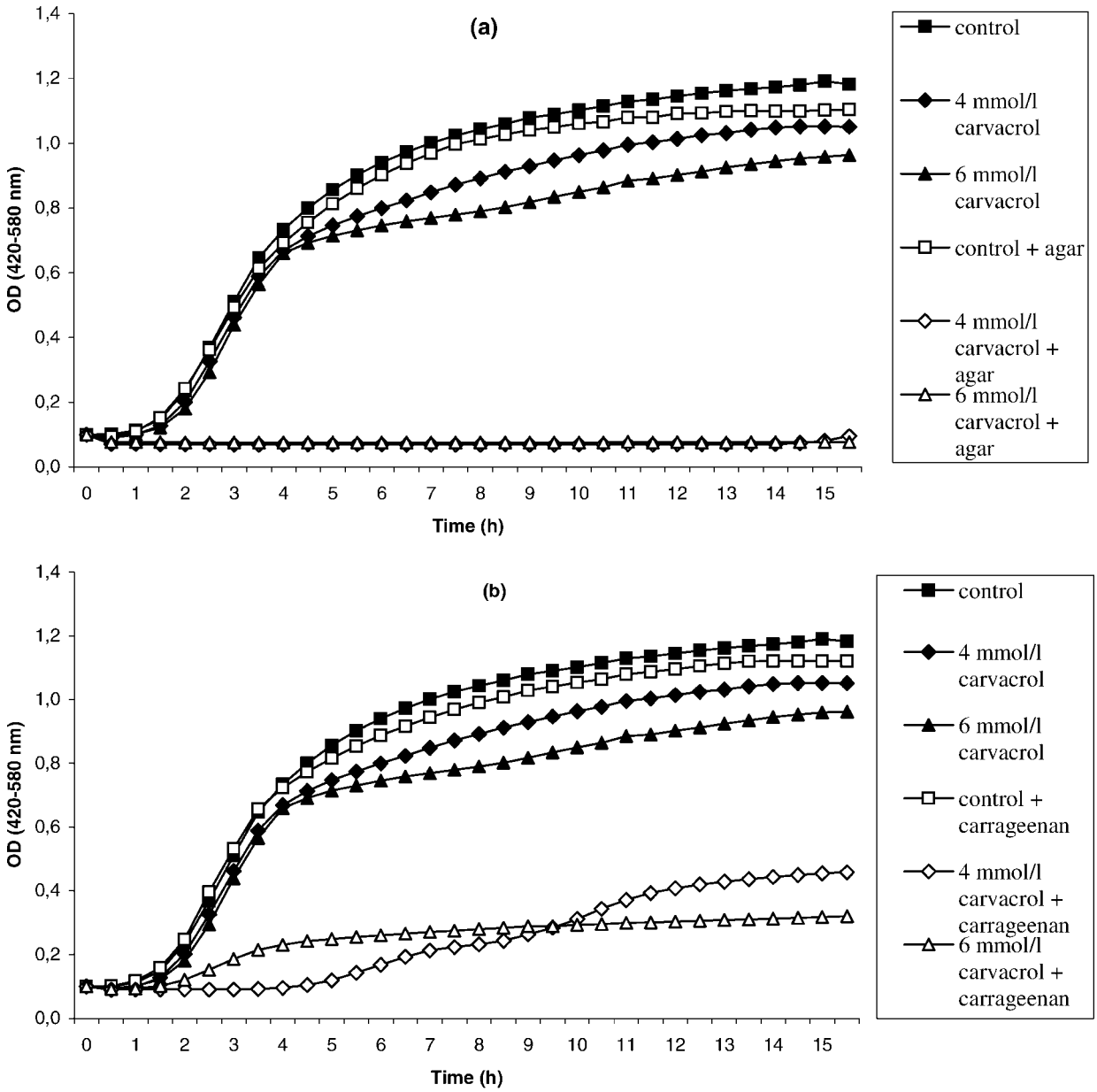

FIGURE 1. *Effect of (a) agar (0 or 0.05%, wt/vol) and (b) carrageenan (0 or 0.125%, wt/vol) as stabilizers on the activity of carvacrol (0, 4, or 6 mmol/liter) against* E. coli *O157:H7 in Mueller-Hinton broth at 37°C. The mean ODs are plotted for the outcomes of three experiments with three replicates each.*

TABLE 3. *Maximum specific growth rate (μ_{max}), time to reach μ_{max}, and final population for* E. coli *incubated in broth containing carvacrol and the stabilizers agar and carrageenan*

Concentration of carvacrol (mmol/liter)	Concentration of stabilizer (%, wt/vol)	μ_{max} (h^{-1})[a]	Time to μ_{max} (h)[a]	Mean population density at 16 h (log CFU/ml)
0 (control)	0 (control)	0.97 ± 0.09 A	2.10 ± 0.21 A	8.6
4	0	0.97 ± 0.03 A	2.33 ± 0.25 A	ND[b]
4	0.05% agar	0.07 ± 0.04 B	8.67 ± 5.47 B	ND
4	0.125% carrageenan	0.42 ± 0.44 C	9.87 ± 4.05 B	2.3
6	0	0.98 ± 0.07 A	2.50 ± 0.00 A	8.3
6	0.05% agar	0.06 ± 0.02 B	11.33 ± 5.31 B	—[c]
6	0.125% carrageenan	0.25 ± 0.37 C	7.07 ± 4.05 B	—[c]

[a] Values are mean ± standard deviation. Means with different letters within a column are significantly different ($P < 0.05$).
[b] ND, not done.
[c] No colonies incubated.

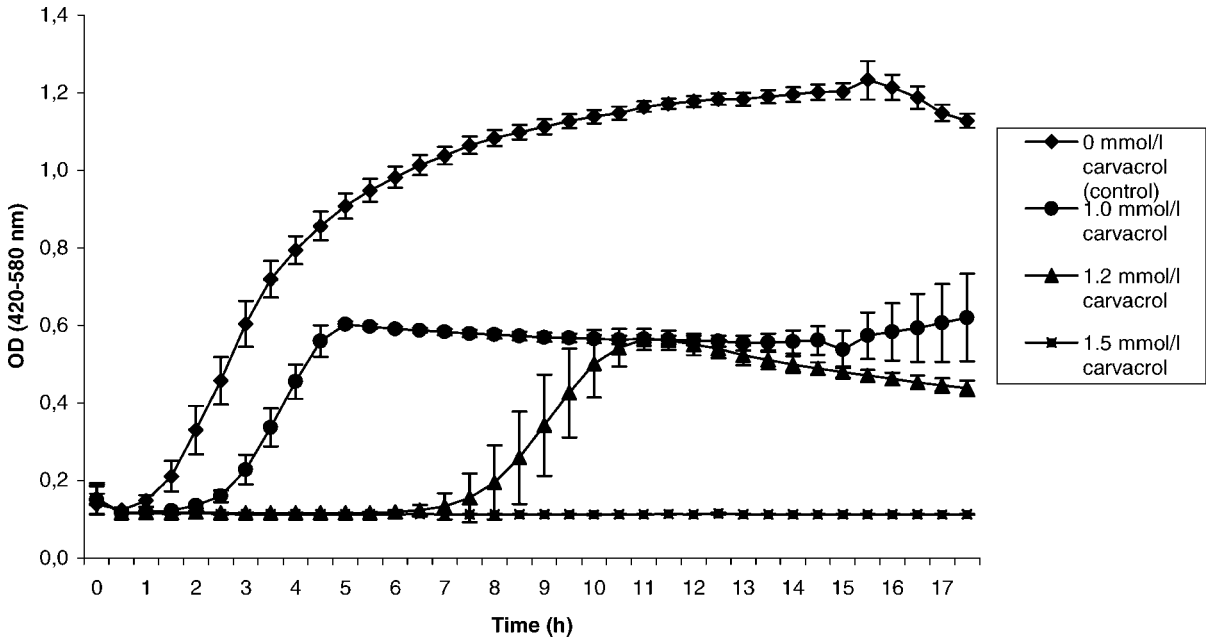

FIGURE 2. *Effect of 0, 1.0, 1.2, and 1.5 mmol/liter carvacrol on the growth of* E. coli *O157:H7 in Mueller-Hinton broth at 37°C after dissolution of the carvacrol in 96% ethanol. Data points represent the mean and standard deviation for two experiments with five replicates each.*

fective than 4 mmol/liter ($P < 0.05$) (Table 3). The use of carrageenan at 0.05% (wt/vol) had no apparent effect on the activity of carvacrol (data not shown).

When the stock preparation of carvacrol was made by solution in 96% ethanol instead of by shaking in distilled water, the antibacterial activity was improved much more than by the action of stabilizers. A concentration-dependent effect was seen (Fig. 2) whereby carvacrol extended the lag phase and reduced both the μ_{max} and the maximum population density in comparison with the untreated control. The mean population densities after 18 h of incubation were 8.6 log CFU/ml for controls, 8.3 log CFU/ml for 1.2 mmol/liter carvacrol, and 2.7 log CFU/ml for 1.5 mmol/liter carvacrol. Population densities for 1.0 mmol/liter carvacrol were not measured. The use of broth containing agar or carrageenan did not further increase the antibacterial activity when carvacrol was dissolved in ethanol (data not shown).

DISCUSSION

The composition of EOs is dependent on many things, including geographical location of the plant, harvesting season, extraction method, and part of the plant used *(1, 10, 17, 26, 28).* This variability is reflected in the range of composition of EOs noted by other workers. Oregano oils have been reported as containing carvacrol, thymol, *p*-cymene, and γ-terpinene in amounts ranging from trace up to 80, 64, 52, and 52%, respectively *(3, 9, 29, 37).* Thyme oils have been reported as containing the same components in ranges of 0 to 11, 10 to 64, 5 to 56, and 2 to 31%, respectively. The proportions of the four components in the oregano oils tested in the present study fall within these ranges. The thymol, *p*-cymene, and γ-terpinene contents of the two thyme oils also were in agreement with reported ranges. Thyme 1 contained more carvacrol than found in previous studies, which is unusual. Thyme 2 lacked carvacrol altogether; this is not common but has been described previously *(3, 9, 18, 29, 37).*

The MICs of the EOs reported here agree very closely with those found by other researchers working with *E. coli* strains in liquid media. MICs of 0.03 to 0.125% (vol/vol equivalent) have been obtained for oils of *O. vulgare (15, 30),* and MICs of 0.03 to 0.08% (vol/vol equivalent) have been obtained for oils of *T. vulgaris (13, 15, 36, 39).*

The MIC for carvacrol is very close to that reported by other workers using strains of *E. coli* in liquid media. When converted from the cited units into mmol per liter, the range is equivalent to 1.5 to 3.3 mmol/liter *(6, 16, 18, 20, 31).* The MIC for thymol obtained in the present study agrees with MICs of 1.2 to 3.0 mmol/liter reported previously *(6, 16, 31).* In another study, the MIC for thymol was between 0.66 and 3.3 mmol/liter *(44).* The range of values in the literature reflects the differences in media composition, methodology, and strains of bacteria used.

To get an impression of the proportion of the antibacterial activity that could be attributed to the carvacrol and thymol content, theoretical MICs for the EOs were calculated based on the carvacrol and thymol content (Table 1). The measured MICs are all slightly higher than the calculated MICs except for oregano 2, which has a much higher MIC (is less antibacterial) than would be expected even based on its low carvacrol plus thymol content. The measured MICs for EOs are slightly higher than the calculated MICs for oregano 1 and both thyme oils because the EOs were shaken in broth before MIC determination and were therefore in suspension, whereas carvacrol and thymol had been dissolved in ethanol and remained in solution when added to the test broth. Droplets of EO in suspension grad-

ually coalesce and separate from the aqueous phase (broth), which limits the activity of the EO. Individual components in solution cannot coalesce and separate and would presumably therefore be in closer contact with the bacteria in the aqueous phase. In oregano 2, the proportion of minor components (i.e., those other than carvacrol, thymol, *p*-cymene, and γ-terpinene) was higher than in the other three oils (58.2% versus 47.5 to 49.5%). In this EO, other minor components may have had an antagonistic effect on carvacrol and/or thymol. This phenomenon has been suggested to occur in certain varieties of thyme and oregano *(23, 25, 32).*

No antagonism or synergism was detected between the four major components. The lack of synergy between carvacrol and *p*-cymene in this study is in contrast to the findings of a study carried out with gram-positive organisms in which a particular combination of carvacrol and cymene was synergistic when tested against *Bacillus cereus* in buffer and on rice *(43).* Carvacrol and *p*-cymene also exhibited a synergistic effect on the viability of *Listeria monocytogenes (33).* Ultee et al. *(41, 43)* postulated that the synergy between carvacrol and cymene when acting on *B. cereus* depends on cymene expanding the cell membrane, resulting in membrane destabilization that allows carvacrol to enter the cell and exert an antibacterial effect. *E. coli* and other gram-negative organisms possess an additional outer cell membrane *(34)* that may inhibit the action of cymene. In most studies, EOs and their components have been more effective against gram-positive than against gram-negative organisms, and cymene does not appear to have an effect on gram-negative bacteria *(3, 11, 18, 19).* The lack of synergy between cymene and carvacrol or thymol in this study could therefore be due to physiological differences between gram-positive and gram-negative bacteria, which could give an indication about their mode of action.

Carvacrol and thymol were additive in their activity against this strain of *E. coli,* as has been reported for *Staphylococcus aureus* and *Pseudomonas aeruginosa (22).* These two components have a very similar chemical structure consisting of a system of delocalized electrons and a hydroxyl group, which makes it likely that they have a similar mechanism of antimicrobial activity *(41).*

To our knowledge, there have been no other studies on the effect of carrageenan on the antibacterial activity of EOs or their components, although the effect of agar on whole EOs has been studied. Agar at 0.2% (wt/vol) was sufficient to achieve a stable dispersion for oregano and clove EOs in MIC determinations with *E. coli, Bacillus megaterium, Salmonella* Hadar, and *S. aureus* and performed better than Tween 80 or ethanol *(35).* A concentration of 0.15% (wt/vol) agar produced a more stable emulsion with tea tree oil than was obtained with Tween 20, Tween 80, ethanol, or dimethyl sulfoxide *(24),* and 0.05% (wt/vol) agar markedly improved the action of oregano and thyme oils *(4).* In the present study, stabilizers significantly reduced the μ_{max} and time to reach μ_{max} for carvacrol. We hypothesized that the flexible three-dimensional matrix formed by the polysaccharide molecules entraps droplets of the hydrophobic EO or EO component and forms a physical barrier that retards their separation from the aqueous phase. This action would extend the period of time that the EO is in contact with bacterial cells, thereby improving the antibacterial activity. This hypothesis is supported by work carried out with gelatin gels and EOs. Surface treatment of cooked ham with up to 6% (vol/vol) cilantro oil was significantly effective against *L. monocytogenes* only when the EO was applied in a 7% (wt/vol) gelatin gel coating. The authors supposed that the immobilization of the cilantro oil in the gel held the oil in contact with the ham and thereby increased the effectiveness of the treatment *(14).* Limitation of diffusion of oregano EO by structural effects caused by gelatin were also thought to have been significant in a study with *Salmonella* Typhimurium. However, the EO was less effective in the gelatin gel than in broth. This reduction in the antibacterial effect was attributed to the limitation of diffusion because of the high concentration of gelatin (10%, wt/vol) *(38).* Perhaps there is an optimum level of structural effect on EOs, i.e., restriction of coalescence in liquid media is useful to increase the antibacterial activity but too much restriction of diffusion, e.g., in solid gels, may be counterproductive.

The large standard deviations in μ_{max} for carrageenan and time to reach μ_{max} for agar and carrageenan (Table 3) may have been caused by the fact that the solution in wells had to be mixed by hand using a pipette to achieve an adequate suspension of carvacrol in the growth medium. Handmixing is difficult to standardize, and because EOs exert their antibacterial effect within 1 to 5 min *(4),* slight variations in mixing can have a large influence on the parameters, therefore increasing the standard deviation.

When carvacrol was dissolved in ethanol before use, the presence of a stabilizer did not further improve the antibacterial activity. The concentration-dependent antibacterial effect of carvacrol when dissolved in ethanol has also been established for *L. monocytogenes* and *B. cereus* and with thymol for *P. aeruginosa (33, 43, 44).*

The result of this study confirm that carvacrol and thymol are the major antibacterial components of oregano and thyme EOs and that they have an additive antibacterial effect on *E. coli* O157:H7 strain rr98089 phage type 34. The activity of the carvacrol and thymol in these oils appears to be related to the antibacterial activity of the whole oil and is not influenced by the presence of the other two major components, *p*-cymene and γ-terpinene, which have no apparent antibacterial effect on this *E. coli* strain. However, other minor components can have an antagonistic effect on the action of carvacrol and thymol.

The addition of agar or carrageenan stabilizers to an aqueous medium markedly improved the antibacterial action of carvacrol by significantly reducing μ_{max}, extending the time to μ_{max}, and reducing the final population density. This effect is presumed to be due to the delay in separation of the hydrophobic carvacrol from the aqueous broth medium and thereby the prolongation of contact between carvacrol and bacterial cells. Dissolution of carvacrol in ethanol before use improved the antibacterial activity even more than the use of stabilizers, presumably because the carvacrol could come into closer association with the bacterial cells in the aqueous phase than was possible in the

presence of stabilizers. Oregano and thyme EOs, carvacrol, and thymol have possible uses as food preservatives. The addition of stabilizers could improve the performance of carvacrol or thymol in liquid foods when the use of ethanol as a solvent is not feasible and where lower concentrations are desirable for organoleptic reasons.

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