ISSN 1330-9862
(FTB-3538)

scientific note

Essential Oil of Common Thyme as a Natural Antimicrobial Food Additive

*Sabina Anžlovar[1], Dea Baričevič[2], Jerneja Ambrožič Avguštin[1] and Jasna Dolenc Koce[1]**

[1]Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia

[2]Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

Received: October 9, 2013
Accepted: April 4, 2014

Summary

Antimicrobial activities of thyme essential oil against selected microorganisms, including *Fusarium* sp., *Armillaria mellea, Bacillus cereus*, *Staphylococcus aureus, Buttiauxella* sp., *Klebsiella pneumoniae, Escherichia coli* K-12, AmpC-producing *E. coli* Z, ESBL-producing *E. coli* strain of KM clonal group ST131, and *E. coli* 1138 were evaluated. The antimicrobial efficacy of thyme essential oil was determined using agar well diffusion assays. The growth of all tested bacteria was inhibited at thyme essential oil fractions higher than 1 %, while a fraction of 10 % was needed to inhibit the growth of fungi. We demonstrate that thyme essential oil has a promising activity against food spoilage bacteria, and also against multiresistant AmpC-producing and ESBL-producing bacterial strains isolated from food, which have recently been recognised as public health concerns. On the basis of our data, the thyme essential oil has a potential for use as a growth inhibitor of multidrug-resistant bacteria, and food spoilage and pathogenic bacteria and fungi, to replace commonly used semi-synthetic antimicrobial products.

Key words: *Thymus vulgaris*, essential oil, antibacterial activity, multidrug-resistant bacteria, antifungal activity, food additive

Introduction

Antimicrobial chemical compounds are used in the food industry for two main reasons: to control natural deterioration processes (food preservation), and to prevent or limit growth of microorganisms (*1*). However, the food industry is now under pressure to reduce the use of synthetic antimicrobial chemical compounds, especially in Western societies, which appear to be experiencing a trend for 'green' consumerism and 'clean-labelling' of food products (*2*). As an alternative to synthetic preservatives, antimicrobial compounds from plants are becoming a positive selling point, thus creating a modern trend towards so-called 'natural' additives and preservatives.

Plants contain a broad pool of secondary metabolites that they use for protection and defence against biotic and abiotic stresses, including microbial infections. The biochemical defences of many plant species are boosted by the presence of antimicrobially active compounds in their leaves, fruits, bulbs and seeds. Although plant oils and extracts have been used for various purposes for centuries, there is a renewed interest in their use as preservatives in food production, as they have generally been recognised as safe and are widely accepted by consumers (*2*).

Essential oils are volatile, natural, complex compounds that are characterised by strong odours and are synthesised by aromatic plants as secondary metabo-

*Corresponding author: Phone: +386 1 320 3325; Fax: +386 1 257 3390; E-mail: jasna.dolenc.koce@bf.uni-lj.si

lites. The chemical compositions of essential oils include complex mixtures of organic substances that have different functional groups, mainly terpenoids (*3*). Essential oils of various plants show sufficient antimicrobial activity to be investigated as possible preservatives for application in the food industry and other consumer-oriented industries (*4–7*).

The genus *Thymus* (thyme) consists of about 215 species of herbaceous perennials and subshrubs that are well adapted to hot and dry climates. This is one of the most widely used genera in folk medicine (*8*). Common thyme (*Thymus vulgaris* L.) is a low-growing herbaceous plant that is native to southern Europe, where it is often cultivated as a culinary herb and used as a spice in many foods. Thyme herb (*Thymi herba*) is produced from the dried leaves and flowering tops of *T. vulgaris*, and it contains tannins, flavonoids, triterpene compounds, and up to 2.5 % of essential oils (*9*). The essential oils of *T. vulgaris* are known to have antiseptic, antiviral and antimicrobial activities. The main components are thymol and *p*-cymene, while other thyme species also contain carvacrol, α-terpinyl acetate, and *cis*-myrtanol (*10*).

The present study reports on the antibacterial and antifungal activities of a common thyme essential oil and its potential for use as a natural food additive, due to its inhibitory effects on the growth of susceptible and multidrug-resistant pathogenic and non-pathogenic food spoilage bacteria and fungi.

Materials and Methods

Plant material

Seedlings of common thyme (*Thymus vulgaris* L. cv. Deutscher winter) were grown from seeds under greenhouse conditions, and transplanted to the experimental field of the Biotechnical Faculty (University of Ljubljana, Slovenia: 46° 2' 53.7'' N, 14° 28' 30.47'' E; altitude 292 m above sea level) in March 2010. The plantation was regularly cultivated and grown in the experimental field until October 2010, when the non-flowering shoots were harvested and processed to extract the essential oils. The fresh and dry mass and the water content in the thyme herb were determined by weighing the plant material before and after drying in a laboratory oven at 105 °C.

Isolation and analysis of the thyme essential oil

Thyme essential oil was isolated by water-steam distillation using a Clevenger apparatus, following the procedures of the European Pharmacopoeia monograph on *Thymi herba* (*11*). Briefly, fresh plant material was placed in an adapted steam distiller that comprised a steam boiler connected to a water condensor. Distillation lasted approx. 2 h. After distillation, the thyme essential oil was collected in dark vials and stored in a refrigerator at 4 °C.

For chemical analysis, the composition of the thyme essential oil was studied as described previously (*12*), with some modifications. Thyme essential oil (1 mL) was dissolved in hexane (1 mL). A gas chromatograph with a QP-2010 mass spectrometry detector (Shimadzu, Kyoto, Japan) was used according to the following characteristics: column Rxi-5Sil MS, 30 m×0.25 mm i.d.; film thickness 0.25 μm (Restek, Bellefonte, PA, USA); temperature programme 50 °C (5 min), 5 °C/min to 200 °C, 200 °C (5 min); injector temperature 250 °C; ion source temperature 200 °C; interface temperature 260 °C; injection volume 1 μL; split 1:100; carrier gas He; carrier gas flow 1 mL/min. The mass spectrometry conditions were: electron impact mode, total ion current record, detector voltage 1 kV. The mass spectra of the compounds that were obtained were compared to the spectra from the NIST08 (National Institute of Standards and Technology, Gaithersburg, MD, USA) and FFNSC 1.3 (Shimadzu, Kyoto, Japan) mass spectral libraries.

Antibacterial activity assay

The antibacterial activity was tested using an agar well diffusion assay (*13*). The Gram-positive and Gram-negative bacteria used in this study were as follows: laboratory strains of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* K-12, AmpC-producing *E. coli* Z isolated from meat, extended-spectrum β-lactamase (ESBL)-producing and fluoroquinolone-resistant *E. coli* 1138 isolated from a sausage, a clinical ESBL-producing *E. coli* KM isolate belonging to the clonal group ST131, a clinical ESBL-producing *Klebsiella pneumoniae* isolate, and a strain of the potential food spoilage bacteria *Buttiauxella* sp. isolated from meat. All of the strains were obtained from the EX Culture Collection (Department of Biology, Biotechnical Faculty and Infrastructural Centre Mycosmo, University of Ljubljana, Slovenia) and the Chair of Molecular Genetics and Microorganisms Biology (Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia).

Precultured bacteria grown in Luria-Bertani (LB) broth (Sigma-Aldrich, St. Louis, MO, USA) were used for the inoculation of the LB agar plates to a final concentration of $5 \cdot 10^5$ cells/L. Four wells were made in each agar plate using a sterile borer (diameter of 7 mm) and then filled with 30 μL of 96 % ethanol (control), or 0.1, 1 or 10 % of thyme essential oil. All of the thyme essential oil solutions were prepared by diluting the concentrated thyme essential oil in 96 % ethanol, which was used as control. The size of the inhibition zone diameters was determined after 24 h of incubation at 37 °C. Samples showing inhibition of the bacterial growth were tested again, and the mean values of three independent replicates were calculated.

Fungal growth inhibition assay

The thyme essential oil was tested for antifungal activity using a hyphal extension inhibition assay (*14*). The antifungal activity was tested against the fungi *Armillaria mellea* (Ascomycetes) and *Fusarium* sp. (Basidiomycetes), both of which were obtained from the fungal collection of the Chair of Botany and Plant Physiology (Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia). The fungal inocula (size 5×5 mm) were placed in the centre of potato dextrose agar plates (Biolife, Milan, Italy). The plates were then sealed, and the fungi left to grow in the dark at room temperature for 2–3 weeks, until the colonies were approx. 3 cm in diameter. Small paper discs were soaked with 30 μL of 96 % ethanol (control), or 0.1, 1 or 10 % thyme essential oil,

prepared as above. The paper discs were placed on the periphery of the developed fungal colonies. The fungal growth was monitored over 1–2 weeks to determine the antifungal activity of thyme essential oil. The size of the inhibition zone of each sample was measured. Samples showing inhibition of fungal growth were tested again, and the mean values of three independent replicates were calculated.

Statistical analysis

Statistical analysis was performed with the GraphPad Prism (Graph Pad Software, Inc., San Diego, CA, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) softwares. Mean values and standard deviations were calculated for the measurements of inhibition zones. The significance of the differences between samples was tested using *t*-test. The level of significance was set at α=0.05 ($p<0.05$).

Results and Discussion

The quality of the plant material and the thyme essential oil was evaluated according to the European Pharmacopoeia criteria (*11*). The water content in the thyme herb was 8.64 %, which is lower than the maximum required, of 10 %. Also, the thyme essential oil content was 27 mL per kg of the fresh mass and 35.9 mL per kg on the dry mass basis, which is within the European Pharmacopoeia quality criteria (in anhydrous drug minimum of 12 mL per kg of essential oil, of which a minimum of 40 % is thymol and carvacrol). Chemical analysis of the thyme essential oil showed that thymol (68.91 %), *para*-cymene (13.61 %), γ-terpinene (7.60 %), ocimene (2.11 %) and carvacrol (1.55 %) were present at the highest fractions (Fig. 1). Lower amounts of following compounds were measured (in %): eucalyptol 0.89, sabinene hydrate 0.80, myrcene 0.76, α-terpinolene 0.69, caryophyllene 0.49, 1-octen-3-ol 0.48, camphor 0.45, thymol methyl ether 0.45, carvacrol methyl ether 0.34, limonene 0.33, α-phellandrene 0.30 and α-pinene 0.24.

Antibacterial activity

The antibacterial activities of thyme essential oil were assayed *in vitro* using an agar well diffusion test against eight bacterial strains belonging to five bacterial species. The data show that at fractions of 1 and 10 %, the thyme essential oil had inhibitory dose-dependent effects against all eight of these bacterial strains (Table 1). The lowest fraction of thyme essential oil (0.1 %) showed only slight inhibitory activity against ESBL-producing *Klebsiella pneumoniae* and *Buttiauxella* sp., while essential oil at the fraction of 1 % and even more pronounced at 10 %, significantly inhibited the growth of all the tested bacterial strains (Table 1). Mean inhibition zone diameters in the presence of 1 % of essential oil were 1.9 mm for *S. aureus* and 3.8 mm for *Buttiauxella* sp. The inhibition zones of the bacterial cultures grown in the presence of 10 % of essential oil were larger, and ranged from 5.6 mm for ESBL-producing *K. pneumoniae* to 10.6 mm for ESBL-producing *E. coli* KM:ST131.

These dose-dependent effects of the antibacterial activities of thyme essential oil are in agreement with other studies (*8,15,16*). Most of these studies investigated the activities of thyme essential oils against bacteria, and they also indicated that, in general, thyme essential oils are more active against Gram-positive than against Gram-negative bacteria (*2*). However, in the present study, thyme essential oil did not show any selective Gram-positive *vs.* Gram-negative antimicrobial activity. Thus, the most (ESBL-producing *E. coli* KM:ST131) and the least (ESBL-producing *K. pneumoniae*) susceptible strains were both Gram-negative bacteria. Similar effects were observed by Sokmen *et al.* (*17*) and Viuda-Martos *et al.* (*16*), who concluded that the essential oils of spices

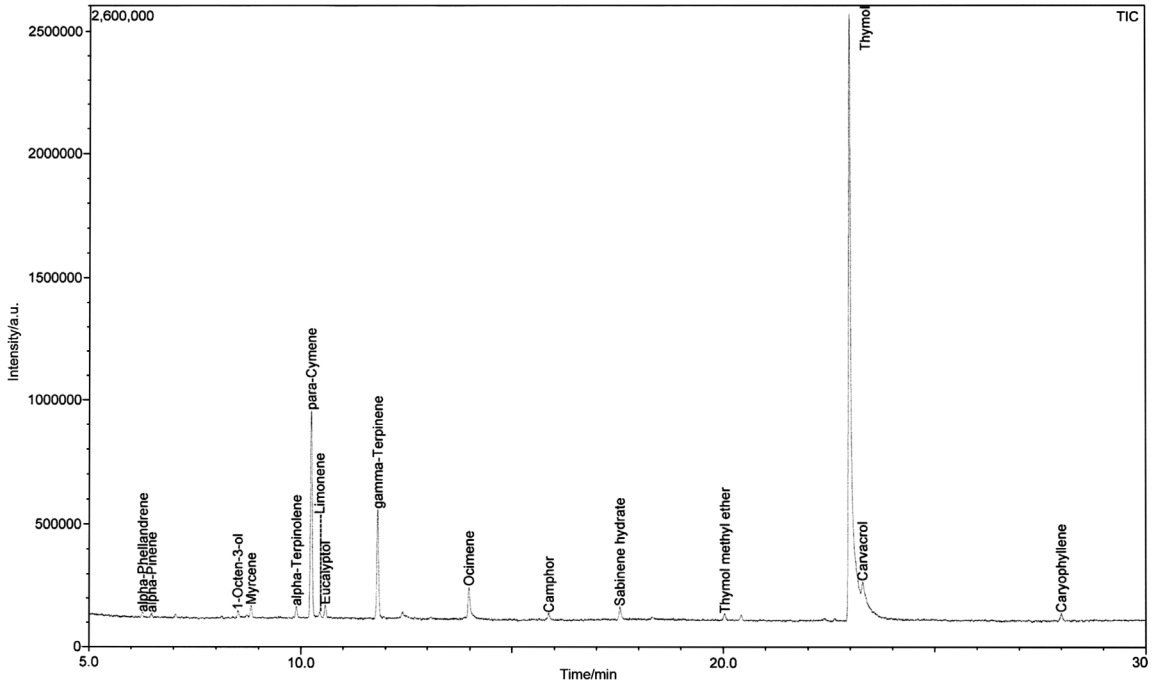

Fig. 1. Gas chromatography-mass spectroscopy spectrum of *Thymus vulgaris* essential oil

Table 1. Antibacterial activities of *Thymus vulgaris* essential oil

	φ(thyme essential oil)/%			
	0	0.1	1	10
Bacterial strain	r(inhibition zone)/mm			
Gram-positive				
S. aureus	n.d.	n.d.	1.9±0.3	7.0±1.3
B. cereus	n.d.	n.d.	3.2±0.4	9.5±0.8
Gram-negative				
E. coli K-12	n.d.	n.d.	3.0±0.4	8.1±0.5
AmpC-producing *E. coli Z*	n.d.	n.d.	2.8±0.5	7.6±0.6
ESBL-producing *E. coli* KM:ST131	n.d.	n.d.	3.1±0.5	10.6±1.0
ESBL-producing *E. coli* 1138	n.d.	n.d.	2.5±0.1	10.3±1.5
ESBL-producing *K. pneumoniae*	n.d.	0.3±0.6	2.3±0.3	5.6±1.2
Buttiauxella sp.	n.d.	0.3±0.6	3.8±0.6	9.4±1.0

Data are mean values±standard deviations of three independent replicates (N=3)
n.d.=growth inhibition not detected

show no selectivity towards bacterial cell walls. Politeo *et al.* (*18*) showed that a volatile oil of *Campanula portenschlagiana* had antimicrobial effects that were more pronounced against Gram-negative bacteria.

It is assumed that the age of the distilled essential oils and their compositions will vary due to the geographic origins and harvesting period, which will affect the degree of activity against Gram-positive and Gram--negative bacteria (2). Similar antimicrobial activities of the essential oil from *T. vulgaris* cultivated in Spain were reported by Viuda-Martos *et al.* (*16*) at 10 % fraction, whereas the 5 % thyme essential oil had no inhibitory effect against any of the tested bacteria. The antibacterial activities of essential oils of several other thyme species have also been studied. De Martino *et al.* (*19*) investigated the antibacterial activities of essential oils from the aerial parts of *Thymus longicaulis* and *Thymus pulegioides* collected in Italy. Both of these thyme essential oils showed substantial antibacterial activities against *S. aureus*, *B. cereus* and four other bacterial strains, at a concentration of 10 mg/mL. Of note, it has generally been shown that higher concentrations of essential oils are needed to achieve the same growth inhibitory and preservation effects in food (2).

The antimicrobial activities were investigated in the present study against standard test strains, including *S. aureus*, *B. cereus* and *E. coli* K-12. Additionally, the antimicrobial activities of thyme essential oil were tested against AmpC-producing and ESBL-producing bacterial strains, as there have been increasing numbers of reports on these multidrug-resistant Gram-negative bacteria isolated from meat and meat products, due to food processing (*20–22*). As a consequence, the emergence of multidrug-resistant pathogenic and non-pathogenic food-spoilage bacteria has recently been recognised as a major health concern.

In the present study, the 10 % thyme essential oil had the strongest antimicrobial activity against the ESBL--producing *E. coli* strain 1138, which was isolated from a sausage, and against a clinical ESBL-producing *E. coli* isolate KM, of the pandemic ST131 clonal linage. Among the foodborne bacteria, the thyme essential oil was least effective against the AmpC-producing *E. coli Z*, which was isolated from meat. The inhibition zone against *Buttiauxella* sp., which was isolated from ground meat, was in the medium range (Table 1). This strain had the largest inhibition zone when the lower, 1 % fraction of thyme essential oil was applied. *Buttiauxella* sp. has been reported to be a food spoilage bacterium and a large problem in the food industry, because it can grow at refrigeration temperatures (*23–25*). Additionally, it has been shown that some *Buttiauxella* strains have AmpC β-lactamases, which can potentially be transfered to other bacteria (*26*). The prevalence of these bacteria in foods is unknown, because it is frequently incorrectly identified as *E. coli* in rapid identification tests (Ambrožič Avguštin, unpublished data).

Antibacterial properties of spice essential oils suggest that they have a potential for use as natural food additives. Ruiz-Navajas *et al.* (*8*) investigated the antimicrobial activities of *Thymus moroderi* and *Thymus piperella* essential oils from southeast Spain against 11 bacteria that are associated with food spoilage. *T. moroderi* essential oil had an inhibitory effect against four of these and *T. piperella* essential oil had an inhibitory effect against five spoilage bacteria.

From the data arising from the present study and other studies, we can conclude that thyme essential oils show antibacterial activities against important pathogenic and non-pathogenic foodborne bacteria.

Antifungal activity

The antifungal activity of thyme essential oil was much weaker than the antibacterial activities, as fungal growth was inhibited only at the highest tested fraction, at 10 % of essential oil. The mean diameters of the growth inhibition zones of these fungi that grew in the presence of 10 % essential oil were 4.1 mm for *Fusarium* sp. and 4.9 mm for *Armillaria mellea* (Table 2).

Pathogenic or spoilage fungi are one of the major economic problems in food production. They can cause food decay, and many of them present very serious risks for consumers, due to the production of dangerous secondary metabolites. In terms of food safety, the fungi of

Table 2. Antifungal activities of *Thymus vulgaris* essential oil

	φ(thyme essential oil)/%			
	0	0.1	1	10
Fungal strain	r(inhibition zone)/mm			
Fusarium sp.	n.d.	n.d.	n.d.	4.1±1.6
Armillaria mellea	n.d.	0.1±0.0	0.1±0.0	4.9±0.2

Data are mean values±standard deviations of three independent replicates (N=3)
n.d.=growth inhibition not detected

the genera *Fusarium*, *Aspergillus* and *Penicillium* are considered important, because they produce mycotoxins (*27*). High antifungal activity of thyme essential oil was measured also by Zabka *et al.* (*28*), who tested 25 essential oils from medicinal plants. All these data show that thyme essential oil has the potential to be used also in antifungal treatments in food production and technology. One of the possible applications could be the superficial treatment of food products with thyme essential oil to prevent microbial growth.

Conclusions

Thyme essential oil showed antibacterial and antifungal activity at fractions of 1 and 10 %, respectively. The antibacterial activity of thyme essential oil was not specific for Gram-positive or Gram-negative bacteria. Thyme essential oil most effectively inhibited the growth of ESBL-producing *Echerichia coli* KM:ST131, and least effectively the growth of ESBL-producing *Klebsiella pneumoniae*. Promising antimicrobial activities were detected against all of the foodborne isolates tested, including AmpC-producing and ESBL-producing *E. coli*, which have recently been isolated more frequently from food products, and mainly from poultry meat.

Acknowledgements

This study was partially financially supported by the Slovenian Research Agency, grant no. P1-0212. The authors thank Andrej Anderlič for technical help, and Dr. Samo Kreft and Dr. Damjan Janeš (University of Ljubljana, Faculty of Pharmacy) for the thyme essential oil analysis.

References

1. M.M. Tajkarimi, S.A. Ibrahim, D.O. Cliver, Antimicrobial herb and spice compounds in food, *Food Control, 21* (2010) 1199–1218.
2. S. Burt, Essential oils: Their antibacterial properties and potential applications in foods – A review, *Int. J. Food Microbiol. 94* (2004) 223–253.
3. F. Bakkali, S. Averbeck, D. Averbeck, M. Idaomar, Biological effects of essential oils – A review, *Food Chem. Toxicol. 46* (2007) 446–475.
4. D.L. Miladinović, B.S. Ilić, T.M. Mihajilov-Krstev, N.D. Nikolić, L.C. Miladinović, O.G. Cvetković, Investigation of the chemical composition–antibacterial activity relationship of essential oils by chemometric methods, *Anal. Bioanal. Chem. 403* (2012) 1007–1018.
5. M. Sienkiewicz, M. Wasiela, Activity of thyme and lavender essential oils against antibiotic resistant clinical bacterial strains of *Pseudomonas aeruginosa*, *Postepy Fitoterapii, 4* (2012) 139–145.
6. M.G. Cattelan, M.B.M. de Castilhos, P.J.P. Sales, F.L. Hoffmann, Antibacterial activity of oregano essential oil against foodborne pathogens, *Nutr. Food Sci. 43* (2013) 169–174.
7. J. Širvaityte, J. Šiugždaite, V. Valeika, E. Dambrauskiene, Application of essential oils of thyme as a natural preservative in leather tanning, *Proc. Estonian Acad. Sci. 61* (2012) 220–227.
8. Y. Ruiz-Navajas, M. Viuda-Martos, E. Sendra, J.A. Perez-Alvarez, J. Fernández-López, Chemical characterization and antibacterial activity of *Thymus moroderi* and *Thymus piperella* essential oils, two *Thymus* endemic species from southeast of Spain, *Food Control, 27* (2012) 294–299.
9. *Herba Thymi*. In: *WHO Monographs on Selected Medicinal Plants, Vol. 1*, World Health Organization, Geneva, Switzerland (1999) pp. 259–266.
10. M.D. Soković, J. Vukojević, P.D. Marin, D.D. Brkić, V. Vajs, L.J.L.D. Van Griensven, Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities, *Molecules, 14* (2009) 238–249.
11. *European Pharmacopoeia*, Council of Europe, Strasbourg, France, EU (2011).
12. D. Janeš, H. Prosen, S. Kreft, Identification and quantification of aroma compounds of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) and some of its milling fractions, *J. Food Sci. 7* (2012) C746–C751.
13. K. Sepčić, S. Kauferstein, D. Mebs, T. Turk, Biological activities of aqueous and organic extracts from tropical marine sponges, *Mar. Drugs, 8* (2010) 1550–1566.
14. W.K. Roberts, C.P. Selitrennikoff, Plants and bacterial chitinases differ in antifungal activity, *J. Gen. Microbiol. 134* (1988) 169–176.
15. N. Solomakos, A. Govaris, P. Koidis, N. Botsoglou, The antimicrobial effect of thyme essential oil, nisin and their combination against *Escherichia coli* O157:H7 in minced beef during refrigerated storage, *Meat Sci. 80* (2008) 159–166.
16. M. Viuda-Martos, Y. Ruiz-Navajas, J. Fernández-López, J.A. Pérez-Álvarez, Antibacterial activity of different essential oils obtained from species widely used in Mediterranean diet, *Int. J. Food Sci. Technol. 43* (2008) 526–531.
17. A. Sokmen, M. Gulluce, H.A. Akpulat, D. Daferera, B. Tepe, M. Polissiou *et al.*, The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of the endemic *Thymus spathulifolius, Food Control, 15* (2004) 627–634.
18. O. Politeo, M. Skocibusic, F. Burcul, A. Maravic, I. Carev, M. Ruscic, M. Milos, *Campanula portenschlagiana* Roem. et Schult.: Chemical and antimicrobial activities, *Chem. Biodivers. 10* (2013) 1072–1080.
19. L. De Martino, M. Bruno, C. Formisano, V. De Feo, F. Napolitano, S. Rosselli, F. Senatore, Chemical composition and antimicrobial activity of the essential oils from two species of *Thymus* grown in southern Italy, *Molecules, 14* (2009) 4614–4624.
20. N. Silva, L. Costa, A. Gonçalves, M. Sousa, H. Radhouani, F. Brito *et al.*, Genetic characterisation of extended-spectrum β-lactamases in *Escherichia coli* isolated from retail chicken products including CTX-M-9 containing isolates: A food safety risk factor, *Br. Poult. Sci. 53* (2012) 747–755.
21. J. Tham, M. Walder, E. Melander, I. Odenholt, Prevalence of extended-spectrum beta-lactamase-producing bacteria in food, *Infect. Drug Resist. 5* (2012) 143–147.
22. E. Ojer-Usoz, D. González, A.I. Vitas, J. Leiva, I. García-Jalón, A. Febles-Casquero, L. Escolano Mde, Prevalence of extended-spectrum β-lactamase-producing Enterobacteriaceae in meat products sold in Navarra, Spain, *Meat Sci. 93* (2013) 316–321.
23. S. Macé, J. Cornet, F. Chevalier, M. Cardinal, M.F. Pilet, X. Dousset, J.J. Joffraud, Characterisation of the spoilage microbiota in raw salmon (*Salmo salar*) steaks stored under vacuum or modified atmosphere packaging combining conventional methods and PCR-TTGE, *Food Microbiol. 30* (2012) 164–172.
24. E. Säde, A. Murros, J. Björkroth, Predominant enterobacteria on modified-atmosphere packaged meat and poultry, *Food Microbiol. 34* (2013) 252–258.
25. D. Ercolini, F. Russo, A. Nasi, P. Ferranti, F. Villani, Mesophilic and psychrotrophic bacteria from meat and their spoil-

age potential *in vitro* and in beef, *Appl. Environ. Microbiol. 75* (2009) 1990–2001.

26. V. Fihman, M. Rottman, Y. Benzerara, F. Delisle, R. Labia, A. Philippon, G. Arlet, BUT-1: A new member in the chromosomal inducible class C β-lactamases family from a clinical isolate of *Buttiauxella* sp., *FEMS Microbiol. Lett. 213* (2002) 103–111.

27. J.P.F. D'Mello, A.M.C. Macdonald, D. Postel, W.T.P. Dijksma, A. Dujardin, C.M. Placinta, Pesticide use and mycotoxin production in *Fusarium* and *Aspergillus* phytopathogens, *Eur. J. Plant Pathol. 104* (1998) 741–751.

28. M. Zabka, R. Pavela, L. Slezakova, Antifungal effect of *Pimenta dioica* essential oil against dangerous pathogenic and toxinogenic fungi, *Industr. Crops Prod. 30* (2009) 250–253.