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# A study of the synergistic antilisterial effects of a sub-lethal dose of lactic acid and essential oils from *Thymus vulgaris* L., *Rosmarinus officinalis* L. and *Origanum vulgare* L.

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**Abstract**

Commercial essential oils of *Thymus vulgaris* L., *Rosmarinus officinalis* L. and *Origanum vulgare* L. were chemically characterized by GC, GC/MS analyses. The antilisterial activity of the oils, and the sub-lethal concentration of lactic acid were established by the agar-well diffusion method. The bactericidal kinetics of the diluted oils (50 ppm, 100 ppm, 200 ppm and 300 ppm) and their mixtures with 50 ppm of lactic acid were determined by optical density ($OD_{600}$) measurements. The results suggest that a sub-lethal dose of lactic acid noticeably increased the antilisterial activity, especially of rosemary and thyme oils, but that the synergistic effects were reduced with higher concentrations of oils.


*Keywords:* Oregano oil; Rosemary oil; Thyme oil; Chemical composition; *L. monocytogenes*; Lactic acid; Synergy

## 1. Introduction

The increased demand for safe and natural food, without chemical preservatives, provokes many researchers to investigate the antimicrobial effects of natural compounds. Numerous investigations have confirmed the antimicrobial action of essential oils (EOs) in model food systems and in real food (Koutsoumanis, Tassou, Taoukis, & Nychas, 1998; Tsigarida, Skandamis, & Nychas, 2000). In addition, many natural compounds found in dietary plants, such as extracts of herbs and fruit extracts, possess antimicrobial activities against *L. monocytogenes* (Cowan, 1999; Hao, Brackett, & Doyle, 1998; Kim, Marshall, & Wei, 1995). Several constituents of EO exhibit significant antimicrobial properties when tested separately (Kim et al., 1995; Lambert, Skandamis, Coote, & Nychas, 2001). However, there is evidence that EOs are more strongly antimicrobial than is accounted for by the additive effect of their major antimicrobial components; minor components appear, therefore, to play a significant role (Lattaoui & Tantaoui-Elaraki, 1994). Since EOs are considered as generally recognized as safe (GRAS) (Kabara, 1991), the possibility of reinforcing their natural antimicrobial effects by the addition of small amounts of other natural preservatives may be a way to attain a balance between sensory acceptability and antimicrobial efficacy.

Many studies have shown that the EOs of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*) and rosemary (*Rosmarinus officinalis*) are among the most active in this respect against a number of food spoilage and pathogen microorganisms (Smith-Palmer, Stewart, & Fyfe, 1998; Hammer, Carson, & Riley, 1999; Mangena & Muyima, 1999). The compositions of EOs from a particular species

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of plant can differ between harvesting seasons (McGimpsey & Douglas, 1994) and between geographical locations (Juliano, Mattana, & Usai, 2000); hence the principal constituents of these oils have to be chemically characterized.

*L. monocytogenes* has been recognized as a causative agent of foodborne illness, numerous outbreaks of which have occurred worldwide (Gahan & Collins, 1991). The ingestion of products contaminated with this organism may be a potential health threat to high-risk populations, such as immune-suppressed patients, children, pregnant women and the elderly. *L. monocytogenes* has the ability to survive adverse conditions, such as vacuum, freezing and ultraviolet rays, and to resist conventional pasteurization (Farber et al., 1988; Yousef & Marth, 1988; Gill & Reichel, 1989). The survivability of *L. monocytogenes* over a wide range of pH (4.4–9) and temperature (1–45 °C), and in foods with a high salt concentration (up to 10%) makes it difficult to control (Seeliger & Jones, 1986).

Lactic acid is widely used for food treatment as it lacks acute and chronic toxicity, which has led to its widespread employment as a food preservative and decontamination agent (Goncalves, Almeida, Alves, & Almeida, 2005). However, *L. monocytogenes* is a relatively acid-tolerant pathogen. Therefore, a high percentage of organic acid is often necessary to either kill or limit the growth of *L. monocytogenes* (Buchanan, Golden, & Whiting, 1993; Young & Foegeding, 1993; Tienungoon, Ratkowsky, McMeekin, & Ross, 2000) which may influence the sensory quality of food.

In this work, a study of the synergistic antilisterial effects of thymus, rosemary and oregano essential oil, in a mixture with a sub-lethal dose of lactic acid, on the population of *L. monocytogenes* IM2002 was, for the first time, undertaken. Essential oils in combination with a safe preservative, such as lactic acid, are expected to become valuable additives as consumer concern with the prevention of *L. monocytogenes* infections increases.

## 2. Materials and methods

### 2.1. Essential oils

The essential oils from oregano (*O. vulgare* L.), thyme (*T. vulgaris* L.) and rosemary (*R. officinalis* L.) were procured from a local company (BEOLAB Co., Belgrade, Serbia). All the essential oils were stored in brown bottles at 4 °C. The sterility of the oils (1 μl) was tested in TSB (10 ml) incubated under normal atmospheric conditions, at 37 °C for 24 h. The contaminating bacterial growth, if at all, was indicated by the presence of a white "pellet" on the well bottom. The oils were characterized by GC and GC–MS analyses.

### 2.2. GC analysis conditions

Gas chromatographic (GC) analysis was performed with a Varian 1400 instrument equipped with a Varian 4270 data integrator. The analysis was carried out using a DB-5 fused-silica capillary column (60 m × 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific, Palo Alto, USA) and a flame ionization detector. Nitrogen was used as the carrier gas at a constant flow rate of 1.0 ml/min. The split/splitless injector temperature was set at 200 °C and the detector temperature at 350 °C. The column temperature was programmed as follows: isothermal at 60 °C for 8 min, ramp to 240 °C at 3 °C/min, isothermal for 10 min and then a ramp to 325 °C at 10 °C/min. The split ratio was 1:60 with a split flow of 15 ml/min. Volume injected was 1 μl diluted in hexane (1:10).

### 2.3. GC/MS analysis conditions

The GC/MS analyses were performed in a Varian 1400 GC coupled with a Saturn II ion trap detector. The column and column temperature programme were the same as above. The interface temperature was 280°C, split ratio 1:100, carrier gas He, flow rate 1.0 ml/min, ionization energy 70 eV, mass range 40–350; volume injected was 0.4 μl diluted in hexane (1:10). Identification of the individual components was based on comparison of their GC retention indices (RI) on polar columns and comparison with the mass spectra of the components by GC/MS.

### 2.4. Bacterial strains and culture conditions

*L. monocytogenes* IM2002 was obtained from the Institute of Meat Hygiene and Technology (Belgrade). The bacteria were maintained in soft Trypton Soy Agar supplemented with 0.6% v/v yeast extract (TSYA—Institute of Immunology and Virology, Torlak, Belgrade) at +4 °C. The growth of listeria for investigation was conducted in Trypton Soy broth or agar, supplemented with yeast extract (TSBY or TSYA).

### 2.5. Antilisterial investigation

#### 2.5.1. Activity of oils

Two methods were used for the determination of the antilisterial activity of the oils. First, screening was performed by the agar-well diffusion method. The study of the bactericidal kinetics of the oils and their mixtures with lactic acid was performed in the broth system.

#### 2.5.2. Agar-well diffusion method

The antimicrobial test was done according to the method of Wan, Wilckok, and Conventry (1998) with some modifications. Briefly, 200 μl of fresh overnight cultures of indicators strains of *L. monocytogenes* IM2002 (density of ca. $10^9$ CFU/ml) were added to 6 ml of soft TSYA. The soft agar was vigorously mixed and poured over Petri plates with previously dried TSYA, on the surface of which sterile tubes (7 mm diameter) were placed. After solidification of the soft agar, the tubes were removed and the obtained wells were filled with 20 μl of oil samples and

lactic acid as the control. All plates were incubated at 37 °C for 24–48 h. The diameters of inhibition zones were measured in millimeters, including the well diameter. To establish the nature of the inhibitory activity of the oils, samples were taken from the clear zones with a loop and surface-plated onto TSYA and incubated under optimal conditions for up to 48 h.

#### 2.5.3. Inhibitory kinetics of the oils

The inhibitory effects of the oils in the broth system (TSYB) were monitored by optical density ($OD_{600}$) measurements, using a UV/Vis spectrophotometer (Shimatsu, 1700). The EOs were diluted to final concentrations of 50 ppm, 100 ppm, 200 ppm and 300 ppm. In a series of dilutions, 50 ppm of lactic acid (DL, 85% v/v, Aldrich) were added and all suspensions were vigorously vortexed at full speed for 1–3 min (pH of the suspensions was $6.80 \pm 0.05$). Assays for antimicrobial activity were conducted in 5 ml of TSBY (control) or TSBY containing different amounts of the essential oils, 50 ppm of lactic acid and mixtures of 50 ppm lactic acid and the appropriate concentration of the oils. The mixture was inoculated with a pathogen to reach ca. $10^4$ CFU/ml ($OD_{600} \sim 0.01$) and incubated at 37 °C for 24 h. The inhibition demonstrated by the essential oils and their mixtures with lactic acid is expressed by the following equation:

$$\text{Inhibition } (\%) = [(\text{ODcont.} - \text{ODt})/\text{ODcont.}] * 100, \quad (1)$$

where ODcont. is the $OD_{600}$ for the negative control (containing no essential oils), and ODt is the $OD_{600}$ for the sample treated with the antimicrobial compounds. All tests were performed in duplicate and the experiment was repeated at least twice to ensure reproducibility.

## 3. Results and discussion

### 3.1. Chemical analyses of the oils

The identified components of the essential oils, as well as the percentages and retention index (RI) of each component are listed in Table 1. GC/GC–MS analysis revealed 29, 22 and 35 compounds of oregano, rosemary and thyme oils, respectively These compounds represent over 90% of the oils.

The oils were characterized with prominent (>10%) concentrations of carvacrol and caryophyllene for oregano, 1,8-cyneol and camphor for rosemary and *p*-cymene, thymol and carvacrol for thyme. The oregano oil was also distinctive in its high concentrations (>5%) of spathulenol, *p*-cymene, germacrene D, thymol and caryophyllene oxide, while piperitone was the single compound in rosemary oil and α-pinene was present in thyme oil within these concentrations. The presence of α-pinene, camphene, β-pinene, myrcene, 1,8-cyneol, camphor, borneol, 4-terpineole, α-terpineole and β-caryophyllene was detected in all oils in different concentrations. Linalol was present in oregano and rosemary oils but not in thyme oil, while *p*-cymene and limonene were present in oregano and thyme oils but not in rosemary oil.

According to various studies, the composition of EOs from a particular species of plant can vary between harvesting seasons (McGimpsey & Douglas, 1994) and between geographical sources (Juliano et al., 2000). As in our results, Hristova, Ristic, Brkic, Stefkov, and Kulevanova (1999), as well as Baranauskiene, Venskutonis, Dewettinck, and Verhe (2006) recognized carvacrol as a major constituent in oregano oil. By contrast, Mockutë, Judþentienë, and Bernotienë (2004) found that the main components of wild *O. vulgare* essential oil from leaf were germacrene D and β-cariophyllene (>10%), while carvacrol was detected only in traces. Atti-Santos et al. (2005) reported that, while the investigated rosemary oils contained large quantities of camphor and 1,8-cineole, the main component was α-pinene. Comparably, the rosemary oil examined in the present study contained 1,8-cyneol, camphor, piperitone and α-pinene in concentrations of 52.20%, 10.08%, 6.68% and 4.21%, respectively. Another study confirmed that 1,8-cineole, camphor and α-pinene were the main constituents of rosemary oil (Mangena & Muyima, 1999). As main constituents, *p*-cymene and thymol were found in a phenotype of thyme oil from Croatia. The content of carvacrol was about 5% (Jukic & Milos, 2005). These results are similar to those found in the present study (Table 1), which might be due to the proximity of the territory. The established chemical content of the oils studied in the present study also agrees with the results of reviewed analyses of different essential oils (Burt, 2004).

### 3.2. Antilisterial activity

In this study, the influence of a small (sub-lethal) concentration of lactic acid (50 ppm) on the antilisterial activities of oregano, rosemary and thyme essential oils was examined. The aim of the preliminary examination was to confirm the antilisterial effect of the oils (20 μl), as well as to estimate the effect of a sub-lethal concentration of lactic acid (20 μl of 50 ppm solution in TSBY) on *L. monocytogenes*. The results of this examination by agar-well diffusion assay (Fig. 1 and Table 2) reveal that all the oils showed good antilisterial activity. The rosemary and thyme oils were slightly more active than was oregano oil and all the oils showed bactericidal activity. In comparison to the oils, lactic acid showed a lower antilisterial activity in the form of a turbid zone roundabout the well and bacteristatic activity against *L. monocytogenes* IM2002.

A similar zone diameter ($17 \pm 1.4$ mm) was obtained with rosemary oil during *L. moncytogenes* ATCC7644 inhibition, when a 6 mm disk and 15 μl of sample were used (Mangena & Muyima, 1999). The investigation of Baydar, Sagdic, Ozkan, and Karadogan (2004) showed that 50 μl of oregano oil on a 5 mm filter paper disk produced a 24.5–31.0 mm zone of *L. monocytogenes* inhibition.

Examinations of bactericidal kinetics of the oils showed that there were similar response effects of the essential oils

Table 1
Chemical composition of the essential oils from *Origanum vulgare* L., *Rosmarinus officinalis* L. and *Thymus vulgaris* L.

| No. | Components | *O. vulgare* L. | | *R. officinalis* L. | | *T. vulgaris* L. | |
|---|---|---|---|---|---|---|---|
| | | RI | % | RI | % | RI | % |
| 1 | Tricyclene | – | – | 928 | 0.17 | 926 | 0.12 |
| 2 | α-Thujene | – | – | – | – | 929 | 0.61 |
| **3** | **α-Pinene** | **939** | **0.27** | **935** | **4.21** | **936** | **7.75** |
| **4** | **Camphene** | **957** | **0.26** | **955** | **4.06** | **954** | **4.83** |
| 5 | 3-Octanone | – | – | 964 | 1.49 | – | – |
| 6 | Sabinene | 977 | 0.10 | 975 | 0.50 | – | – |
| **7** | **β-Pinene** | **985** | **0.26** | **988** | **0.28** | **983** | **0.86** |
| 8 | 1-Octen-3-ol | – | – | – | – | 980 | 1.99 |
| 9 | 3-Octanone | – | – | – | – | 985 | 0.45 |
| 10 | 3-Octanol | – | – | – | – | 995 | 0.12 |
| **11** | **Myrcene** | **995** | **0.10** | **992** | **2.61** | **997** | **1.22** |
| 12 | α-Phellandrene | – | – | – | – | 1002 | 0.10 |
| 13 | *o*-Cymene | – | – | 1012 | 0.59 | – | – |
| 14 | α-Terpinene | 1019 | 0.92 | – | – | 1017 | 1.00 |
| **15** | **1,8-Cineole** | **1021** | **0.52** | **1022** | **52.20** | **1029** | **0.54** |
| **16** | ***p*-Cymene** | **1032** | **6.68** | – | – | **1032** | **15.3** |
| 17 | Limonene | 1038 | 2.56 | – | – | 1038 | 1.08 |
| 18 | *cis*-β-Ocimene | – | – | – | – | 1040 | 0.20 |
| 19 | *trans*-β-Ocimene | 1052 | 0.48 | – | – | 1050 | 0.31 |
| 20 | γ-Terpinene | 1062 | 0.61 | – | – | 1059 | 1.10 |
| 21 | *trans*-Sabinene hydrate | 1078 | 0.11 | – | – | – | – |
| 22 | Terpinolene | – | – | – | – | 1088 | 1.73 |
| 23 | *cis*-Sabinene hydrate | 1093 | 0.62 | – | – | – | – |
| 24 | Terpineol | 1090 | 1.10 | – | – | – | – |
| **25** | **Linalol** | **1101** | **3.48** | **1099** | **3.37** | – | – |
| 26 | *cis*-Thujone | – | – | – | – | 1101 | 3.48 |
| 27 | *trans*-Thujone | – | – | – | – | 1114 | 0.10 |
| 28 | Myrcenol | – | – | 1105 | 0.32 | | |
| **29** | **Camphor** | **1148** | **0.42** | **1146** | **10.08** | **1145** | **1.93** |
| 30 | Isoborneol | 1153 | 0.28 | – | – | – | – |
| **31** | **Borneol** | **1164** | **0.64** | **1165** | **3.75** | **1167** | **2.29** |
| **32** | **4-Terpineol** | **1183** | **0.10** | **1184** | **1.71** | **1185** | **0.53** |
| 33 | Verbinone | – | – | 1185 | 1.86 | – | – |
| **34** | **α-Terpineol** | **1190** | **1.96** | **1188** | **0.80** | **1188** | **0.78** |
| 35 | Thymol methyl ether | – | – | – | – | 1235 | 0.16 |
| 36 | Carvacrol methyl ether | – | – | – | – | 1244 | 1.21 |
| **37** | **Piperitone** | – | – | **1245** | **6.68** | – | – |
| 38 | Geraniol | – | – | – | – | 1253 | 8.63 |
| 39 | Geranial | – | – | – | – | 1266 | 0.12 |
| 40 | Bornyl acetate | – | – | 1284 | 1.46 | 1288 | 0.56 |
| **41** | **Thymol** | **1301** | **5.67** | – | – | **1300** | **17.37** |
| **42** | **Carvacrol** | **1316** | **33.51** | – | – | **1218** | **12.10** |
| 43 | α-Terpynyl acetate | – | – | – | – | 1348 | 0.75 |
| 44 | Geranyl acetate | – | – | | | 1380 | 3.54 |
| **45** | **β-Caryophyllene** | **1431** | **0.14** | **1434** | **1.36** | **1436** | **0.56** |
| **46** | **Caryophyllene** | **1421** | **10.29** | – | – | – | – |
| 47 | *cis*-β-Farnesene | – | – | 1449 | 0.26 | – | – |
| 48 | α-Caryophyllene | 1459 | 3.26 | – | – | – | – |
| **49** | **Germacrene D** | **1491** | **6.20** | **1490** | **0.13** | – | – |
| 50 | Bicyclogernacrene | 1504 | 2.52 | – | – | – | – |
| **51** | **Spathulenol** | **1582** | **8.44** | – | – | **1582** | **0.19** |
| 52 | Cariophyllene oxide | 1588 | 5.19 | – | – | – | – |
| 53 | α-Bisabolol | – | – | 1672 | 0.18 | – | – |
| | **Total**[a] | | **96.15** | | **98.07** | | **93.61** |

(RI)—Kovats retention indices (Kovats, 1958).
[a] Other components were found at less than 0.1%.

in broth systems during the following period of incubation (Figs. 2–4, a). However, the applied doses (50 ppm, 100 ppm, 200 ppm and 300 ppm) were not sufficient for complete inhibition of listerial growth. According to the observation of Burt (2004), the usual values for complete inhibition of *L. monocytogenes* varied around 0.2 μl/ml (the recalculated value is 200 ppm) and 0.156–0.450 μl/ml (156–450 ppm) for rosemary and thyme oil, respectively.

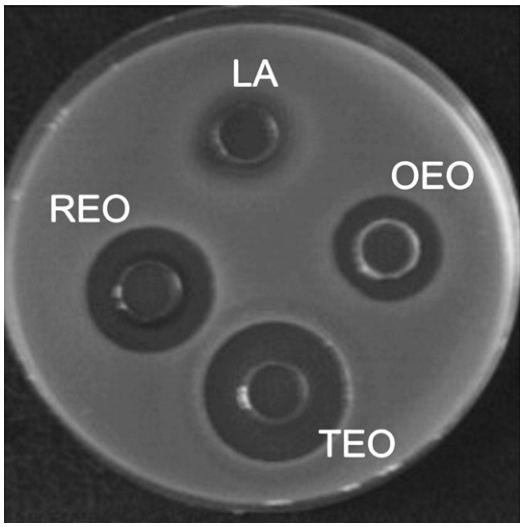


Fig. 1. Inhibition zone of LA—lactic acid; OEO—oregano oil; TEO—Thyme oil and REO—rosemary oil against *L. monocytogenes* IM200.

Marino, Bersani, and Com (1999) found that concentrations of 400 and 800 ppm of different thyme oils irreversibly damaged cells of *Listeria innocua*. In a study of the minimal concentrations which inhibited 10 different microorganisms, these values for oregano, rosemary and thyme oils ranged from 0.12% to 0.25% v/v, 0.5% to 1.0% v/v and 0.12% to 0.5% v/v, respectively (Hammer et al., 1999).

Table 2
Antibacterial properties of essential oils against *L. monocytogenes* using the agar-well diffusion method

| Inhibitory substances | Diameter of inhibition zone (mm) (mean ± SD) | Nature of the inhibitory activity |
|---|---|---|
| Lactic acid | 13 ± 0.2[a] | BS |
| Oregano | 18 ± 0.2[b] | BC |
| Thyme | 23 ± 0.1[b] | BC |
| Rosemary | 21 ± 0.1[b] | BC |

The diameter of the zone of inhibition includes the wells (7 mm).
BS—bacteriostatic, BC—bactericidal.
[a] Turbid.
[b] Clear.

Comparable inhibitory action was observed for thyme and rosemary oils in the applied concentration, while oregano oil showed a lower antilisterial activity, even at the highest concentration (Figs. 2–4, a). However, the scale of inhibition decreased, especially after 4 h of incubation, for all samples. This suggests that *L. monocytogenes* IM2002 becomes accustomed to the presence of the oils and exhibits growth during the remaining incubation period.

When lactic acid was added to the oils, a notable increase of the antilisterial activity, especially for the thyme and rosemary oils (Figs. 2–4, b), was observed. In a mixture of lactic acid with 300 ppm and 200 ppm of rosemary and thyme oils, respectively, complete inhibition of *L. monocytogenes* was achieved. In contrast to the results obtained in the absence of lactic acid, inhibition also remained high during longer incubation times.

It is known that organic acids can interact with other preservatives to enhance their effects. For example, acetic and lactic acids enhance the antilisterial effects of monolaurin (Monk, Beuchat, & Hathcox, 1996). Lactic acid also increased the susceptibility of *L. monocytogenes* to heat shock in culture media (Jorgensen, Hansen, & Knochel, 1999).

It is notable that the applied concentration of LA (50 ppm) also showed a high percent inhibition (67.78%) at the beginning which was reduced to 35.76% at the end of the incubation period (Figs. 2–4, b). Ahamad and Marth (1989) reported that lactic acid, as well as acetic acid, in concentrations as low as 0.1%, when incorporated into tryptose broth, inhibited the growth of *L. monocytogenes*.

The percentages of inhibition were calculated for a period of 4–18 h of incubation. This period was selected because, after 4 h of incubation, the cells of *L. monocytogenes* went

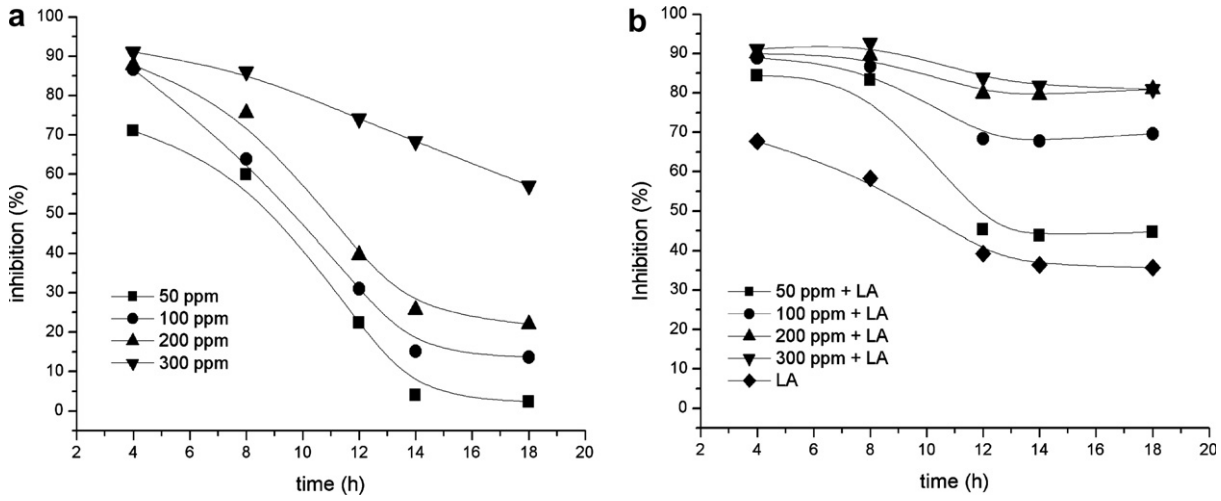


Fig. 2. The percent inhibition of *L. monocytogenes* IM2000 growth in TSYB with (a) oregano oil—OEO and (b) oregano oil plus 50 ppm of lactic acid (LA).

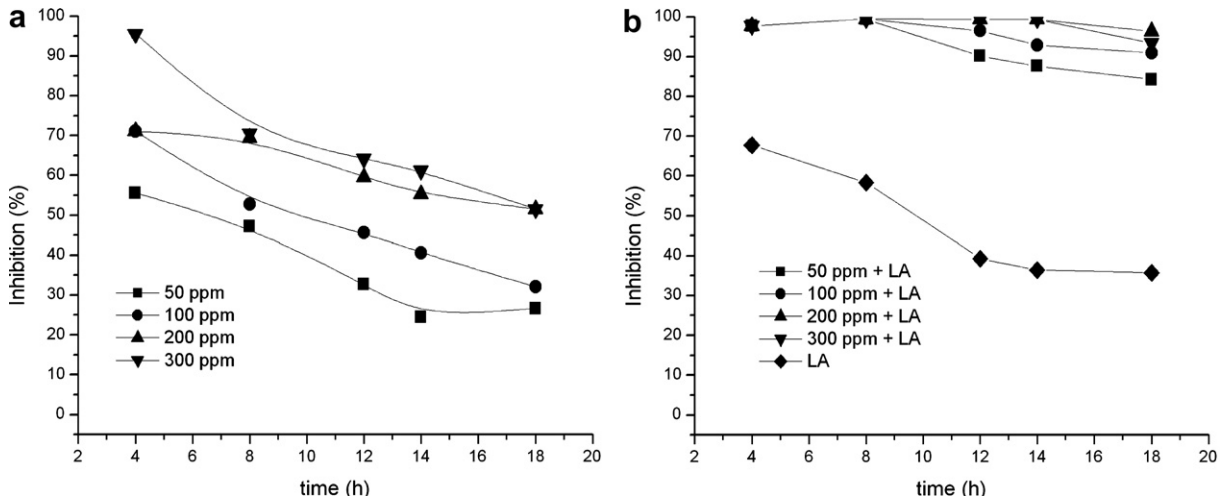


Fig. 3. The percent inhibition of *L. monocytogenes* IM2000 growth in TSYB with (a) rosemary oil—REO and (b) rosemary oil plus 50 ppm of lactic acid (LA).

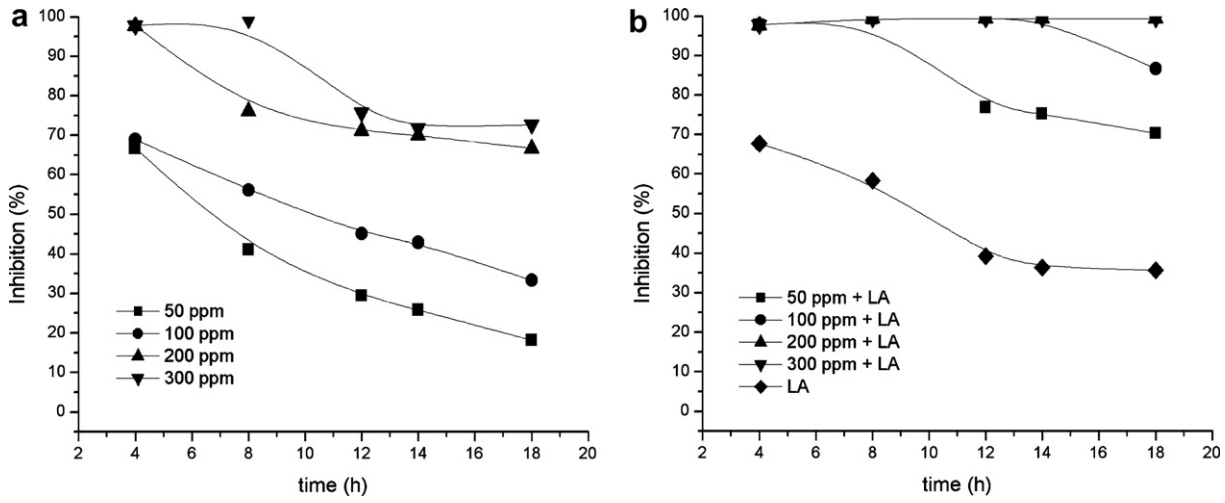


Fig. 4. The percent inhibition of *L. monocytogenes* IM2000 growth in TSYB with (a) thyme oil—TEO and (b) thyme oil plus 50 ppm of lactic acid (LA).

into an exponential phase of growth in the samples with the oil alone, as well as with only lactic acid. In the control sample (without antimicrobials), the exponential phase of growth of *L. monocytogenes* commenced after 1 h of incubation. After 8 h, the *L. monocytogenes* entered into a stationary phase of growth and subsequently the $OD_{600}$ values remained almost constant until the end of the incubation (data not shown). Since the inhibition rate differs within a selected period of incubation, the mean values of the percent inhibition during the period of 4–18 h were calculated for all samples. The obtained values are plotted against concentration on a linear scale. Table 3 and Figs. 5–7 represent the comparative mean values of inhibition for the individual oils and the oil mixtures with lactic acid in dependence on concentration.

The results obtained in this study show that, according to the diameter of the inhibition zone (Fig. 1) as well as to the overall percent inhibition (Table 3), thyme oil has a stronger antilisterial activity than have rosemary and oregano oils. However, the results presented in Table 3 show that the overall percent inhibition of rosemary oil was higher than that of thyme oil at the smallest concentration employed (50 ppm of oil as well as mixture with lactic acid). The antilisterial activity of oregano oil was inferior except at the highest employed concentration (300 ppm), when the percent overall inhibition of *L. monocytogenes* growth was higher than that for rosemary oil (Table 3). The percent overall inhibition of lactic acid was 47.53%, which is similar to the inhibition of 100 ppm of the oils (Table 3).

It has been reported that there is a relationship between the chemical composition of the tested oil and the antimicrobial activity. The phenolic compounds are widely reported to possess high levels of antimicrobial activity (Baydar et al., 2004; Dorman & Deans, 2000; Lambert et al., 2001). The antimicrobial natures of the studied essential oils of thyme and oregano are apparently related to their high phenolic contents, particularly carvacrol and

Table 3
The mean value of inhibition (%) of *L. monocytogenes* IM2000 growth during 4–18 h of incubation in dependence on the concentration of the essential oils (EOs) and mixtures of the EOs and 50 ppm of lactic acid (LA)[a]

| Concentration (ppm) | Mean value (4–18 h)[b] of inhibition (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | EO50 | EO50 + LA | EO100 | EO100 + LA | EO200 | EO200 + LA | EO300 | EO300 + LA |
| Oregano | 32.00 | 60.33 | 42.09 | 76.28 | 50.15 | 83.96 | 75.38 | 86.12 |
| Thyme | 36.26 | 83.93 | 49.27 | 96.52 | 76.33 | 99.08 | 83.48 | 99.08 |
| Rosemary | 37.33 | 91.86 | 48.45 | 95.52 | 61.38 | 98.49 | 68.59 | 97.89 |

[a] Concentration of lactic acid is the same in all samples (50 ppm). The mean value of inhibition of 50 ppm LA during the time of incubation was 47.53%.
[b] Period for which the mean value of % of inhibition was calculated.

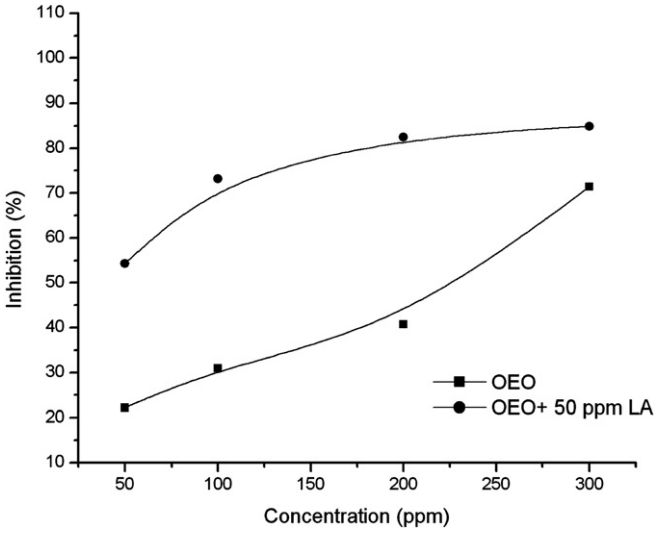


Fig. 5. The mean value of inhibition (%) of *L. monocytogenes* IM2000 growth during 4–18 h of incubation in dependence on the concentration of oregano oil (OEO) and a mixture of OEO and 50 ppm of lactic acid (OEO + 50 ppm LA).

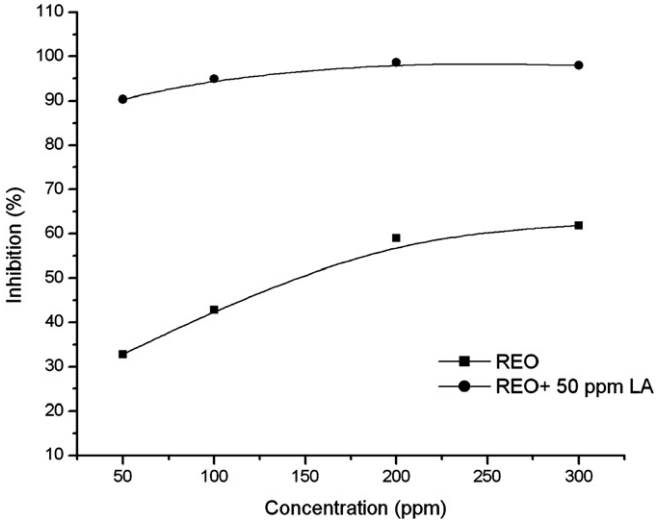


Fig. 6. The mean value of inhibition (%) of *L. monocytogenes* IM2000 growth during 4–18 h of incubation in dependence on the concentration of rosemary oil (REO) and a mixture of REO and 50 ppm of lactic acid (REO + 50 ppm LA).

thymol (Table 1). Mourey and Canillac (2002) found that the constituents of EOs, such as monoterpenes (pinene, limonene, and cineole), contribute to the antimicrobial

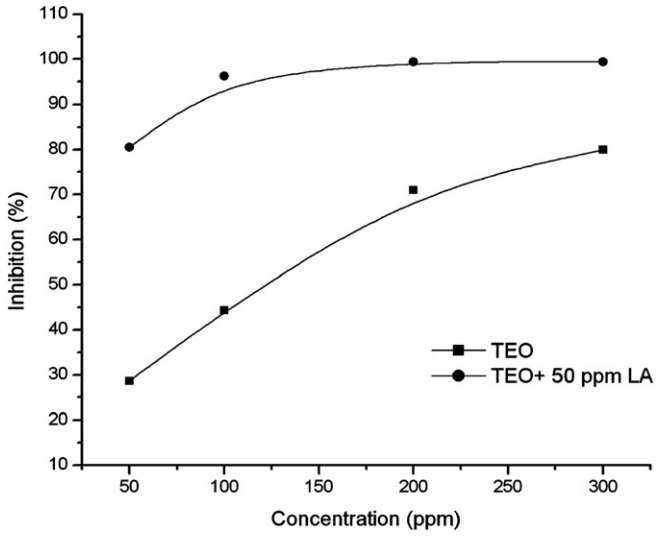


Fig. 7. The mean value of inhibition (%) of *L. monocytogenes* IM2000 growth during 4–18 h of incubation in dependence on concentration of thyme oil (TEO) and a mixture of TEO and 50 ppm of lactic acid (TEO + 50 ppm LA).

effect, particularly against *L. monocytogenes*. These compounds are revealed in high content in the essential oil of rosemary (Table 1).

Leistner and Gorris (1995) suggested that food preservation, by multiple preservatives in small amounts, was superior to preservation by a large amount of a single preservative in order to secure both microbial stability and safety and maintain the sensory, nutritive and economic properties of the foods. A synergistic effect between essential oils and other antimicrobial substances has been demonstrated (Blaszyk & Holley, 1998; Knowles & Roller, 2001) and it has been noted that the activities of the essential oil constituents (e.g. carvacrol and thymol) are enhanced by the presence of nisin (Pol & Smid, 1999; Ettayebi, Yamani, & Rossi-Hassani, 2000). However, little information exists about the use of EOs and LA antimicrobial combination in foods. In the present study, the antimicrobial activity of small amounts of the tested EOs, especially of thyme and oregano oils, was found to be enhanced by sub-lethal dose of lactic acid. An increased antibacterial effect, after lactic acid addition (at pH 6) to a mixture of oregano oil and cranberry extract, was reported by Lin, Labbe, and Shetty (2004) Lin, Labbe, and Shetty (2005). In work of Naveena, Muthukumar, Sen, Babji, and Murthy (2006),

the usage of mixed LA and clove oil significantly extended buffalo meat display life at 4 °C.

It is difficult to understand what exactly is the mechanism of synergistic action of investigated EOs and LA. Most of the studies on the mechanism of phenolic compounds focussed on their effects on the cellular membrane, altering its function and in some instances structure, causing swelling and increasing its permeability. Lin et al. (2005) postulated that phenolic-substances may create a low pH microenvironment due to proton donation and cell membrane disruption, due to stacking. On the other hand, the inhibition of growth by a weak acid preservative has been proposed to be due to a number of actions, including membrane disruption, inhibition of essential metabolic reactions, stress on intracellular pH homeostasis and the accumulation of toxic anions (Brul & Coote, 1999). Combination of these effects might lead to synergy between EOs or some components of the EOs and LA.

However, in this study the synergistic effect of the mixtures decreased with increasing concentration of the oils (Figs. 5–7). The best synergy effect was achieved in mixtures of both thyme oil (50 ppm) and rosemary oil (50 ppm) with LA. To the best of our knowledge, it seems that this phenomenon has not been noticed before. There are not many reports about combinations between sublethal acidic and aromatic compounds present simultaneously in the growth medium. However, this work might be an interesting and promising approach for the optimization of food preservation, considering both economical aspects and the sensory quality of food. The potential interest of this topic requires further studies using model system to investigate the effects of certain combinations of EOs or their pure components and LA, at various levels, against pathogenic and spoilage micro-organisms, in order to expand the knowledge on usage of such natural additives in industrial practice.

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