academicJournals

Vol. 7(26), pp. 3396-3404, 25 June, 2013
DOI: 10.5897/AJMR12.1618
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Effects of thyme (*Thymus vulgaris* L.) and rosemary (*Rosmarinus officinalis* L.) essential oils on growth of *Brochothrix thermosphacta*

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Accepted 17 June, 2013

The objective of the study was to investigate the chemical composition of Polish rosemary and thyme oils and to quantify the effects of these oils against *Brochothrix thermosphacta*. The major constituents of the thyme oil investigated were monoterpenes: thymol (29.4%) and p-cymene (21.6%). The rosemary oil represents 1,8-cineole chemotype (1,8-cineole 27.6%, limonene 13.5% and β-pinene 13.0%). The minimum inhibitory concentration (MIC) of thyme oil for *B. thermosphacta* is 0.05% and that of rosemary oil 0.5%. MIC values are not dependent on the temperature of the cultures or *B. thermosphacta* strain. However, the most significant influence of both oils on *B. thermosphacta* growth was observed at 4°C than at 25°C.

Key words: *Brochothrix thermosphacta*, thyme oil, rosemary oil.

INTRODUCTION

For economical reasons, it has become necessary for food manufacturers to use new technologies to extend the shelf lives of food. Implementation of such technologies is a difficult task because of increasing consumer demand for foods that are minimally processed and virtually free of preservatives. Because meat is an excellent habitat for numerous microorganisms, the meat industry is increasingly searching for natural solutions to inhibit the microbial growth. Modified Atmosphere Packaging (MAP) offers multiple advantages to the meat industry owing to its potential to extend the shelf-life of fresh meat. Compositions of modified atmospheres play a crucial role in selection of specific spoilage microorganisms (Labadie, 1999). When meat is exposed to air, the dominating microbial species are bacteria of the genus *Pseudomonas*, the most widespread being *Pseudomonas fragi.* Apart from the aforementioned meat-spoiling species, some other bacteria such as *Carnobacterium piscicola*, *Carnobacterium divergens*, *Lactobacillus sakei*, *Lactobacillus curvatus* and *Leuconostoc carnosum* contaminate meat samples in the presence of increased CO_2 content in modified atmospheres (Labadie, 1999; Ercolini et al., 2006; McMillin, 2008).

Essential oils are regarded as natural alternatives to chemical preservatives and can be used as additional methods of controlling spoilage microbiota. The sensitivity of spoilage organisms to selected essential oils and their components is presented in Table 1. High-oxygen MAP (comprising 70 to 80% O_2 and 20 to 30% CO_2) is widely used for the preservation of meat. Under high oxygen conditions, *Brochothrix thermosphacta* is the most commonly present microorganism in meat. According to Leistner's concept of hurdles, appropriate

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Table 1. Sensitivity of meat spoilage organisms to essential oils and their components.

Bacterial species	Essential oils/components	References
Pseudomonas fragi	Oils: oregano, savory, thyme, clove, pimento, mastic gum from *Pistacia lentiscus* var. *chia* Constituents: thymol, eugenol, carvacrol	Lebert et al., 2007; La Storia et al., 2011; Tassou and Nychas, 1995; Girova et al., 2010
Carnobacterium piscicola	Oils: cinnamon, clove, cumin, garlic, oregano, black pepper, pimento, rosemary	Ouattara et al., 1997
Carnobacterium divergens	Oils: rosemary, grape fruit seed, Constituents: allyl isothiocyanate, cinnamaldehyde thymol	Schirmer and Langsrud, 2010
Lactobacillus sakei	Oils: cinnamon, clove, cumin, garlic, oregano, black pepper, pimento, rosemary, thyme, lemon balm, marjoram, sage, orange, lemon, grapefruit, mandarin Constituents: eugenol, carvacrol, thymol	Ouattara et al., 1997; Fernandez-Lopez et al., 2005; Blaszczyk and Holley, 1998; Rias et al., 2010; Gutierrez et al., 2009; Gill and Holley, 2006; Gill and Holley, 2004; Viuda-Martos et al., 2008a, 2008b
Lactobacillus curvatus	Oils: cinnamon, clove, cumin, garlic, oregano, black pepper, pimento, rosemary, garlic, thyme, sage, orange, lemon, grapefruit, mandarin, sweet basil	Ouattara et al., 1997; Fernandez-Lopez et al., 2005; Viuda-Martos et al., 2008a, 2008b; Lachowicz et al., 1998
Leuconostoc carnosum	Oils: rosemary, grape fruit seed, Constituents: allyl isothiocyanate, cinnamaldehyde, thymol	Schirmer and Langsrud, 2010

combination of preservation methods can achieve microbial stability and safety of food (Leistner, 2000). The combination of MAP and antimicrobial packaging materials containing essential oils could be very promising.

Our previous studies showed that the application of thyme and rosemary oils as components of modified atmospheres do not, however, bring the expected results because the oils are not effective against *B. thermosphacta* at concentrations of 2MIC, while they considerably affect the organoleptic qualities of meat (Nowak et al., 2012). Perhaps it will be possible to use essential oils as an additive to the meat products or packaging materials. It is necessary to know the impact of these oils on the growth parameters of *B. thermosphacta* to determine the possibility of extending the shelf life of products.

Our research had two main objectives: First, to investigate the chemical composition of rosemary and thyme oils, and second to evaluate and quantify the effects of these oils against *B. thermosphacta*.

MATERIALS AND METHODS

Bacterial strains

Three strains of *B. thermosphacta* bacteria were used in the bioassays. Two were isolated from meat packed in a modified atmosphere (MMAP4 and MMAP20) and one was obtained from the American Type Culture Collection (ATCC 11509). All three strains were genetically identified by 16S rRNA gene sequencing (Nowak, 2012). The sequences were deposited in the GenBank Database under the following accession numbers: HQ890943.1 – MMAP4, HQ890944.1 – MMAP20 and HQ890942.1 – ATCC 11509.

Essential oils

Two essential oils were tested: thyme (*Thymus vulgaris*) oil (Avicenna-Oil, Poland) and rosemary (*Rosmarinus officinalis*) oil (Semifarm, Poland).

Composition of the essential oils

GC-FID-MS analysis

The composition of essential oils was analyzed using GC-FID-MS method described previously (Nowak et al., 2012).

Fourier transform infrared spectroscopy (FTIR)

A Nicolet 6700 FTIR spectrometer (ThermoScientific) with a diamond ATR crystal was used. Measurements were recorded in the range of 4000 to 500 cm^{-1}, with an interval of 4 cm^{-1}. The final spectrum of the sample was achieved using on average 32 scans with 2 µL of the essential oil placed on the surface of the diamond ATR crystal. Chemometric analyses of the ATR-FTIR spectra were performed using Omnic software.

Determination of the effects of thyme and rosemary oils on *B. thermosphacta* growth

The antimicrobial activity of thyme and rosemary oils against

Table 2. Composition of thyme oil.

[%]	RI	RI ref	Component
3.8	933	936	α-Pinene
0.1	946	950	Camphene
0.8	972	978	β-Pinene
0.1	986	987	Myrcene
0.1	997	1002	α-Phellandrene
3.0	1005	1010	3-Carene
0.2	1010	1013	α-Terpinene
21.6	1015	1015	p-Cymene
9.8	1022	1024	1,8-Cineole
6.6	1024	1025	Limonene
0.3	1053	1051	γ-Terpinene
0.7	1086	1086	Linalool
0.2	1129	1126	Cis-limonene oxide
0.5	1143	1142	Isoborneol
2.0	1152	1150	Borneol
2.1	1175	1176	α-Terpineol
0.7	1181	1188	γ-Terpineol
1.6	1243	1239	Linalyl acetate
29.4	1274	1267	Thymol
4.2	1281	1278	Carvacrol
0.2	1406	1411	Longifolene
2.8	1419	1421	β-Caryophyllene
0.2	1452	1455	α-Humulene

RI– Retention indices; RI ref– reference retention indices.

B. thermosphacta strains was evaluated with a microtiter plate assay. The cultures were grown in liquid TSB medium (Merck) with 1% Tween 80. The oils were initially dissolved in 99.5% DMSO (Sigma-Aldrich) prior to adding them to the growth medium. An initial solution of 1% thyme or rosemary oil was added to the growth medium to make the working solutions of 0.05, 0.01, 0.005, 0.001 and 0.0001% (v/v) for thyme oil and 0.5, 0.4, 0.3, 0.2 and 0.1% (v/v) for rosemary oil, respectively. The media were inoculated with the *B. thermosphacta* strain and 200 µL of the culture was added to each well in five independent replicates. The control consisted of a culture without essential oils. Polypropylene microtiter plates with 96 wells (Sigma-Aldrich) were used for the assay. The plates were incubated for 96 h at 25°C and for 360 h at 4°C. Following incubation, the optical absorbance of each well at 600 nm (OD_{600}) was recorded using an Asys UVM340 microtiter plate reader (Biogenet). The minimum inhibitory concentration (MIC) was defined as the lowest concentration inhibiting visible growth of the tested organism (Burt, 2004). The OD_{600} values were converted to cfu mL^{-1} using a calibration curve. The cell numbers were fitted to the Gompertz equation using an Excel add-in, DMFit 2.1 (Institute of Ford Research, Norwich, UK):

$$L(t) = A + C \exp\{-\exp[-B (t - M)]\}$$

The following growth parameters were estimated: maximum specific growth rate $\mu_{max} = BC/e$; generation time $g = (\ln 2)e/BC$; lag time $t_{Lag} = M - (1/B)$; and maximum population density $\log(N_{max}) = A + C$.

Statistical methods

Mean values and standard deviations were determined with OriginPro 7.5 software. The occurrence of differences (ANOVA tests) and the effect of essential oil concentration on growth parameters of *B. thermosphacta* (linear regression) were analyzed using STATISTICA 10 PL software.

RESULTS AND DISCUSSION

Composition of the essential oils

The two major constituents of the studied thyme oil were thymol (29.4%) and p-cymene (21.6%) (Table 2). According to Burt (2004), thyme oil consists of 10 to 64% thymol and 10 to 56% p-cymene. Another two major components reported by Burt are carvacrol (2 to 11%) and γ-terpinene (2 to 31%). The former constituted 4.2% of the essential oil used in this study, while the content of the latter diverged from the above-mentioned range, being 0.3%. Also Nhu-Trang et al. (2006) found only trace amounts of γ-terpinene in thyme essential oil. All the above-mentioned compounds are monoterpenes. In general, monoterpenes are the most representative molecules constituting 90% of essential oils (Bakkali et al., 2008). The rosemary essential oil used in this study also consisted mostly of monoterpenes: 1,8-cineole, limonene, and α-pinene, constituting 27.6, 13.5 and 13.0% of the essential oil, respectively (Table 3). Burt (2004) reported that 1,8-cineole constitutes 3 to 89% of thyme oil, while not including limonene or β-pinene among the major components of the oil. In rosemary essential oil analyzed by Graber et al. (2010) the content of the latter two compounds was 3.54 to 4.73% and 5.36 to 8.08%, respectively, being dependent on the method of essential oil production and the time of rosemary harvest. Flamini et al. (2002) classified rosemary oil into two chemotypes: the α-pinene chemotype with the main compounds being α-pinene (20.6%) and 1,8-cineole (6.6%) and the 1,8-cineole chemotype with the major components being 1,8-cineole (40.2%) and α-pinene (13.2%). The Polish rosemary examined by Szumny et al. (2010) was classified as the α-pinene chemotype as it contained 33.3% α-pinene and 12.3% 1,8-cineole.

A high proportion of limonene (21.7%) in Serbian rosemary oil was reported by Bozin et al. (2007). The rosemary oil used in our study was obtained from a commercial source, so its method of production and time of harvest are not known. We also analyzed infrared spectra by the ATR-FTIR method in our study. The obtained spectra are shown in Figures 1 and 2. The ATR-FTIR spectrum of thyme oil (Figure 1), which shows an intense band at 807.18 cm^{-1}, reflects the complex composition of this essential oil: 29.4% thymol and 21.6% p-cymene. This band arises from the overlapping of thymol and p-cymene bands (804 and 813 cm^{-1}) (Schulz et al., 2005). The band can be attributed to out-of-plane CH wagging vibrations, which are the most important signals used in distinguishing different types of aromatic ring substitution. In the ATR-FTIR spectrum of rosemary essential oil (Figure 2), the following key bands are present: 1374.59, 1214.69, 1079.50, 985.24 and

Table 3. Composition of rosemary oil.

[%]	RI	RI ref	Component
0.2	921	915	Tricyclene
5.8	934	936	α-Pinene
0.1	945	941	α-Fenchene
3.3	946	950	Camphene
0.5	969	973	Sabinene
13.0	974	978	β-Pinene
0.6	984	987	Myrcene
0.2	997	1002	α-Phellandrene
0.5	1004	1006	3-Carene
3.1	1010	1013	α-Terpinene
2.0	1013	1015	p-Cymene
27.6	1024	1024	1,8-Cineole
13.5	1026	1025	Limonene
0.3	1030	1030	(*Z*)-β-Ocimene
2.5	1052	1051	γ-Terpinene
0.1	1063	1052	Trans-sabinene hydrate
0.7	1077	1077	p-Mentha-2,4(8)-diene
4.9	1081	1082	Terpinolene
0.3	1083	1082	Cis-sabinene hydrate
0.1	1087	1087	Linalool
0.1	1098	1099	α-Fenchol
8.9	1123	1123	Camphor
0.3	1128	1137	Cis-β-terpineol
1.1	1143	1142	Isoborneol
1.8	1152	1150	Borneol
0.2	1164	1164	Terpinen-4-ol
3.0	1175	1176	α-Terpineol
0.6	1181	1188	γ-Terpineol
0.1	1267	1267	β-Terpinyl acetate
2.8	1272	1276	Bornyl acetate
0.7	1333	1335	α-Terpinyl acetate
0.1	1405	1411	Longifolene

RI– Retention indices; RI ref– reference retention indices.

843.01 cm^{-1}. These bands indicate 1,8-cineole as the main component of rosemary oil and are attributed to the vibration of $CH_3(CO)$, C-O-C and CH_2 groups (Schulz et al., 2005).

Activity of the essential oils against *B. thermosphacta*

The Gompertz function used for experimental data analysis appropriately describes the growth of *B. thermosphacta*, which is confirmed by the high values of the R^2 coefficient, ranging from 0.9665 to 0.9998 (Tables 4 and 5).

The present study found that the MIC of thyme essential oil was 0.05% irrespective of the *B. thermosphacta* strain and the temperature of the culture (Table 4). As far as concentrations below the MIC are concerned, while at 25°C thyme essential oil did not influence the growth parameters of *B. thermosphacta* in a statistically significant manner (as compared to the control sample without the oil) at concentrations below 0.005%, at 4°C it was effective at a concentration of 0.0001%. The maximum specific growth rate (μ_{max}) was most significantly reduced in the MMAP4 strain culture with the addition of 0.01% thyme oil at 4°C. The longest lag phase was also observed in this strain (at 25°C with 0.01% thyme oil).

The final population density was most decreased in the MMAP4 strain culture at 4°C with 0.01% of the oil. *B. thermosphacta* bacteria are part of the typical microflora of meat and meat products, especially under modified atmospheres with increased oxygen content. The present results indicate that the application of thyme oil even at concentrations below the MIC may lead to inhibition of the growth of these microbes in refrigerated products. The world literature contains many reports on the influence of essential oils on *B. thermosphacta*. The sensitivity of these bacteria to thyme oil has been studied by Girova et al. (2010). Using the disc diffusion method, they found that the minimum inhibitory concentration of thyme oil for *B. thermosphacta* was ≤0.02%. The susceptibility of *B. thermosphacta* to thymol, which is the main component of thyme oil, has been examined by Schirmer and Langsrud (2010), Rivas et al. (2010), and Szczepaniak et al. (2011). Schirmer and Langsrud (2010) reported that the MIC of this compound for *B. thermosphacta* was 250 µg mL^{-1}. The same figure was given by Rivas et al. (2010) as the minimum bactericidal concentration (MBC) of thymol for *B. thermosphacta.* Szczepaniak et al. (2011) reported that a mixture of thymol, carvacrol and cymene inhibited the growth rate of *B. thermosphacta* by 25.7% and extended their lag phase during an *in situ* study using minced pork.

The MIC of rosemary oil is 0.5%, irrespective of the *B. thermosphacta* strain and growth temperature (Table 5). As in the case with thyme oil, the concentrations of rosemary oil at which statistically significant changes in μ_{max} appeared were lower at 4°C than at 25°C. The growth rate was most considerably reduced, by a factor of 3.9, when the MMAP4 strain was grown in medium with the addition of 0.4% thyme oil. We found that the lag phase was longer in a statistically significant manner at the lowest concentration of rosemary oil used, irrespective of the temperature at which the bacteria were grown, while the changes were more pronounced at 25°C. These differences were greatest in the MMAP4 culture, where the lag phase was 43.8 times longer than that of control at 25°C but only 3.4 times longer at 4°C. The final population density of *B. thermosphacta* biomass in the culture carried out at 25°C with the addition of rosemary oil did not change, but a lower population density was observed at 4°C. The influence of rosemary extracts and essential oils on *B. thermosphacta* has also

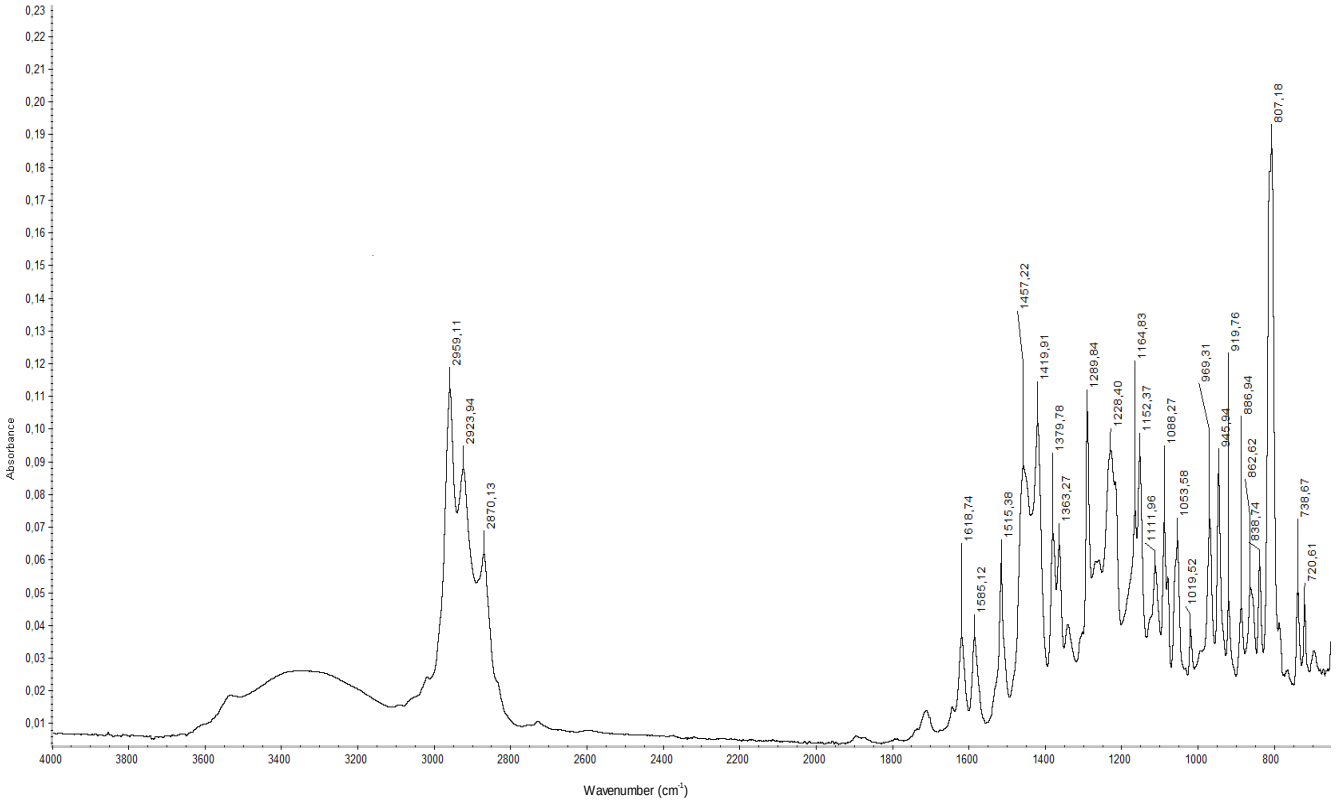

Figure 1. FTIR analysis of thyme oil.

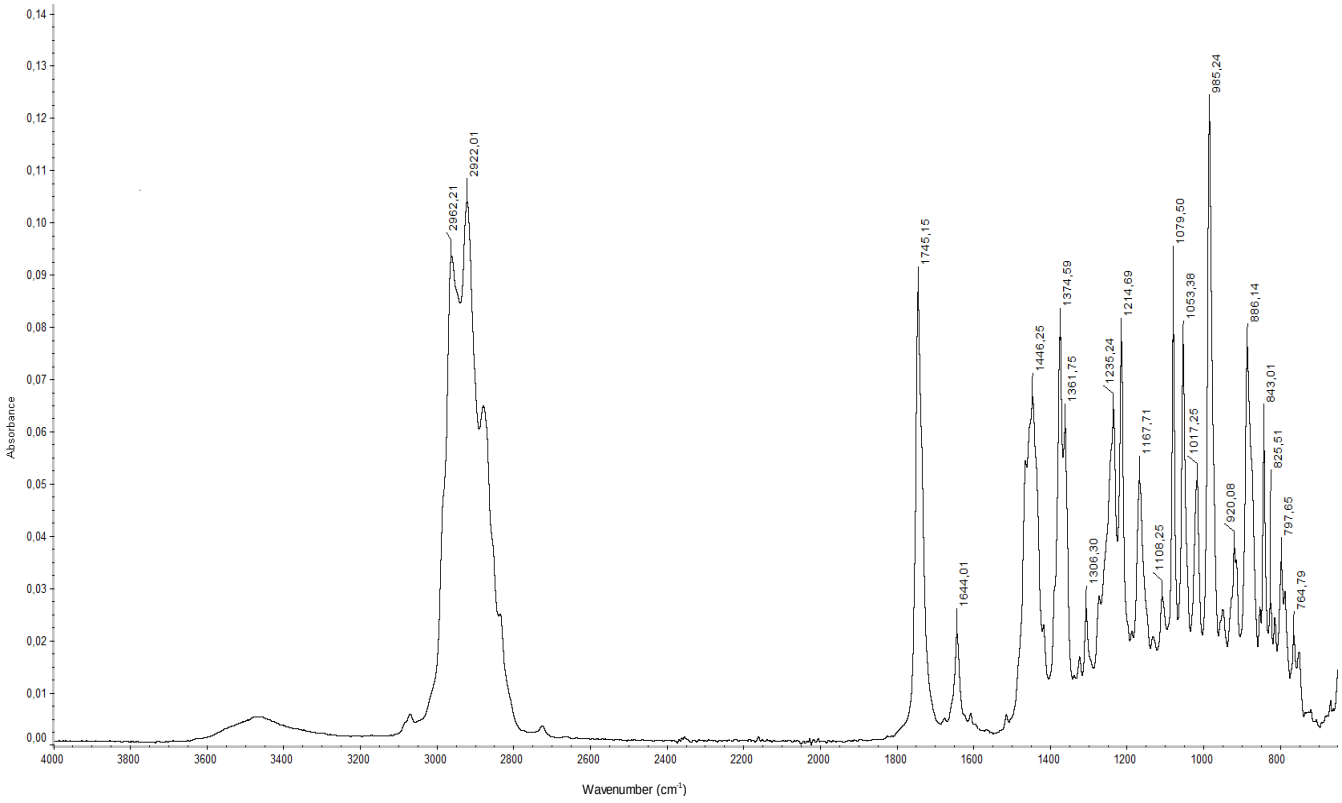

Figure 2. FTIR analysis of rosemary oil.

Table 4. Thyme oil effects on *B. thermosphacta* growth parameters (mean±SD).

Strain	T[°C]	c_{oil} [%]	μ_{max} [h^{-1}]	G [h]	t_{Lag} [h]	log(N_{max})	R^2
MMAP4	25	0	0.275±0.025^{a}	2.5±0.2^{a}	5.9±0.4^{a}	8.63±0.13^{a}	0.9900±0.0085
		0.0001	0.272±0.034^{a}	2.6±0.3^{a}	6.2±0.5^{a}	8,63±0.13^{a}	0.9937±0.0079
		0.001	0.270±0.010^{a}	2.6±0.1^{a}	5.9±0.6^{a}	8,54±0.19^{a}	0.9948±0.0020
		0.005	0.262±0.002^{a}	2.6±0.1^{a}	12.1±0.1^{b}	8.19±0.12^{b}	0.9960±0.0006
		0.01	0.192±0.018^{b}	3.6±0.3^{b}	21.2±1.2^{c}	8.06±0.22^{b}	0.9990±0.0010
		0.05	nd	nd	nd	nd	nd
	4	0	0.220±0.023^{a}	3.2±0.3^{a}	51.8±0.3^{a}	8.91±0.11^{a}	0.9980±0.0009
		0.0001	0.073±0.006^{b}	9.5±0.8^{b}	78.1±2.1^{b}	8.06±0.48^{b}	0.9974±0.0001
		0.001	0.070±0.011^{b,c}	10.1±1.5^{b}	81.4±2.0^{b}	7.09±0.49^{c}	0.9914±0.0028
		0.005	0.068±0.001^{b,c}	10.2±0.1^{b}	117.7±3.3^{c}	7.25±0.53^{c}	0.9946±0.0043
		0.01	0.065±0.001^{c}	10.7±0.1^{c}	119.0±4.7^{c}	7.28±0.17^{c}	0.9941±0.0087
		0.05	nd	nd	nd	nd	nd
MMAP20	25	0	0.315±0.026^{a}	2.2±0.2^{a}	6.2±1.0^{a}	7.58±0.14^{a}	0.9978±0.0014
		0.0001	0.303±0.005^{a}	2.3±0.1^{a}	6.9±0.4^{a}	7.73±0.28^{a}	0.9972±0.0018
		0.001	0.291±0.021^{a,b}	2.4±0.2^{a,b}	7.7±0.8^{a}	7.73±0.14^{a}	0.9949±0.0038
		0.005	0.275±0.008^{b}	2.5±0.1^{b}	11.9±0.8^{b}	7.50±0.12^{a}	0.9665±0.0166
		0.01	0.130±0.013^{c}	5.3±0.5^{c}	20.9±0.9^{c}	7.19±0.61^{a}	0.9955±0.0010
		0.05	nd	nd	nd	nd	nd
	4	0	0.136±0.011^{a}	5.1±0.4^{a}	39.7±0.3^{a}	8.52±0.53^{a}	0.9969±0.0005
		0.0001	0.085±0.005^{b}	8.2±0.5^{b}	75.4±3.4^{b}	8.15±0.28^{a}	0.9971±0.0005
		0.001	0.081±0.005^{b,c}	8.5±0.5^{b,c}	82.9±0.6^{c}	6.81±0.29^{b}	0.9916±0.0032
		0.005	0.077±0.001^{c}	9.0±0.1^{c}	107.2±3.6^{d}	6.99±0.35^{b}	0.9975±0.0010
		0.01	0.069±0.009^{c}	10.1±1.5^{c}	130.0±5.0^{e}	6.41±0.40^{b}	0.9834±0.0035
		0.05	nd	nd	nd	nd	nd
ATCC 11509	25	0	0.264±0.032^{a}	2.7±0.4^{a}	18.3±1.9^{a}	8.86±0.07^{a}	0.9981±0.0011
		0.0001	0.251±0.008^{a}	2.8±0.1^{a}	20.3±0.7^{a}	8.83±0.17^{a}	0.9988±0.0009
		0.001	0.236±0.019^{a,b}	3.0±0.2^{a,b}	19.9±0.7^{a}	8.48±0.04^{b}	0.9978±0.0014
		0.005	0.205±0.023^{b}	3.4±0.4^{b,c}	29.5±3.9^{b}	7.73±0.09^{c}	0.9984±0.0022
		0.01	0.179±0.014^{c}	3.9±0.3^{c}	53.4±13.8^{c}	7.59±0.03^{d}	0.9994±0.0001
		0.05	nd	nd	nd	nd	nd
	4	0	0,105±0.011^{a}	6.7±0.7^{a}	44.5±0.7^{a}	8.94±0.15^{a}	0.9891±0.0019
		0.0001	0.052±0.003^{b}	13.4±0.7^{b}	117.4±3.1^{b}	7.22±0.17^{d}	0.9971±0.0018
		0.001	0.048±0.001^{b}	14.3±0.4^{b}	119.6±3.4^{b}	7.58±0.12^{c}	0.9990±0.0005
		0.005	0.047±0.002^{b}	14.7±0.6^{b}	147.6±2.6^{c}	7.97±0.07^{b}	0.9984±0.0010
		0.01	0.047±0.004^{b}	15.0±1.3^{b}	159.6±2.8^{c}	8.06±0.16^{b}	0.9985±0.0007
		0.05	nd	nd	nd	nd	nd

μ_{max} – Maximum specific growth rate; g– generation time; t_{Lag}– lag time; log(N_{max})– maximum population density; nd– not detected. Differences were assessed by ANOVA test. Different superscript letters associated with values of the same growth parameter, strain and temperature indicate statistically significant differences (P<0.05).

been examined by Ouattara et al. (1997), Fernandez-Lopez et al. (2005), and Schirmer and Langsrud (2010). Ouattara et al. (1997) reported the inhibition of *B. thermosphacta* growth on Petri dishes containing rosemary oil diluted 1:100. Fernandez-Lopez et al. (2005) studied the sensitivity of these bacteria to rosemary oil extract and rosemary water extract by the disc diffusion method. The strains which they tested were susceptible to both types of extracts, while the growth inhibition zones were larger for the rosemary oil extract. Schirmer and Langsrud (2010) determined the MIC of rosemary extract for *B. thermosphacta* at 150 µg mL^{-1}.

Table 5. Rosemary oil effects on B. thermosphacta growth parameters (mean±SD).

Strain	T [°C]	c_{oil} [%]	μ_{max} [h^{-1}]	g [h]	t_{Lag}[h]	log(N_{max})	R^2
		0	0.275±0.025^{a}	2.5±0.2^{a}	5.9±0.4^{a}	8.63±0.13^{a}	0.9900±0.0085
		0.1	0.256±0.012^{a}	2.7±0.1^{a}	61.2±0.3^{b}	8.68±0.06^{a}	0.9993±0.0001
	25	0.2	0.248±0.033^{a}	2.8±0.4^{a}	121.9±1.1^{c}	8.49±0.25^{a}	0.9897±0.0045
		0.3	0.135±0.042^{b}	5.4±1.6^{b}	166.2±6.7^{d}	8.38±1.07^{a}	0.9997±0.0003
		0.4	0.120±0.021^{b}	5.8±0.6^{b}	256.9±4.4^{e}	8.41±0.37^{a}	0.9976±0.0006
		0.5	nd	nd	nd	nd	nd
MMAP4							
		0	0.220±0.023^{a}	3.2±0.3^{a}	51.8±0.3^{a}	8.91±0.11^{a}	0.9980±0.0009
		0.1	0.191±0.062^{a}	4.0±1.2^{a}	120.3±1.5^{b}	8.22±0.13^{b}	0.9938±0.0100
	4	0.2	0.111±0.037^{b}	6.2±0.5^{b}	143.0±0.5^{c}	8.12±0.33^{b}	0.9977±0.0019
		0.3	0.073±0.007^{c}	9.5±0.9^{c}	154.9±7.6^{c}	8.05±0.26^{b}	0.9996±0.0001
		0.4	0.056±0.002^{d}	12.4±0.6^{d}	176.8±2.8^{d}	7.84±0.36^{b}	0.9993±0.0001
		0.5	nd	nd	nd	nd	nd
		0	0.315±0.026^{a}	2.2±0.2^{a}	6.2±1.0^{a}	7.58±0.14^{a}	0.9978±0.0014
		0.1	0.290±0.003^{a}	2.4±0.1^{a}	113.2±0.1^{b}	7.39±0.08^{a}	0.9994±0.0014
	25	0.2	0.294±0.012^{a}	2.4±0.4^{a}	154.8±7.1^{c}	7.42±0.12^{a}	0.9963±0.0032
		0.3	0.139±0.002^{b}	5.0±0.1^{b}	174.4±0.2^{d}	7.49±0.01^{a}	0.9998±0.0001
		0.4	0.105±0.005^{c}	6.6±0.3^{c}	198.0±5.1^{e}	7.52±0.21^{a}	0.9895±0.0076
		0.5	nd	nd	nd	nd	nd
MMAP20							
		0	0.136±0.011^{a}	5.1±0.4^{a}	39.7±0.3^{a}	8.52±0.53^{a}	0.9969±0.0005
		0.1	0.131±0.014^{a}	5.3±0.6^{a}	109.4±4.3^{b}	8.60±0.06^{a}	0.9953±0.0029
	4	0.2	0.097±0.012^{b}	7.1±0.9^{b}	114.0±2.7^{b}	8.48±0.22^{a}	0.9974±0.0004
		0.3	0.062±0.027^{c}	12.5±4.4^{c}	132.1±8.1^{c}	7.35±0.40^{b}	0.9954±0.0027
		0.4	0.045±0.003^{c}	15.4±0.7^{c}	153.9±1.1^{d}	7.13±0.13^{b}	0.9986±0.0005
		0.5	nd	nd	nd	nd	nd
		0	0.264±0.032^{a}	2.7±0.4^{a}	18.3±1.9^{a}	8.86±0.07^{a}	0.9981±0.0011
		0.1	0.263±0.058^{a}	2.7±0.6^{a}	55.0±10.3^{b}	8.60±0.18^{a}	0.9993±0.0004
	25	0.2	0.246±0.021^{a}	2.8±0.3^{a}	87.9±4.1^{c}	8.85±0.15^{a}	0.9974±0.0009
		0.3	0.238±0.006^{a}	2.9±0.1^{a}	102.4±4.8^{d}	8.67±0.20^{a}	0.9960±0.0049
		0.4	0.185±0.011^{b}	3.7±0.3^{b}	149.8±6.6^{e}	8.53±0.31^{a}	0.9870±0.0056
		0.5	nd	nd	nd	nd	nd
ATCC 1159							
		0	0.105±0.011^{a}	6.7±0.7^{a}	44.5±0.7^{a}	8.94±0.15^{a}	0.9891±0.0019
		0.1	0.074±0.006^{b}	9.4±0.7^{b}	82.4±1.2^{b}	8.88±0.09^{a}	0.9955±0.0005
	4	0.2	0.070±0.005^{b}	9.9±0.7^{b}	104.9±5.4^{c}	8.69±0.11^{b}	0.9956±0.0021
		0.3	0.056±0.015^{b,c}	13.0±2.7^{b,c}	116.2±5.3^{d}	8.76±0.09^{b}	0.9952±0.0013
		0.4	0.049±0.009^{c}	14.1±2.1^{c}	139.9±2.7^{e}	7.43±0.17^{c}	0.9979±0.0005
		0.5	nd	nd	nd	nd	nd

μ_{max} – Maximum specific growth rate; g– generation time; t_{Lag}– lag time; log(N_{max})– maximum population density; nd– not detected. Differences were assessed by ANOVA test. Different superscript letters associated with values of the same growth parameter, strain and temperature indicate statistically significant differences (P<0.05).

According to the results, growth parameters of *B. thermosphacta* were correlated with concentration of both investigated oils. Very weak linear correlation was observed only for thyme oil concentration and maximum population density of ATTC strain at 4°C and for rosemary oil concentration and MMAP20 strain

Table 6. Pearson's r coefficient for linear regression of essential oil concentration on growth parameters of *B. thermosphacta.*

Oil	Strain	T [°C]	μ_{max}	g	t_{Lag}	log(N_{max})
Thyme	MMAP4	25	-0.9918[a]	0.8955[a]	0.9919[a]	0.9681[a]
		4	-0.6203[b]	0.5262[c]	0.8617[a]	0.5548[c]
	MMAP20	25	-0.9289[a]	0.9119[a]	0.9916[a]	0.9369[a]
		4	-0.8871[a]	0.8351[a]	0.8570[a]	0.7709[b]
	ATCC 11509	25	-0.9903[a]	0.9898[a]	0.9790[a]	0.9320[a]
		4	-0.7812[b]	0.5471[c]	0.7390[b]	0.0623[d]
Rosemary	MMAP4	25	-0.9304[a]	0.9097[a]	0.9935[a]	0.8827[a]
		4	-0.9754[a]	0.9800[a]	0.9387[a]	0.8998[a]
	MMAP20	25	-0.9165[a]	0.9101[a]	0.8689[a]	0.0017[d]
		4	-0.9795[a]	0.9497[a]	0.9247[a]	0.8924[a]
	ATCC 11509	25	-0.8965[a]	0.8245[a]	0.9799[a]	0.3961[c]
		4	-0.9494[a]	0.9814[a]	0.9628[a]	0.6245[b]

μ_{max}– Maximum specific growth rate; g– generation time; t_{Lag}– lag time; log(N_{max})– maximum population density. The correlation strength (according to Evans, 1996): a– very strong, b- strong, c- moderate, d-very weak.

at 25°C (Table 6).

Conclusions

B. thermosphacta bacteria are the prevalent microflora of meat refrigerated in modified atmospheres with high oxygen content. These bacteria are sensitive both to thyme and rosemary essential oils. The application of thyme and rosemary oil even at concentrations below the MIC may lead to inhibition of the growth of these microbes in refrigerated products.

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