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Solid- and Vapor-Phase Antimicrobial Activities of Six Essential Oils: Susceptibility of Selected Foodborne Bacterial and Fungal Strains

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The antimicrobial activity of essential oils (EOs) of cinnamon (*Cinnamon zeylanicum*), clove (*Syzygium aromaticum*), basil (*Ocimum basillicum*), rosemary (*Rosmarinus officinalis*), dill (*Anethum graveolens*), and ginger (*Zingiber officinalis*) was evaluated over a range of concentrations in two types of contact tests (solid and vapor diffusion). The EOs were tested against an array of four Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, and *Listeria monocytogenes*), four Gram-negative bacteria (*Escherichia coli*, *Yersinia enterocolitica*, *Salmonella choleraesuis*, and *Pseudomonas aeruginosa*), and three fungi (a yeast, *Candida albicans*, and two molds, *Penicillium islandicum* and *Aspergillus flavus*). The rationale for this work was to test the possibility of creating a protective atmosphere by using natural compounds that could extend the shelf life of packaged foodstuffs while minimizing organoleptic alterations. In the solid diffusion tests, cinnamon and clove gave the strongest (and very similar) inhibition, followed by basil and rosemary, with dill and ginger giving the weakest inhibition. The fungi were the most sensitive microorganisms, followed by the Gram-positive bacterial strains. The Gram-negative strain *P. aeruginosa* was the least inhibited. The composition of the atmosphere generated by the EOs, and their minimum inhibitory concentrations (MICs), were determined using a disk volatilization method, in which no inhibition from rosemary or basil was observed. Cinnamon and clove, once again, gave similar results for every microorganism. As a general rule, MIC (fungi) ≪ MIC (bacteria) with no clear differences between Gram-positive or -negative strains except for *P. aeruginosa*, which was not inhibited by any of the EOs in the vapor phase. The atmosphere generated from the EOs was analyzed by means of solid-phase microextraction combined with gas chromatography−ion trap mass spectrometry. Differences among the volatiles in the EOs, which may be responsible for the differences in their antimicrobial performances, were found.

KEYWORDS: Essential oils; comparative evaluation; vapor-phase antimicrobial activity; minimum inhibitory concentration; atmospheric composition

INTRODUCTION

Many food products are perishable and require protection from microbial spoilage during their shelf life. Traditional food preservation techniques or the use of refrigeration alone cannot ensure the quality and safety of all foods, and alternative preservation techniques, such as modified atmosphere packaging (MAP), the use of pulsed light, electric, or magnetic fields, high pressure, irradiation, or a wide range of food-grade chemicals, are being applied or investigated for their preservative potential (*1*−*5*). Nevertheless, food processors and consumers have expressed a desire to reduce the use of both aggressive techniques and synthetic chemicals in food, mainly because the presence of chemical residues in foods and labeling of preservatives in food packages are major concerns nowadays. Therefore, there is a clear need for new methods of preserving food using natural additives, and a very interesting option is the use of essential oils (EOs) as antimicrobial additives, because they are rich sources of biologically active compounds.

EOs are mainly obtained by steam distillation from various plant sources. The antimicrobial activity of EOs has been extensively studied and demonstrated against a number of microorganisms, mostly in vitro rather than in tests with food, and usually using a direct-contact antimicrobial assay. In these tests EOs are brought into contact with the selected microorganisms, and their inhibition is monitored by means of direct inspection or by measuring a physical property that is directly

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related with microorganism growth, such as optical density, impedance, or conductance (*6*−*11*).

Screening methods mainly consist of adding a known volume of an undiluted or diluted (mostly with ethanol or methanol) EO directly to a test tube or well containing an appropriate medium and the selected microorganism (*12*) or to sterile media and then adding the microorganism (*13*). A very popular development of this procedure is known as the disk diffusion test, in which EOs are added to blank, sterile disks of known size (*14*). Spiked disks are usually added after inoculation of the medium, after which the inhibition zones are measured, giving an indication of the antimicrobial strength.

In solid disk diffusion tests, the contact between the antimicrobial agent and the inoculated media (or foodstuff) takes place on the surface, thus simulating real-life situations better than tube or well approaches. Nevertheless, there is still an underlying assumption that EOs are to be added to the foodstuff per se rather than to the packaging, and even if they are added to the packaging, they will exert their effects solely via direct contact, rather than via the volatiles in the vapor phase, which may not always be the case. There is a general lack of scientific information concerning the antimicrobial effectiveness of EOs in the vapor phase compared with direct contact, although some degree of inhibition by volatile components of EOs has been demonstrated (*15*−*18*).

It is also potentially interesting to correlate the composition of the atmosphere generated by EOs with their antimicrobial behavior. Solid-phase microextraction (SPME) is a convenient and completely solvent-free technique in which a small fused-silica fiber with a polymeric coating is used to extract analytes from sample matrices. SPME methods have been developed for a wide range of vapor-monitoring applications, and they can be regarded as passive and diffusive sampling methods (*19*, *20*), thus avoiding the need for the air-sampling pumps or aspirators that are typically used in dynamic or active methods.

Thus, the work presented in this contribution had three main aims: first, to check the effectiveness of selected EOs against different microbial strains in the solid phase by means of the disk diffusion method; second, to test the EOs' activities in the vapor phase to evaluate their effectiveness when there is no direct contact with food; third, to characterize the atmosphere generated by the most effective EOs in the disk diffusion tests, to find correlations with their antimicrobial performance.

MATERIALS AND METHODS

Microbial Cultures. The following foodborne microbial strains were selected for their relevance in the food industry: the Gram-positive bacteria *Staphylococcus aureus* (American Type Culture Collection, ATCC 29213), *Bacillus cereus* (Colección Española de Cultivos Tipo, CECT 495), *Enterococcus faecalis* (ATCC 29212), and *Listeria monocytogenes* (ATCC 7644); the Gram-negative bacteria *Escherichia coli* (ATCC 29252), *Yersinia enterocolitica* (CECT 4315), *Salmonella choleraesuis* (CECT 4000), and *Pseudomonas aeruginosa* (ATCC 27853); the yeast *Candida albicans* (ATCC 64550); and the molds *Penicillium islandicum* (CECT 2762NT) and *Aspergillus flavus* (CECT 2687).

The strains were stored at −18 °C in sterilized skimmed milk and subcultured as follows. Gram-positive bacteria were subcultured in Mueller−Hinton agar at 30 °C for 48 h except for *B. cereus*, which was subcultured in Mueller−Hinton blood agar. Gram-negative bacteria were grown in Mueller−Hinton agar at 30 °C for 24 h. Fungi were subcultured in Sabouraud cloramphenicol agar at either 30 °C for 48 h (the yeast *C. albicans*) or 36.5 °C for 7 days (the molds *P. islandicum* and *A. flavus*).

Essential Oils and Chemicals. The essential oils were supplied by ARTIBAL (Sabiñánigo, Spain). Oils from the following plant species were tested in this work: *Cinnamon zeylanicum* [cinnamon, Chemical Abstracts Service (CAS) Registry number 805-91-6], *Syzygium aromaticum* (clove, CAS 801-98-25), *Ocimum basilicum* (basil, CAS 8015-73-4), *Rosmarinus officinalis* (rosemary, CAS 8000-25-7), *Anethum graveolens* (dill, CAS 8006-75-5), and *Zingiber officinalis* (ginger, CAS 8007-08-7).

The oil constituents were identified by comparing mass spectra obtained from them with those reported in the National Institute of Standards and Technology (NIST) gas chromatography−ion trap mass spectrometry (GC-IT-MS) database. In addition, the identity of the main constituents was confirmed by GC-IT-MS analysis of standard compounds. The compounds used and their corresponding CAS numbers were the following: *trans*-cinnamaldehyde 99% (14371-10-9), β-caryophyllene 99.5% (87-44-5), bornyl acetate 95% (5655-61-8), estragol 98% (140-67-0), borneol 98% (464-43-7), α-pinene 98% (80-56-8), thymol 99.5% (89-83-8), 1,8-cineole 99% (470-82-6), D-limonene 97% (5989-27-5), camphor 96% (76-22-2), benzyl benzoate 99% (126-51-4), linalool 97% (78-70-6), eugenol 99% (97-53-0), β-pinene 99% (8172-673), camphene 95% (79-92-5), α-humulene 99.5% (6753-98-6) supplied by Sigma-Aldrich (Bellefonte, PA); α-cubenene 97% (17699-14-8), α-copaene 90% (3856-25-5), (−)-verbenone 97% (1196-01-6), γ-terpinene 97% (99-85-4), α-terpinolene 97% (582-67-9), α-phellandrene 95% (4221-98-1), α-terpinene 95% (99-86-5) supplied by Fluka (Bellefonte, PA), and α-terpineol 98% (562-74-3) supplied by Chem Service (West Chester, PA).

Antimicrobial Activity Tests. *Solid Diffusion Assays.* A plastic Petri dish (90 mm diameter) containing the appropriate solidified medium was inoculated with 100 μL of a physiological saline solution containing 10^5 colony-forming units (CFU)/mL of the microorganism under study. Three microliters of the undiluted EO was added to a 5 mm diameter sterile blank filter disk, placed on top of the cultured media. After incubation under optimal conditions (temperature and time), the average diameter of two different zones was measured: first, the zone where no growth of the microorganism was detected, called total inhibition, and then the zone where growth of the microorganism was significantly reduced in terms of amount of colonies when compared to blank assays. All analyses were carried out in triplicate.

Vapor Diffusion Assays. Solidified medium was inoculated as described above, that is, with 100 μL of the physiological saline solution containing 10^5 CFU/mL of the microorganism under study. Each pure essential oil was diluted in ethyl ether (GC quality, Merck, Darmstadt, Germany) to obtain serial dilutions down to 1% (v/v). Then, 10 μL of each dilution was added to 10 mm sterile blank filter disks and placed on the medium-free cover of each Petri dish. The Petri dishes were then sealed using sterile adhesive tape (Deltalab, Rubí, Spain). No hermetic sealing was needed because experiments were designed to simulate a worst-case situation, when leaking of the active components to the atmosphere can occur, thus increasing probability for microorganism contamination. Blanks were prepared by adding 10 μL of ethyl ether to the filter disks and had no effect on the viability of any of the tested organisms.

After the incubation period, the minimal inhibitory concentration (MIC, expressed as microliters of EO per volume unit of atmosphere above the organism growing on the agar surface) that caused apparent inhibition by comparison with control tests was measured. Following these measurements, the dishes were incubated for a further 35 days under the same temperature conditions either unchanged, that is, without removing the antimicrobial atmosphere generated (to check whether the protective effects were temporary or prolonged), or following removal of the antimicrobial atmosphere generated by replacing the filter disk and cover with another sterile cover (to check whether the effects were static or cidal). If microorganisms start to grow after removal, there was a static effect, whereas if no growth occurs (i.e., the inhibition percentage remains constant with time), the effect was cidal.

The plates were checked for signs of growth every 7 days. All tests were performed in triplicate.

Solid-Phase Microextraction. Fully retracted SPME fibers (Supelco, Bellefonte, PA), coated with a 100 μm layer of polydimethylsiloxane (PMDS) for cinnamon, basil, and rosemary EOs or with an 85 μm layer of polyacrylate (PA) for clove EO, were used in all of the

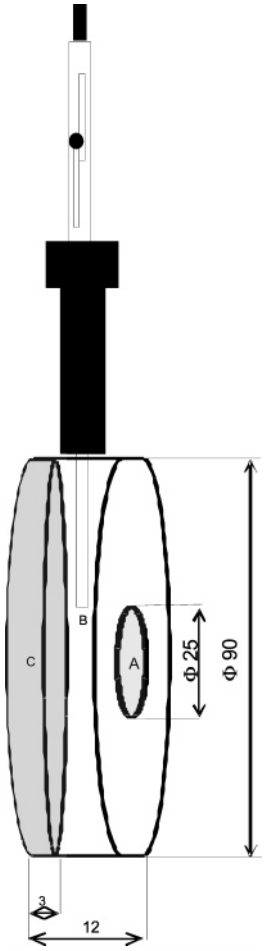

Figure 1. Scheme of the experimental setup to sample the atmosphere generated by the EOs: A, paper disk impregnated with 10 μL of EOs; B, fully retracted SPME fiber; C, solid agar medium. All dimensions are in millimeters.

experiments based on the results of previous studies (*21*). In these preliminary studies the potential utility of polydimethylsiloxane/divinylbenzene (PDMS/DVB) and Carbowax fibers was also investigated.

The atmosphere generated in the vapor diffusion assays was measured by placing a fully retracted SPME fiber into the headspace of the Petri dish, as shown in **Figure 1**. Sampling was performed over 24 h. The volume of the atmosphere sampled on every occasion was 57.3 cm^3.

Gas Chromatography−Ion Trap Mass Spectrometric (GC-IT-MS) Analysis. GC-IT-MS analyses were carried out using a Varian CP 3800 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with a VF-5 MS (Varian) column (60 m × 0.25 mm, 0.25 μm film thickness) coupled to a Saturn 2000 ITMS detector, a split−splitless injector (operated in splitless mode, splitless time = 2 min) with a 0.8 mm i.d. SPME specific liner (Varian), and a MS version 6.03 Chemstation. The carrier gas was helium (C-50, Carburos Metálicos, Zaragoza, Spain) at a constant flow rate of 1.0 mL/min.

Two different sets of chromatographic conditions were used, according to a previous study (*21*). For cinnamon, rosemary, and basil EOs, the injector temperature was held at 250 °C, whereas the oven temperature was initially held at 45 °C for 1 min, linearly increased by 2 °C/min to 85 °C, by 5 °C/min to 170 °C, then finally by 15 °C/min to 200 °C, and held for 2 min. For clove analysis, the injector temperature was 265 °C, and the oven program was as follows: initial temperature, 45 °C, held for 1 min; 15 °C/min to 90 °C; 5 °C/min to 170 °C; then 5 °C/min to 200 °C, and held for 15 min.

The MS was operated in electron impact (EI) ionization mode, and complete scans from 40 to 350 amu (atomic mass units) were recorded. Compounds were identified by matching their mass spectra in the NIST commercial library (purity criterion, >85%). When available, the retention times and fragmentation spectra of pure standards (>95%) were obtained for confirmation. All analyses were carried out in triplicate.

Statistical Analysis. The triplicate data obtained are presented in **Figure 2** as error bars and in **Tables 1** and **3** as means ± standard deviation (SD). Significant differences between average MICs for each individual microorganism were determined by Student's *t* test at the 95% significance level using WinStat 2.0 (R. Fitch Software, Staufen, Germany).

RESULTS AND DISCUSSION

In a first series of assays, the antimicrobial activities of the EOs under study were screened by means of solid diffusion tests. **Table 1** shows the results obtained. The antibacterial activities of the clove and cinnamon EOs were the highest, and similar, followed by rosemary and basil, whereas dill and ginger gave little or no protection. As for microorganisms, molds were the most strongly (completely) inhibited, followed by yeast, and the bacteria were the least inhibited. Gram-negative strains were somewhat less inhibited than Gram-positive strains, but the differences were not statistically significant, as shown in **Table 1**.

Following these tests, cinnamon, clove, basil, and rosemary EOs were selected for further evaluation in the vapor phase as described under Materials and Methods. Pure EOs of rosemary and basil showed no inhibitory activity toward any of the microorganisms in the vapor phase tests. Therefore, rosemary and basil EOs have antimicrobial activity only in direct contact, so subsequent studies were performed with cinnamon and clove EOs. **Table 2** shows the results obtained. The MIC, defined as the lowest concentration inhibiting visible growth of the test organism (*22*, *23*), was calculated in microliters of essential oil per liter of the atmosphere sampled. Cinnamon and clove EOs inhibited all of the organisms in these tests except *P. aeruginosa.* The lower susceptibility of *P. aeruginosa* may be due to the distinctive properties of its outer membrane (*24*−*26*).

As a general rule, inhibition in the vapor phase was weaker than in the solid diffusion tests. Good correlations between the results of the solid diffusion and vapor phase tests were found for all of the Gram-positive bacteria except *E. faecalis*. For this organism the cinnamon EO gave a stronger inhibition than the clove EO in the vapor tests (with MICs of 52.4 and 87.3 μL/L, respectively), although the two EOs gave similar results in the solid diffusion tests. For Gram-negative strains similar results were obtained with *E. coli*, because the cinnamon EO was more inhibitory than clove EO in the vapor phase (with MICs of 17.5 and 26.2 μL/L, respectively), although the two EOs were equally inhibitory in the solid diffusion tests. Surprisingly, however, clove EO inhibited *S. choleraesuis* and *Y. enterocolitica* more strongly than cinnamon EO (with MICs of 52.4 versus 131 μL/dm^3 and 8.73 versus 17.5 μL/L, respectively) in the vapor phase, although the two EOs were equally inhibitory in the solid phase. For fungi, results obtained from both tests were highly correlated. Inhibition curves (i.e., the percentage of inhibition versus EO concentration) were recorded for each microorganism, as shown in **Figure 2**. The percentage of inhibition means the percentage of growth compared with control treatment without oil, that is, % inhibition = $100 - (T/C \times 100)$, where T is colony diameter after exposure to the vapors generated by essential oils and C is control, exposed only to vapor generated by diethyl ether. Despite the differences in MICs mentioned above, similar curves were obtained, as confirmed by statistical studies: no significant differences were found at the 95% significance level (using Student's *t* test) between clove and cinnamon EOs for *A. flavus* and *S. aureus*.

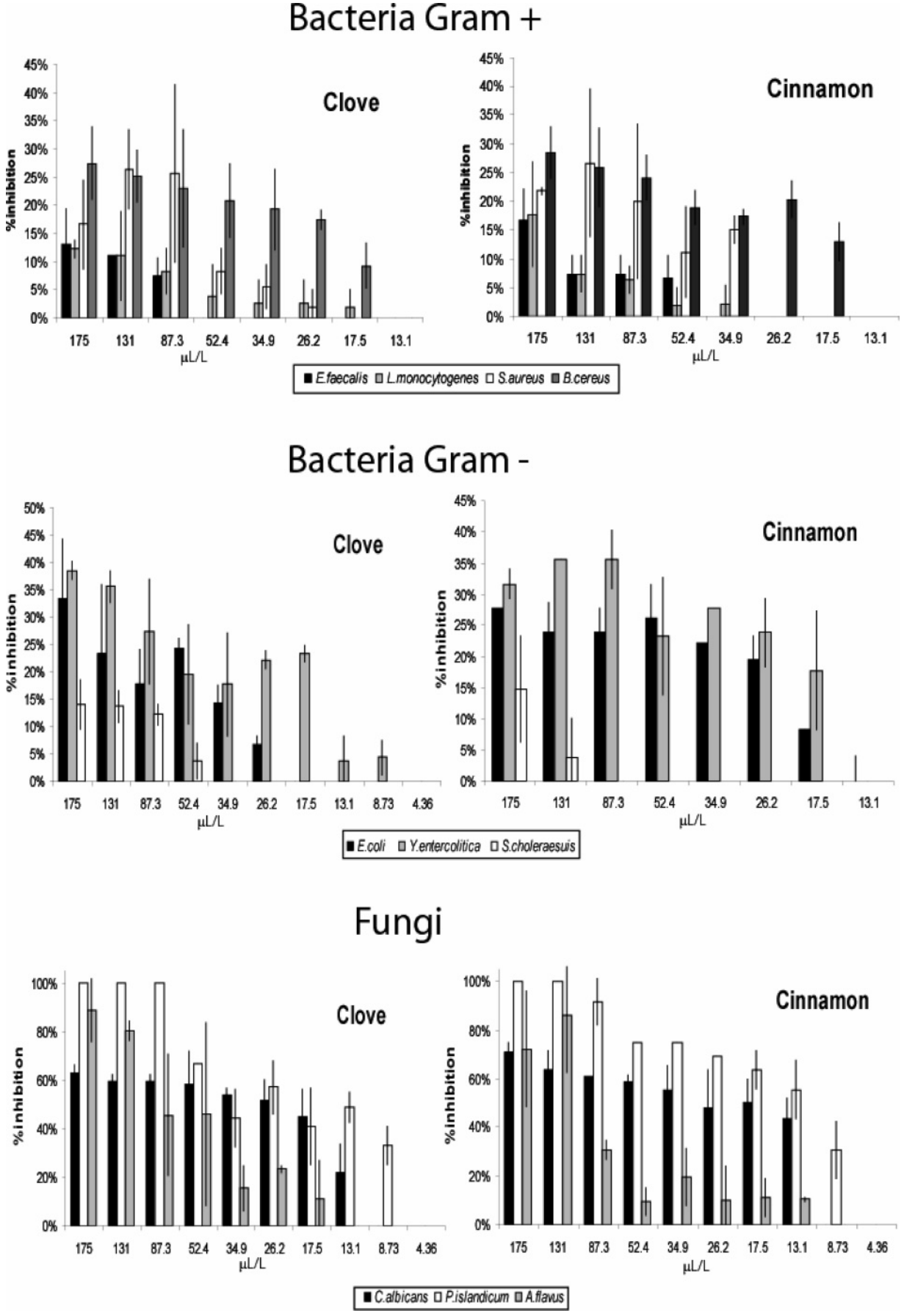

Figure 2. Effect of essential oils in the vapor phase on the test organisms as a function of concentration ($n = 3$). Y-error bars, standard deviation; % inhibition $= 100 - (T/C \times 100)$.

Table 1. Antimicrobial Activities of Pure Essential Oils, Solid Diffusion Test: Total Inhibition, mm (Delay Effect, mm)[a,b]

	microorganism	clove	cinnamon	rosemary	basil	ginger	dill
Gram-postive	*S. aureus*	20 (25)	19 (23)	10 (13)	12 (15)	10 (12)	15 (17)
		5 (7)	*2 (4)*	*4 (4)*	*4 (4)*	*3 (5)*	*2 (2)*
	L. monocytogenes	23 (25)	18 (20)	13 (15)	16 (17)	0	0
		9 (8)	*6 (8)*	*5 (3)*	*6 (4)*	*0*	*0*
	E. faecalis	15 (17)	15 (17)	7 (9)	12 (14)	0	0
		0 (2)	*2 (1)*	*4 (3)*	*2 (3)*	*0*	*0*
	B. cereus	22 (25)	23 (25)	7 (9)	0	6 (7)	0
		4 (6)	*4 (5)*	*0 (3)*	*0*	*1 (1)*	*0*
Gram-negative	*E. coli*	15 (17)	15 (18)	7 (10)	9 (10)	0	0
		2 (3)	*2 (2)*	*1 (3)*	*1 (3)*	*0*	*0*
	Y. enterocolitica	20 (25)	18 (20)	11 (15)	12 (15)	0	8 (10)
		4 (6)	*3 (2)*	*3 (3)*	*2 (3)*	*0*	*1 (3)*
	S. choleraesuis	16 (19)	16 (19)	7 (9)	10 (12)	0	0
		4 (7)	*4 (7)*	*3 (4)*	*2 (1)*	*0*	*0*
	P. aeruginosa	11 (13)	11 (13)	7 (7)	8 (8)	0	0
		2 (3)	*1 (2)*	*2 (2)*	*2 (2)*	*0*	*0*
Yeast	*C. albicans*	40 (45)	39 (45)	14 (16)	10 (15)	12 (15)	12 (15)
		1 (3)	*10 (10)*	*7 (9)*	*2 (3)*	*1 (3)*	*0 (2)*
Molds	*A. flavus*	90	90	0	0	0	0
		0	*0*	*0*	*0*	*0*	*0*
	P. islandicum	90	90	0	0	0	0
		0	*0*	*0*	*0*	*0*	*0*

[a] Total inhibition means no growth of microorganism. Delay means colonies affected by the EO compared to blank tests. An inhibition of 90 mm represents total inhibition (Petri dish diameter); 0 mm represents no inhibition (inhibition zone is included in the delay zone reported). [b] Both inhibition and delay diameters are the means of three different observations taken from three different experiments. Standard deviations of both are given in italics.

Table 2. Antimicrobial Activity of the Atmosphere Generated by Clove and Cinnamon Essential Oils: Minimum Inhibitory Concentration (MIC, $\mu L_{EO}/L_{headspace}$)[a]

		MIC (μL/L)	
	microorganism	clove	cinnamon
Gram-positive	*S. aureus*[b]	26.2	34.9
	L. monocytogenes[c]	17.5	34.9
	E. faecalis[c]	87.3	52.4
	B. cereus[b]	17.5	17.5
Gram-negative	*E. coli*[c]	26.2	17.5
	Y. enterocolitica[c]	8.73	17.5
	S. choleraesuis[c]	52.4	131
	P. aeruginosa[b]	no inhibition	no inhibition
Yeast	*C. albicans*[b]	13.1	13.1
Molds	*A. flavus*[b]	17.5	13.1
	P. islandicum[b]	8.73	8.73

[a] Comparison was performed between means of MICs obtained with clove and cinnamon EOs for each microorganisms by using Student's t test at 95% ($p < 0.05$) significance level. [b] No significant differences. [c] Significant differences.

Gram-negative strains are generally more resistant than Gram-positive strains in solid diffusion tests, and this trait has been attributed to the external lipopolysaccharide wall that surrounds the peptidoglycan cell wall of the former. However, this does not seem to be a major factor in vapor-phase inhibition, because there were no significant differences overall in inhibition between Gram-positive and Gram-negative bacteria in the vapor-phase tests. Furthermore, *E. faecalis* (Gram-positive) was the most resistant to clove oil, whereas *S. choleraesuis* (Gram-negative) was least inhibited by cinnamon oil.

Whether the observed inhibition was static or cidal was elucidated as described under Materials and Methods. As shown in **Figure 3**, the effect was found to be cidal (inhibition percentage remains constant with time after removal of the antimicrobial atmosphere) for all of the organisms except *A. flavus*. In this case, a reduction in percentage inhibition during the second week was observed, after which it remained constant for the rest of the testing period. To check the ability of EOs toprovide prolonged protection, tests were prolonged over 35

Table 3. Composition of the Atmosphere Generated by the Essential Oils (Expressed as Percentages of Total Ion Counts Identified, $n = 3$)

peak no.	compd name[a]	essential oil: cinnamon	basil	rosemary	clove	identification
1	α-pinene	1.1 ± 0.4	0.1 ± 0.0	3.8 ± 0.7		*b*
2	camphene	0.4 ± 0.2		1.8 ± 0.4		*b*
3	β-pinene	0.8 ± 0.2	0.3 ± 0.0	4.8 ± 0.9		*b*
4	β-myrcene		0.2 ± 0.0	0.6 ± 0.1		*b*
5	α-phellandrene	1.2 ± 0.6				*b*
6	*p*-cymene	1.2 ± 0.1		1.3 ± 0.6		*b*
7	limonene	0.9 ± 0.5	0.2 ± 0.0	1.0 ± 0.4		*b*
8	1,8-cineole	1.2 ± 0.1	6.5 ± 0.7	48 ± 9.1		*b*
9	(*Z*)-ocimene		0.9 ± 0.2			*c*
10	γ-terpinene			0.3 ± 0.0		*b*
11	β-terpineol			0.1 ± 0.1		*c*
12	α-terpinolene			0.2 ± 0.0		*b*
13	L-fenchone		0.3 ± 0.1	-		*c*
14	linalool	3.0 ± 0.4	1.6 ± 0.4	1.6 ± 0.6		*b*
15	fenchol		0.2 ± 0.1			*c*
16	camphor	0.4 ± 0.3	1.1 ± 0.4	17 ± 4.0		*b*
17	menthone		0.2 ± 0.0			*c*
18	citronellal	0.2 ± 0.1				*c*
19	borneol	0.2 ± 0.0	0.2 ± 0.0	2.9 ± 1.5		*b*
20	isopinocamphone			0.3 ± 0.1		*c*
21	menthol		1.6 ± 0.0			*c*
22	1-terpinen-4-ol	0.3 ± 0.2		1.0 ± 0.5		*c*
23	α-terpineol	0.5 ± 0.1		2.1 ± 1.3		*b*
24	estragol	1.7 ± 0.5	82 ± 1.2	1.5 ± 0.4	0.2 ± 0.1	*b*
25	verbenone			0.1 ± 0.0		*b*
26	fenchyl acetate		0.2 ± 0.0			*c*
27	pulegone		0.1 ± 0.0			*c*
28	(*Z*)-anethole		0.1 ± 0.0			*c*
29	*(E)-cinnamaldehyde*	0.4 ± 0.0				*b*
30	bornyl acetate		0.2 ± 0.0	1.7 ± 0.2		*b*
31	(*E*)-anethole		0.4 ± 0.3			*c*
32	safrole	2.8 ± 0.9	0.1 ± 0.0			*c*
33	**thymol**	0.1 ± 0.0			0.1 ± 0.0	*b*
34	*(E)-cinnamyl alcohol*	0.1 ± 0.0				*c*
35	α-cubebene	0.1 ± 0.0		0.1 ± 0.0		*b*
36	eugenol	67 ± 12	0.5 ± 0.0	0.1 ± 0.0	82 ± 2.2	*b*
37	benzenepropylactetate	0.1 ± 0.0				*c*
38	α-copaene	2.1 ± 0.7		0.4 ± 0.2		*b*
39	methyl ether eugenol		0.2 ± 0.1			*c*
40	β-caryophyllene	8.6 ± 1.7	0.1 ± 0.0	6.8 ± 3.7	10 ± 2.0	*b*
41	α-bergamotene		2.0 ± 0.1			*c*
42	α-aromadendrene	0.1 ± 0.0		0.1 ± 0.0		*c*
43	*(E)-cinnamyl acetate*	0.7 ± 0.1				*c*
44	α-humulene	1.7 ± 0.6	0.1 ± 0.0	0.8 ± 0.1	2.9 ± 0.5	*b*
45	α-alloaromadendrene			0.2 ± 0.0		*c*
46	β-cubebene		0.1 ± 0.1			*c*
47	γ-muurolene	0.2 ± 0.0				*c*
48	ledene	0.2 ± 0.0				*c*
49	α-muurolene	0.1 ± 0.1				*c*
50	γ-cadinene		0.2 ± 0.0	0.2 ± 0.0		*c*
51	eugenol acetate	0.6 ± 0.5		0.2 ± 0.0	0.5 ± 0.3	*c*
52	δ-cadinene	0.3 ± 0.1		0.3 ± 0.1	0.4 ± 0.1	*c*
53	**(*Z*)-calamenene**	0.1 ± 0.0			0.3 ± 0.0	*c*
54	benzyl benzoate	1.1 ± 0.4				*b*
	not identified	0.5 ± 0.2	1.1 ± 0.1	0.6 ± 0.8	0.5 ± 0.2	
	total	100 ± 1.2	101 ± 1.0	99 ± 1	100 ± 3.3	

[a] Compounds that appear in only cinnamon and clove EO headspace are shown in bold characters, whereas those appearing in only cinnamon are shown in italics. [b] Identification was based on comparison of the compounds' MS spectra with spectra in the NIST library, along with those of standard compounds. [c] Identification was based on comparison of the compounds' MS spectra with spectra in the NIST library.

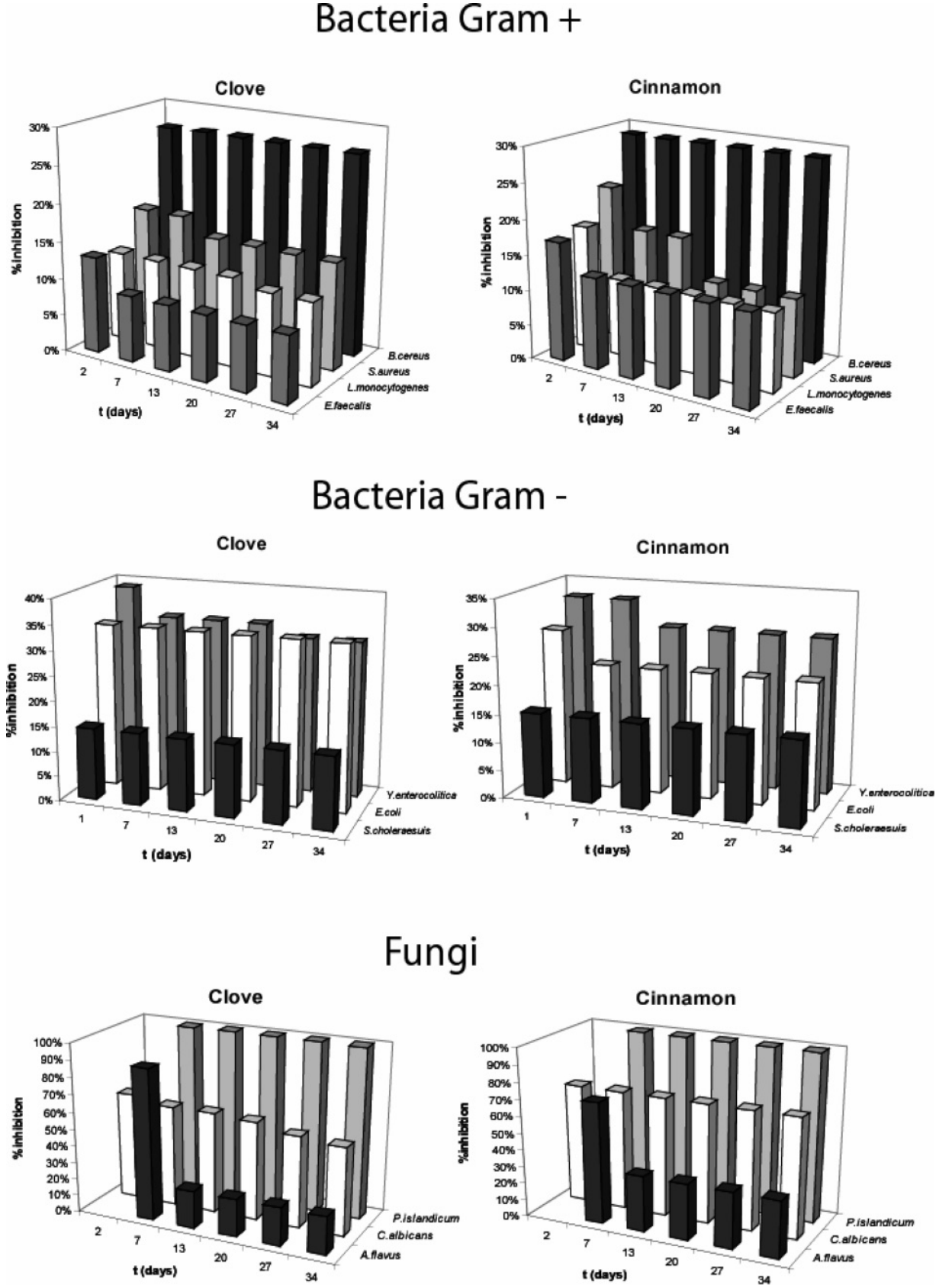

Figure 3. Results of testing whether the effects were static or cidal, showing percentage of inhibition as a function of test time ($n = 3$). % inhibition $= 100 - (T/C \times 100)$.

days. No growth was observed for any of the microorganisms except *A. flavus*, confirming the static hypothesis for the latter organism.

Figure 4 and **Table 3** show the results of SPME analysis of the headspace over the different essential oils. As can be seen, a number of potentially interesting chemicals, especially terpenes

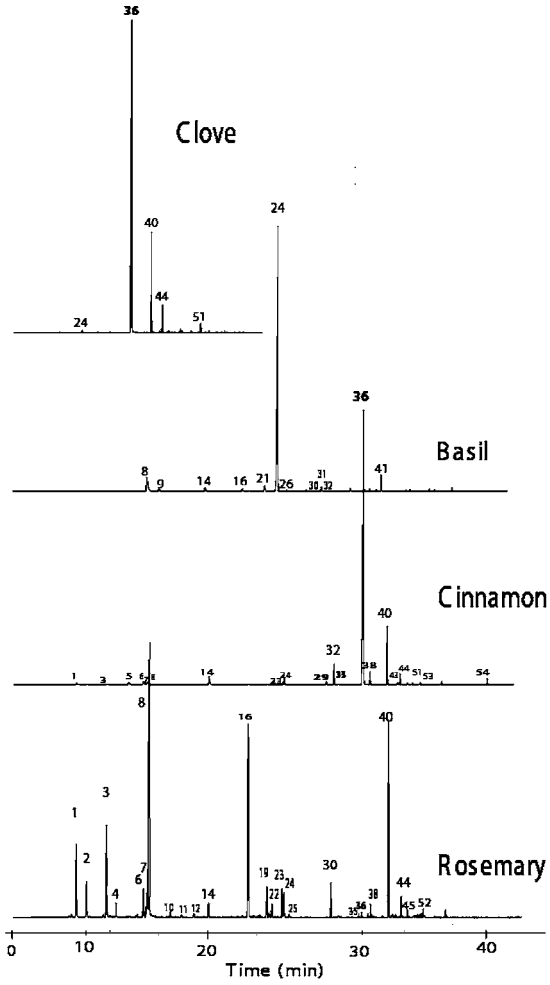

Figure 4. GC-IT-MS chromatograms of four essential oils evaluated in this study as antimicrobial agents in the vapor phase: clove, basil, rosemary, and cinnamon. For peak identification, see **Table 3**.

and phenol derivatives with well-known antimicrobial activity (*27*−*30*), were detected in the generated atmospheres, and the differences in their composition are clearly of interest.

Because the clove and cinnamon oils were the most effective, there should be some differences in the atmosphere they generate compared to the other EOs. As shown in **Table 3** and **Figure 4**, two compounds were exclusively detected in these EOs, that is, calamenene and thymol, which have been described as potentially interesting antimicrobial compounds. Given its high relative concentration in these EOs compared to those in the basil and rosemary EOs, eugenol seems to be the most relevant compound, although minor compounds have been reported to have very important antimicrobial effects in some cases (*6*, *10*). It is also interesting that estragol had no relevance in the antimicrobial effects of the tested EOs in the vapor phase, as demonstrated by basil being totally ineffective. Other relevant compounds found in the cinnamon and clove EOs are β-caryophyllene and α-humulene. Their relative concentrations in both of these EOs are similar to those in rosemary, which generated an atmosphere with no apparent antimicrobial effect. Therefore, according to literature data (*31*), the antimicrobial activity of these compounds is lower than that of eugenol.

A number of interesting compounds have been detected in cinnamon but not in clove. Among them, (*E*)-cinnamaldehyde and two derivatives, alcohol and acetate, as well as α-phellandrene and citronellal were detected in only cinnamon. Cinnamaldehyde is well-known to have antimicrobial activity (*32*), so it could be responsible for differences in the effects of the two EOs, but further experiments are needed to test this hypothesis.

In this study, cinnamon and clove oils in the vapor phase were shown to have significant antimicrobial activity. Thus, the vapor-phase approach appears to be promising as a control protocol and could be applied in active packaging, creating a protective atmosphere with minimum organoleptic alteration of the packaged foodstuffs. For this purpose, further studies related to the absorbance of volatile compounds in food matrices are required. Cidal effects were observed for all of the test organisms, except *A. flavus*, for which the effects were static, and the effects were shown to last for at least 35 days.

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