BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Food Microbiology

Antimicrobial activities of six essential oils commonly used as condiments in Brazil against *Clostridium perfringens*

Marcela Radaelli[a], *Bárbara Parraga da Silva*[a], *Luciana Weidlich*[a], *Lucélia Hoehne*[b], *Adriana Flach*[c], *Luiz Antonio Mendonça Alves da Costa*[c], *Eduardo Miranda Ethur*[b,*]

[a] *Centro de Ciências Biológicas e da Saúde, Centro Universitário UNIVATES Lajeado, RS, Brazil*
[b] *Centro de Ciências Exatas e Tecnológicas, Centro Universitário UNIVATES, Lajeado, RS, Brazil*
[c] *Departamento de Química, Universidade Federal de Roraima Boa Vista, RR, Brazil*

ARTICLE INFO

Article history:
Received 12 August 2014
Accepted 2 October 2015
Available online 2 March 2016

Associate Editor: Mariza Landgraf

Keywords:
Food-borne disease
Antimicrobial activity
Clostridium perfringens
Spices
Essential oils

ABSTRACT

Despite recent advances in food production technology, food-borne diseases (FBD) remain a challenging public health concern. In several countries, including Brazil, *Clostridium perfringens* is among the five main causative agents of food-borne diseases. The present study determines antimicrobial activities of essential oils of six condiments commonly used in Brazil, viz., *Ocimum basilicum* L. (basil), *Rosmarinus officinalis* L. (rosemary), *Origanum majorana* L. (marjoram), *Mentha* × *piperita* L. var. Piperita (peppermint), *Thymus vulgaris* L. (thyme) and *Pimpinella anisum* L. (anise) against *C. perfringens* strain A. Chemical compositions of the oils were determined by GC–MS (gas chromatography–mass spectrometry). The identities of the isolated compounds were established from the respective Kováts indices, and a comparison of mass spectral data was made with those reported earlier. The antibacterial activity was assessed from minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the microdilution method. Minimum inhibitory concentration values were 1.25 mg mL^{-1} for thyme, 5.0 mg mL^{-1} for basil and marjoram, and 10 mg mL^{-1} for rosemary, peppermint and anise. All oils showed bactericidal activity at their minimum inhibitory concentration, except anise oil, which was only bacteriostatic. The use of essential oils from these common spices might serve as an alternative to the use of chemical preservatives in the control and inactivation of pathogens in commercially produced food systems.

* *Corresponding author.*
E-mail: eduardome@univates.br (E.M. Ethur).
http://dx.doi.org/10.1016/j.bjm.2015.10.001

Introduction

Food-borne disease (FBD) is characterized by diverse symptoms that arise from consumption of contaminated foods or beverages. Despite the recent advances in food production technology and processing, FBD remains a major cause of morbidity and mortality, constituting both an important public health concern and a significant economic problem at a global level.[1–4]

One of the most common causes of FBD is the Gram-positive, anaerobic, spore-forming bacterium *Clostridium perfringens* (family Clostridiaceae), which is widely distributed in the environment and in foodstuffs. The spores of this bacterium, compared to the vegetative cells, are very robust and resistant to heating, drying, pH and certain toxic compounds. This allows the microorganism to persist until conditions become favorable for germination and growth. There are five strain-types of *C. perfringens*, designated as A–E. Each of them produces a unique spectrum of exotoxins. Type A strains of the bacterium cause food poisoning in the classical form, while the strains of type C cause necrotic enteritis – a disorder that can be severe and is often fatal but, fortunately, its occurrence is rare. The microorganism prefers substrates, such as meat products, poultry, and sauces, which contain high level of moisture and rich in protein. These are the main classes of food materials involved in occurrence of this disease. Specific factors that favor the spread of this agent are prolonged cooling and non-refrigerated storage, wherein sausage, canned fish, pate, cheese and fermented oyster provide ideal conditions for the development of the bacterium.[1,2,5]

Many of the spices commonly used in food not only improve palatability and flavor but also assist in the preservation of the food itself. The antimicrobial activity of such spices is bestowed mainly by the essential oils that contain terpenoids hydrocarbons, alcohols, aldehydes, ketones, phenolics and their derivatives. The quantity, quality, and chemical profiles of the essential oils derived from a single plant species can vary considerably according to the geographic origin, climatic conditions, soil composition, the part of the plant used, and the age and season when the material is collected. Besides, drying process and storage time can alter, quantitatively, and qualitatively, the essential oil composition.[6] While the antimicrobial activities of essential oils are well reported, the mechanisms of their action are not yet fully understood, although there might be several different microbial target sites.[7,8]

In the present study, the essential oils of some of the spices most commonly used in Brazil, namely, *Ocimum basilicum* L. (basil), *Rosmarinus officinalis* L. (rosemary), *Origanum majorana* L. (marjoram), *Mentha* × *piperita* L. var. Piperita (peppermint), *Thymus vulgaris* L. (thyme) and *Pimpinella anisum* L. (anise), were assessed with regard to their antimicrobial activities against *C. perfringens* strain A. The criteria for selection of plants were their popular use as spices, availability to common people, and ensuring that organic production methods have been used in accordance with the Law: 10,831.[9] The chemical compositions of the essential oils were determined by GC–MS (gas chromatography–mass spectrometry), and minimum inhibitory and bactericidal concentrations were determined using the microdilution method.

Materials and methods

Plant materials

Dried, fragmented leaves of basil, rosemary, marjoram, peppermint, and thyme and dried fruits of anise were purchased commercially in São Paulo, Brazil, in October 2013. All samples were acquired in accordance with the terms of expiry mentioned on the labels.

Extraction of essential oils

Dried leaves of basil, marjoram, peppermint, rosemary and thyme, and the dried fruits of anise were powdered (10–20 mesh) and samples were extracted by hydro-distillation (plant:water ratio 1:10, w/v) for 3.5 h in a modified Clevenger apparatus. The oily phase was removed, dried over anhydrous sodium sulfate, and stored in a freezer at –20 °C. Microbiological analyses were performed seven days after the extraction.

Chemical compositions of essential oils

Essential oil samples were submitted for GC–MS analysis to the Laboratory of Chromatography, Energy Research Center, Federal University of Roraima, Boa Vista, RR, Brazil. The analyses were performed using Shimadzu GC2010 system with an autoinjector AOC-20i and Plus mass detector QP2110, and equipped with an HP5-MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm). The chromatographic conditions were as follows: carrier gas, helium at a flow rate of 1.02 mL min^{-1}; oven temperature programmed initially at 60 °C and increased to 310 °C at a ramp of 3 °C min^{-1}; injector temperature, 220 °C; injector mode in split ratio of 1:20 with 2 mL min^{-1} purge; MS interface temperature, 280 °C; ion source temperature, 260 °C; and ionization energy, 70 eV. The oil samples (15 mg) were dissolved in 1.5 mL of purified ethyl acetate and 1 μL volume of that was injected for analysis. The isolated compounds were identified by their respective Kováts retention indices determined in reference to a series of *n*-alkanes, and verified by a comparison of mass spectral data with those obtained using pure standards and with those reported in the literature,[10] and eventually by comparing their mass spectra with the GC–MS spectral library (Wiley 8 and FFNSC 1.2 libraries).

For GC-FID, HP-5 MS column (30 m × 0.25 × 0.25 μm) at same temperature as that of GC–MS, using hydrogen and nitrogen carrier gas, was used. The FID temperature was 260 °C. The relative compositions of the oils were calculated from the peak areas (uncorrected for specific response factors) of the isolated compounds.

Antimicrobial activities of essential oils

The minimum inhibitory concentration (MIC) and the MBC of the oil samples were assessed against type A strain of

C. perfringens ATCC 13124 (a gas gangrene isolate) using the microdilution method.

Standardization of inoculum

C. perfringens (ATCC 13124) was revived according to standard procedures in tryptose sulfite-cycloserine agar (Oxoid) with D-cycloserine (Sigma) under anaerobic conditions at 36 ± 1 °C for 24 h.[11] The bacterial concentration in the inoculum was standardized at 0.5 on the McFarland turbidity scale, equivalent to 10^8 CFU mL^{-1}. An aliquot (1 mL) of this suspension was transferred to a sterile tube and the volume was adjusted to 10 mL with sodium chloride solution (0.8%, w/v) to obtain a concentration of 10^7 CFU mL^{-1}. Working inoculums were prepared by transferring 200 μL aliquots of this suspension to three test tubes and adjusting the volumes to 10 mL with reinforced clostridial medium (RCM; Oxoid) to give final concentrations of 2.0×10^5 CFU mL^{-1}.[12]

Minimal inhibitory concentrations

All microbiological assays were performed under anaerobic conditions. MIC determinations were performed in 96-well microplates according to procedures described by the Clinical and Laboratory Standards Institute.[13] Each essential oil (200 mg) was dissolved in dimethyl sulfoxide (40 μL) and the volume was made to 5 mL with sterile RCM containing 1% Tween 80 to provide a stock solution containing 40 mg mL^{-1} of oil. Serial twofold dilutions of each essential oil stock were made with RCM to yield final concentrations ranging from 20 to 0.625 mg mL^{-1}. The diluted samples (100 μL) were transferred to microplate wells and mixed well with the micropipette. Chloramphenicol was employed as a positive control in the concentration range 0.1–0.003125 mg mL^{-1}, while the negative controls comprised sterile RCM either alone or with dimethyl sulfoxide (at concentrations used in the dilutions). In order to ascertain aseptic conditions, the control wells contained sterile RCM but without inoculum. The inoculated microplates were incubated at 36 ± 1 °C for 48 h under anaerobic conditions; and the bacterial growth was confirmed by adding 10 μL of a sterile 0.5% aqueous solution of triphenyltetrazolium chloride (TTC, Sigma–Aldrich) and incubating at 36 °C for 30 min.[14] The viable bacterial cells reduced the yellow TTC to pink/red 1,3,5-triphenylformazan (TPF). All assays were performed in triplicate.

Minimum bactericidal concentrations

MBCs were determined by inoculating the assay mixtures from the wells showing no microbial growth onto the surface of sterile Shahidi-Ferguson Perfringens agar medium as recommended by the Ministério da Agricultura, Pecuária e Abastecimento.[15] The plates were incubated under anaerobic conditions for 24 h in an oven maintained at 36 ± 1 °C and subjected to visual inspection. The presence of microbial growth on the medium indicated that the essential oil possessed bacteriostatic activity, while the absence of the growth implied bactericidal activity of the oil sample.

Results and discussion

Yields and chemical profiles of the essential oils

The yields of the essential oils were 0.24% for basil, 1.57% for rosemary, 0.47% for marjoram oil, 0.49% for peppermint, 0.97% for thyme and 1.29% for anise.

As shown in Table 1, the most abundant compounds in all of the essential oils were oxygenated compounds, especially oxygenated monoterpenes and phenylpropanoids. The combination of oxygenated monoterpenes and sesquiterpenes, phenylpropanoids and alcohols accounted 82.17% of the oxygenated compounds for *O. basilicum*, 96.56% for *R. officinalis*, 69.39% for *O. majorana*, 96.05% for *Menta* × *piperita*, 92.96% for *T. vulgaris* and 97.87% for *P. anisum*.

In the analyzed oil samples, most of the compounds were identified unambiguously from the Kováts index and mass spectral data (Table 1). For basil oil, the major compounds were linalool, methyl chavicol and 1,8-cineole, with trace amounts of α-*trans*-bergamotene and *epi*-α-cadinol. Hussain, Anawar, Sherazi, and Przybylski analyzed the essential oils from the aerial parts of *O. basilicum* harvested in different seasons and found significant variations in the major compounds including linalool (56.70–60.60%), α-bergamotene (7.60–9.20%), γ-cadinene (3.20–5.40%) and *epi*-α-cadinol (8.60–12.40%).[16] Linalool was reported as the major component (66.40%) of the oil extracted from one of the three botanical varieties and cultivars of *O. basilicum*, while the other two contained α-*trans*-bergamotene (6.84–7.96%).[17]

Rosemary oil contains two major compounds, 1,8-cineole and camphor, together with borneol and α-terpineol in much smaller amounts. In a recent evaluation of the antimicrobial activity of *R. officinalis*, Jiang et al. identified 1,8-cineole (26.54%), α-pinene (20.14%), camphor (12.88%), camphene (11.38%) and β-pinene (6.95%) as the main constituents of the essential oil. In marjoram oil, the major components were terpinen-4-ol, *trans*-sabinene hydrate, γ-terpinene and α-terpineol.[18] Busatta et al. evaluated the antimicrobial activity of the essential oil from dried leaves of *O. majorana* used in a processed food, and found main constituents as terpinen-4-ol (30.41%), γ-terpinene (13.94%), *cis*-sabinene hydrate (9.64%), and α-terpineol (4.47%).[19]

In peppermint oil, the major constituents are menthol and carvone with minute amounts of menthone and menthyl acetate. Tyagi and Malik reported the main constituents of *M. piperita* oil as menthol (19.10%), iso-menthone (14.80%), menthone (14.80%), limonene (10.60%), iso-menthol (8.80%), menthyl acetate (6.60%), β-pinene (5.60%) and α-pinene (4.80%).[20] The essential oil of thyme analyzed herein contained borneol and α-terpineol as the major compounds, together with trace amounts of thymol and carvacrol. Rota, Herrera, Martinez, Sotomayor, and Jordan determined the chemical composition and antimicrobial activity of the essential oil of *T. vulgaris* (thymol chemotype) and identified the main constituents as thymol (57.70%), *p*-cymene (18.70%) and carvacrol (2.80%).[21]

The oil derived from the dried fruits of anise contained almost entirely (95.59%) *E*-anethole. Tepe et al. studied the antioxidative and antimicrobial activities of *P. anisetum* and

Table 1 – Chemical composition of the essential oils obtained from the spices.

Compounds[a]	Kováts index		*Ocimum basilicum* (area %)[d]	*Rosmarinus officinalis* (area %)	*Origanum majorana* (area %)	*Mentha* × *piperita* (area %)	*Thymus vulgaris* (area %)	*Pimpinella anisum* (area %)
	KI_{exp}[b]	KI_{lit}[c]						
Sabinene	981	975			3.14			
β-Pinene	984	979	0.56	0.44	0.23	0.43	0.19	
Myrcene	993	991		0.42	0.55			
3-Octanol	997	991				0.18		
α-Phellandrene	1007	1003			0.19			
α-Terpinene	1017	1017		0.11	4.84			
p-Cymene	1024	1025		1.39	1.56		0.93	
β-Phellandrene	1029	1030			1.51			
Limonene	1029	1029				0.32	0.25	
1,8-Cineole	1031	1031	9.55	57.39	0.19	2.13		
γ-Terpinene	1059	1060			8.27		0.41	
cis-Sabinene hydrate	1067	1070			2.62			
Terpinolene	1089	1089			1.56			
trans-Sabinene hydrate	1100	1098			10.14			
Tetrahydrolinalool	1101	1099	0.83					
Linalool	1104	1099	42.10				2.33	
trans-Pinene hydrate	1122	1123			2.52			
cis-Pinene hydrate	1140	1144			1.64			
Camphor	1144	1146	0.59	27.94			0.23	
Camphene hydrate	1149	1150		0.12				
Menthone	1154	1153				6.22		
Iso-menthone	1164	1163				1.90		
Borneol	1166	1169	0.55	5.74			47.59	
Neo-menthol	1166	1166				3.16		
Menthol	1178	1172				35.33		
Terpinen-4-ol	1178	1177	0.65	0.82	40.85	0.85	1.48	
Iso-menthol	1185	1182				0.30		
α-Terpineol	1192	1189	1.19	3.99	7.25	0.36	25.04	
cis-Piperitol	1197	1196			0.66			
Dihydrocarveol	1198	1194				2.19		
cis-Dihydrocarvone	1199	1193				3.17		
Safranal	1199	1197						1.50
Methyl chavicol	1201	1196	11.85					
trans-Dihydrocarvone	1205	1201				0.66	0.24	
trans-Piperitol	1209	1208			0.80			
trans-Carveol	1221	1217				0.18		
cis-Carveol	1234	1229				0.40		
Pulegone	1240	1237				2.60		
Carvone	1244	1243	0.26			28.39		
Carvacrol methyl ether	1245	1245					1.10	
trans-Sabinene hydrate acetate	1253	1256			0.32			
Z-Anethole	1254	1253						0.07
Piperitone	1255	1253				0.61		
Linalyl acetate	1256	1257			1.56			
2-Phenylethyl acetate	1256	1258						0.71
Neo-menthyl acetate	1276	1274				0.25		
Isobornyl acetate	1286	1286	0.81	0.56			2.19	
E-Anethole	1287	1285				1.04		95.59
Menthyl acetate	1295	1295				4.46		
Thymol	1296	1290					5.31	
Carvacrol	1306	1298					5.52	
Z-Methyl cinnamate	1306	1300	0.50					

Table 1 – (*Continued*)

Compounds[a]	Kováts index		*Ocimum basilicum* (area %)[d]	*Rosmarinus officinalis* (area %)	*Origanum majorana* (area %)	*Mentha* × *piperita* (area %)	*Thymus vulgaris* (area %)	*Pimpinella anisum* (area %)
	KI_{exp}[b]	KI_{lit}[c]						
Eugenol	1359	1359	0.60					
E-Methyl cinnamate	1384	1379	2.00					
β-Boubornene	1385	1388				0.37		
β-Elemene	1393	1391	0.59					
Methyl eugenol	1407	1404	3.19					
E-Caryophyllene	1420	1419	0.97	0.25	4.70	1.21	3.66	
α-*trans*-Bergamotene	1437	1435	6.89					
Aromadendrene	1440	1441	0.39					
α-Humulene	1454	1455	0.62					
β-Chamigrene	1478	1478						0.20
Bicyclogermacrene	1497	1494			2.18			
α-Bulnesene	1506	1510	0.44					
Germacrene D	1515	1485					0.30	
α-Cadinene	1515	1514	2.89					
β-Cadinene	1525	1523					0.25	
Spathulenol	1578	1578	0.61		0.84	0.62		
Caryophyllene oxide	1584	1583					1.12	
Viridiflorol	1592	1590				0.88		
α-Cadinol	1656	1654				0.17		
epi-α-Cadinol	1663	1640	6.89				0.81	
Grouped components (%)								
Monoterpene hydrocarbons			0.56	2.36	21.85	0.75	1.78	
Oxygenated monoterpenes			56.53	96.56	68.55	93.16	91.03	1.50
Sesquiterpene hydrocarbons			12.79	0.25	6.88	1.58	4.21	0.20
Oxygenated sesquiterpenes			7.50		0.84	1.67	1.93	
Alcohols						0.18		
Phenyl-propanoids			18.14			1.04		96.37
Total hydrocarbons compounds			13.35	2.61	28.73	2.33	5.99	0.20
Total oxygenated compounds			82.17	96.56	69.39	96.05	92.96	97.87
Total			95.52	99.17	98.12	98.38	98.95	98.07

[a] All compounds were identified by Kováts index and mass spectral data by comparing values reporter in the literature.
[b] Kováts index experimental.
[c] Kováts index literature.[10]
[d] Relative proportions as percentage of the total peak area.

found *E*-anethole as the main constituent, accounting for 82.80% of the total oil.[22]

The differences between the essential oil profiles reported herein and those in other studies can be attributed to the use of commercially available dried plant materials rather than the fresh plant parts.

Antimicrobial activities of essential oils

Incubation of the assay plates for 48 h was sufficient for all negative controls to show significant microbial growth (in the form of well-dispersed colonies), while the wells with positive control (chloramphenicol) and the system control (RCM without inoculum) remained clear with no observable colony formation. The presence of viable microorganisms in microplate wells via indication of colony formation was verified by reduction of yellow TTC to pink TPF; this color change was not observed in the wells without any microbial growth. In this way, the exact concentration of each of the essential oils able to inhibit the growth of *C. perfringens* was determined. The values obtained for MIC and MBC are shown in Table 2.

The essential oil from leaves of *T. vulgaris* showed the lowest MIC and MBC values (1.25 mg mL^{-1}) against *C. perfringens*, since both values are similar, the oil must be considered to possess strong bactericidal activity. Al-Bayati reported an MIC value of 0.5 mg mL^{-1} for thyme oil against *Klebsiella pneumoniae*, 0.25 mg mL^{-1} against *Salmonella typhi*, 0.125 mg mL^{-1} against *S. typhimurium*, 0.625 mg mL^{-1} against *Escherichia coli* and *Proteus*

Table 2 – Minimal inhibitory concentration (MIC) and minimal bactericide concentration (MBC) of essential oils against *Clostridium perfringens*.[a]

Essential oils	MIC ($mg\,mL^{-1}$)	MBC ($mg\,mL^{-1}$)
Rosmarinus officinalis	10.0	10.0
Mentha × piperita	10.0	10.0
Origanum majorana	5.0	5.0
Ocimum basilicum	5.0	5.0
Thymus vulgaris	1.25	1.25
Pimpinella anisum	10.0	20.0

[a] Chloramphenicol was employed as positive control. The MBC to chloramphenicol was 0.003125 $mg\,mL^{-1}$ in all experiments.

mirabilis, and 0.312 $mg\,mL^{-1}$ against *Staphylococcus aureus* and *P. vulgaris*.[23]

The essential oils from leaves of *O. basilicum* and *O. majorana* showed MIC and MBC values of 5.0 $mg\,mL^{-1}$ against *C. perfringens*, indicating that these oils possess bactericidal properties. Lv, Liang, Yuan, and Li determined MIC values of 1.25 $mg\,mL^{-1}$ for basil oil against *E. coli* and *S. aureus*, and 0.625 $mg\,mL^{-1}$ against *Bacillus subtilis*.[24] In a study on marjoram oil, Busatta et al. determined MIC values of 2.3 $mg\,mL^{-1}$ against *Enterococcus faecalis*, *Serratia* sp. and *Streptococcus mutans*, 0.92 $mg\,mL^{-1}$ against *Aeromonas* sp., *E. coli*, *K. pneumoniae* and *Salmonella choleraesuis*, 0.782 $mg\,mL^{-1}$ against *Shigella flexneri* and *Staphylococcus aureus*, and 0.069 $mg\,mL^{-1}$ against *B. subtilis*.[19]

The MIC and MBC values for *R. officinalis* and *M. piperita* oils against *C. perfringens* were considerably higher (10 $mg\,mL^{-1}$) than the other samples tested suggesting that these oils possess much weaker bactericidal activities. In the case of rosemary oil extracted from fresh plant material, Okoh, Sadimenko, and Afolayan reported an MIC of 3.75 $mg\,mL^{-1}$ and an MBC of 7.5 $mg\,mL^{-1}$ against *S. aureus*, an MIC of 7.5 $mg\,mL^{-1}$ and an MBC > 7.5 $mg\,mL^{-1}$ against *E. coli*, and an MIC of 1.88 $mg\,mL^{-1}$ and an MBC of 7.5 $mg\,mL^{-1}$ against *B. subtilis*.[25] For peppermint oil, Tyagi and Malik found an MIC of 1.13 $mg\,mL^{-1}$ and an MBC of 4.5 $mg\,mL^{-1}$ against *E. coli*, an MIC value of 2.25 $mg\,mL^{-1}$ and an MBC value of 9.0 $mg\,mL^{-1}$ against *Pseudomonas aeruginosa* and *P. fluorescens*, and an MIC value of 1.13 $mg\,mL^{-1}$ and an MBC value of 2.25 $mg\,mL^{-1}$ against *B. subtilis* and *S. aureus*.[20]

For the essential oil from fruits of *Pimpinella anisum*, the MBC value (20 $mg\,mL^{-1}$) was twofold higher than the MIC value indicating only bacteriostatic activity of the oil at a concentration of 10 $mg\,mL^{-1}$. Al-Bayati reported MIC values of 0.125 $mg\,mL^{-1}$ for anise oil against *S. aureus* and *Proteus mirabilis*, 0.25 $mg\,mL^{-1}$ against *Salmonella typhimurium*, 0.5 $mg\,mL^{-1}$ against *S. typhi* and values > 0.5 $mg\,mL^{-1}$ against *K. pneumoniae*, *Pseudomonas aeruginosa* and *E. coli*.[23]

Although the mechanisms associated with the antimicrobial activities of essential oils are not fully understood; numerous modes of action have been proposed involving, for example, degradation of the bacterial cell wall, modification of proteins of the cytoplasmic membrane, alteration of membrane permeability, inactivation of extracellular enzymes, reduction of intracellular ATP, leakage of cellular contents, coagulation of cytoplasm, and interruption of electron flow and active transport.[7,8,26] However, some studies have reported the specific mechanism of action for some oil constituents. Thymol and carvacrol are believed to act by increasing the permeability of cell membranes.[27] In this context, Ultee, Bennik, and Moezelaar showed that carvacrol accumulates in the lipid phase of the membrane by changing the conformation of the phospholipid bilayer, which causes expansion of the membrane and leakage of ions, thereby increasing membrane fluidity and permeability.[28] *p*-Cymene also accumulates in large amounts and acts by causing expansion of the membrane phospholipids by increasing spaces through which ion leakage might occur.[28] In the case of carvone, the compound is believed to be partitioned in the lipid membrane, thereby disturbing selective barrier function and the conservation of metabolic energy.[29] Terpinen-4-ol, on the other hand, has been shown to inhibit cellular respiration and to damage the structure of the cell membrane attenuating its role as a permeable barrier.[30] However, in general, it is believed that the antimicrobial efficacy of an essential oil is not associated exclusively with a specific constituent but rather a synergistic effect of all of the constituents contained.

Conclusions

The results obtained in this study demonstrate that the essential oils of basil, rosemary, marjoram, peppermint, thyme and anise exhibit *in vitro* antimicrobial activities against *C. perfringens*. The essential oil from *T. vulgaris* showed the lowest MIC value among all of the oils tested and was the most effective bactericide against one of the main causes of food poisoning in Brazil. The use of essential oils from commonly employed spices clearly offers an alternative to the chemical preservatives in the control and inactivation of pathogens in food, but further studies are needed in order to verify direct application in commercially produced food systems. The results suggest that the oxygenated compound, especially oxygenated monoterpenes and phenylpropanoids, might be responsible for the antimicrobial activity against *C. perfringens*, but the synergistic effects of these chemicals with other minor constituents of the essential oil should also be considered.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgement

The authors would like to thank the Microbiology Laboratory at Univates for the *C. perfringens* strain and technical assistance with microbiological assays.

REFERENCES

1. Forsythe SJ. *Microbiologia da Segurança Alimentar*. Porto Alegre, RS: Artmed; 2002 [chapters 3 and 5].
2. Franco BDGM, Landgraf M. *Microbiologia dos Alimentos*. São Paulo, SP: Atheneu; 2008 [chapter 4].
3. Ministério da Saúde. *Doenças Transmitidas por Alimentos – Descrição da Doença*. Brasília: Portal da Saúde, Sistema Único

de Saúde (SUS); 2013. http://portal.saude.gov.br/portal/saude/profissional/visualizar_texto.cfm?idtxt=31756 Accessed 05.03.13.
4. World Health Organization. *Food Safety. Foodborne Disease*. Geneva: WHO; 2013. http://www.who.int/foodsafety/foodborne_disease/en/ Accessed 04.03.13.
5. Germano PML, Germano MIS. *Higiene e Vigilância Sanitária de Alimentos*. 4th ed São Paulo, SP: Manole; 2011 [chapter 12].
6. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – a review. *Food Chem Toxicol*. 2008;46:446–475.
7. Burt S. Essential oils: their antibacterial properties and potential applications in foods – a review. *Int J Food Microbiol*. 2004;94:233–253.
8. Holley RA, Patel D. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiol*. 2005;22:273–292.
9. Brasil. Lei n° 10.831, de 23 de dez. 2003. Dispõe sobre a agricultura orgânica e dá outras providências. Diário Oficial [da] República Federativa do Brasil, Brasília, DF, 24 de dez. 2003.
10. Adams RP. *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*. Allured Publishing Corporation: Carol Stream, IL; 2001.
11. British Standards Institute. *BS EN ISO 7937:2004. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration of Clostridium perfringens – Colony-count Technique*; 2004. http://img.21food.cn/img/biaozhun/20100108/181/11285427.pdf Accessed 05.03.13.
12. Rosa OPS, Torres SA, Ferreira CM, Ferreira FBA. In vitro effect of intracanal medicaments on strict anaerobes by means of the broth dilution method. *Pesqui Odontol Bras*. 2002;16:31–36.
13. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard*. seventh ed. Wayne: CLSI; 2006. http://isoforlab.com/phocadownload/csli/M7-A7.pdf Accessed 05.03.13.
14. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med*. 1998;64:711–713.
15. *Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa SDA no. 62, de 26 de agosto de 2003. Oficializa os métodos analíticos oficiais para análises microbiológicas para controle de produtos de origem animal e água, com seus respectivos capítulos e anexos, em conformidade com o anexo desta Instrução Normativa, determinando que sejam utilizados no Sistema de Laboratório Animal do Departamento de Defesa Animal*. Brasília: Diário Oficial da União; 2003. http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do?operacao=visualizarandid=2851 Accessed 03.03.13.
16. Hussain AI, Anwar F, Sherazi STH, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chem*. 2008;108:986–995.
17. Carovic-Stanko K, Orlic S, Politeo O, et al. Composition and antibacterial activities of essential oils of seven *Ocimum* taxa. *Food Chem*. 2010;119:196–201.
18. Jiang Y, Wu N, Fu Y, et al. Chemical composition and antimicrobial activity of the essential oil of Rosemary. *Environ Toxicol Pharmacol*. 2011;32:63–68.
19. Busatta C, Vidal RS, Popiolski AS, et al. Application of *Origanum majorana* L. essential oil as an antimicrobial agent in sausage. *Food Microbiol*. 2008;25:207–211.
20. Tyagi AK, Malik A. Antimicrobial potential and chemical composition of *Mentha piperita* oil in liquid and vapour phase against food spoiling microorganisms. *Food Control*. 2011;22:1707–1714.
21. Rota MC, Herrera A, Martinez RM, Sotomayor JA, Jordan MJ. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis*, and *Thymus hyemalis* essential oils. *Food Control*. 2008;19:681–687.
22. Tepe B, Akpulat HA, Sokmen M, et al. Screening of the antioxidative and antimicrobial properties of the essential oils of *Pimpinella anisetum* and *Pimpinella flabellifolia* from Turkey. *Food Chem*. 2006;97:719–724.
23. Al-Bayati FA. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. *J Ethnopharmacol*. 2008;116:403–406.
24. Lv F, Liang H, Yuan Q, Li C. *In vitro* antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Res Int*. 2011;44:3057–3064.
25. Okoh OO, Sadimenko AP, Afolayan AJ. Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. *Food Chem*. 2010;120:308–312.
26. Souza EL, Lima EO, Naraim N. Especiarias: uma alternativa para o controle da qualidade sanitária e de vida útil de alimentos, frente às perspectivas da indústria alimentícia. *Hig Aliment*. 2003;17:38–42.
27. Lambert RJW, Skandamis PN, Coote PJ, Nychas G-JE. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J Appl Microbiol*. 2001;91:453–462.
28. Ultee A, Bennik MHJ, Moezelaar R. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Appl Environ Microbiol*. 2002;68:1561–1568.
29. Oosterhaven K, Poolman B, Smid EJ. S-Carvone as a natural potato sprout inhibiting, fungistatic and bacteriostatic compound. *Ind Crop Prod*. 1995;4:23–31.
30. Cox SD, Mann CM, Markham JL, et al. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J Appl Microbiol*. 2000;88:170–175.