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Essential Oils of *Lamiaceae* Family Taxa as Natural Preservatives of Food Preparations

Nikos G. Chorianopoulos • George-John E. Nychas • Serkos A. Haroutounian*

Agricultural University of Athens, Iera odos 75, Athens 11855, Greece

Corresponding author: * **sehar@aua.gr**

ABSTRACT

The recent negative consumer perception against artificial food preservatives has shifted the research interest towards the development of alternatives that consumers conceive as naturals. In this context, the essential oils (EOs) of *Lamiaceae* family taxa constitute intriguing candidates, since numerous *in vitro* studies have established well their bactericidal activities against common foodborne pathogens such as *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* ser. Enteritidis, *Salmonella* ser. Typhimurium and *Staphylococcus aureus*. Main components of these EOs such as carvacrol, thymol and α-terpineol, have also been identified as effective antibacterials displaying minimum inhibitory concentrations (MICs) of 0.005-0.05% v/v. Their mode of action involves several targets in bacterial cells, while their hydrophobicity permits their partition in cell membrane lipids leading to the leakage of the cell content. Thus, many of EO components have been legally registered as flavourings in the European Union and the USA. Similar experiments on food products have established the necessity of using larger amounts of EO. More specifically, studies on meat and dairy products, fisheries, vegetables, rice and fruits indicated that concentrations higher than 0.5% v/v or v/w are required in order to display a significant antibacterial effect. On the other hand, low pH, temperature and oxygen levels act synergistically promoting greatly their activity. Finally, it is feasible to eliminate the undesired organoleptic effects of EOs through the selection of the appropriate EO with regard to the preserved food.

Keywords: active packaging, antibacterial activity, biofilm, carvacrol, food preservation, food safety, food-borne pathogens, thymol
Abbreviations: AP, active packaging; **EO**, essential oil; **MAP**, modified atmosphere packaging; **MBC**, minimum bactericidal concentration; **MIC**, minimum inhibitory concentration; **NIC**, non-inhibitory concentration; **PEF**, pulsed electric fields; **UHP**, ultra high pressure; **VC**, viable counts

CONTENTS

INTRODUCTION

Various preservative agents are being used to ensure that manufactured foods remain safe and unspoiled. The rising number and severity of food poisoning outbreaks worldwide has increased public awareness for food safety issues. It is estimated that every year nearly 80 million people are food poisoned causing – according to the Center for Disease Control and Prevention (CDC) – 5,000 deaths annually in the United States. In Japan, the food poisoning outbreak incidents were 3,059 for 1998 implicating a total of 44,645 cases. In this regard, public concern has particularly been stimulated especially as a result of recent scares of BSE in many European countries and *Escherichia coli* O157:H7 in the USA, Japan, Australia and Scotland. The annual health care costs, traced to selected food-borne pathogens such as *Listeria monocytogenes*, *E. coli* O157:H7 and *Salmonella* spp. is estimated at 5-6 billion € per year (WHO 2002). On the other hand, the excessive use of chemical preservatives, many of which are believed to exert potential carcinogenic and/or teratogenic activities as well as residual toxicity, has resulted in mistrust among European consumers. The increasing resistance of various food-borne pathogens against antimicrobials has also become a source of major concern. To remedy the aforementioned problems, the food industry and the European authorities have to present an increased vigilance towards all quality and safety issues. Since consumers need to feel reassured that they consume safe foods,

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Review

an increasing pressure on food manufacturers and authorities is applied with respect to: i) the elimination of harmful chemical preservatives from food preparations, ii) strengthening the research activity towards the discovery of alternative − more effective, non toxic, natural or synthetic − preservatives. Since the development and approval of novel safer and more potent synthetic chemical preservatives is time consuming, the research interest has focused on the development and application of more "natural" means in food preservation. In this context, the use of essential oils (EOs) of edible and medicinal plants, herbs and spices presents an intriguing case, since they constitute a class of very potent natural antibacterial agents (Nychas *et al*. 2003). Their use in food systems may be considered as an additional intrinsic determinant to increase the safety and shelf-life of foods.

The EOs have been known for many years, since Greek and Roman historians have mentioned the essential oil of turpentine (Guenther 1948). The latter was prepared by distillation, a method developed by eastern civilisations (Egypt, India and Persia) (Guenther 1948) and improved in the 9th century by the Arabs (Bauer *et al*. 2001), still constitutes the main method for EO preparation. During the renaissance, many EOs were produced in pharmacies and their pharmacological effects were gradually included in pharmacopoeias (Bauer *et al*. 2001). For example, the use of tea tree oil for medical purposes was well documented at the end of the 18th century, after the colonisation of Australia (Carson and Riley 1993). The first experimental determination of the bactericidal properties of EO vapours was carried out by de la Croix in 1881 (Boyle 1955). However, in the course of the 19th and 20th centuries, the role and applications of EOs in medicine gradually diminished, as compared to their uses for flavour and aroma purposes (Guenther 1948). Currently, EOs are used in food preparations mainly as flavouring agents and by cosmetic and pharmaceutical industries as fragrances and functional additives (Bauer and Garbe 1985). Individual components of the EOs − either extracted from plant material or synthetically manufactured − are also used as food flavourings (Oosterhaven *et al*. 1995). These natural substances have been suggested for use in foodstuffs (Farag *et al*. 1989) because they were found to display a wide range of antimicrobial properties, e.g. against bacteria, fungi and mycobacteria (Conner and Beuchat 1984a, 1984b; Galli *et al*. 1985). For the time being, only a limited number of preservatives containing EOs are commercially available such as the 'DMC Base Natural', a food preservative produced by DOMCA S.A., Alhendin, Granada, Spain. This preservative comprises by 50% from a mixture of the EOs of rosemary, sage and citrus and 50% by glycerol (Mendoza-Yepes *et al*. 1997). Other examples of such natural preparations are 'Protecta One' and 'Protecta Two', which constitute blended herb extracts produced by Bavaria Corp. Apopka, FL, USA. These preparations have been classified and recognized in the US as safe (GRAS) food additives.

The main objective of this review is to provide an overview of the published data on the antibacterial activity of *Lamiaceae* EOs (and their components) and present the methodologies in use for their *in vitro* and *in situ* effectiveness evaluation. Moreover, the challenges that food industry is facing with respect to the use of EOs as antibacterial means in food safety and spoilage prevention will also be discussed. Finally, the mode of action of natural preservatives and the stress response mechanisms that they induce in food-borne pathogens will be reviewed.

MAIN COMPONENTS OF *LAMIACEAE* ESSENTIAL OILS

Steam distillation is the most commonly used method to produce EOs on a commercial basis. Extraction by means of liquid carbon dioxide, under low temperature and high pressure, is a more expensive alternative that provides a more natural organoleptic profile (Burt *et al*. 2004). This differentiation of their organoleptic profiles also reflects the differences in chemical composition between oils obtained by distillation and those produced by solvent extraction. These chemical composition differences also influence their antimicrobial properties, since literature reports have indicated that EOs extracted from herbs with hexane exhibit superior antimicrobial activities as compared to their corresponding EOs that obtained by steam distillation (Burt *et al*. 2004). It is evident, that in order to prevent compositional changes occurred during their storage, all EOs have to be stored in airtight containers to avoid the evaporation of lower boiling temperature possessing components (Mockutë *et al*. 2005). Finally, their maintenance in dark environment is essential in order to avoid the considerable compositional changes caused by various light induced chemical transformations of terpenoids (Misharina *et al.* 2003; Schwob 2004; Misharina *et al.* 2005).

Literature abounds with chemical composition analyses of EOs obtained from plants of the *Lamiaceae* family. All these assays have been performed by gas chromatography and mass spectrometry of the respective EO or its headspace (Salzer 1977; Juliano *et al*. 2000; Daferera *et al*. 2000; Jerkovic *et al*. 2001; Delaquis *et al*. 2002; Szentmihályi *et al*. 2006). A typical EO scan comprises more than sixty individual components (Senatore 1996; Russo *et al*. 1998). The major components account for up to 85%, whereas the minor components are present only in trace amounts (Senatore 1996; Bauer *et al*. 2001). The major component content of many economically interesting EOs has been reviewed by Bauer *et al*. (2001), while the main constituents of *Lamiaceae* EOs that possess significant antibacterial activities are presented in **Table 1**. Despite the presence of many major constituents that display antibacterial activity, the antibacterial properties of *Lamiaceae* EOs are mainly attributed on their content of phenol monoterpenes (Cosentino *et al*. 1999). Furthermore, there is much experimental evidence that minor constituents also play a critical role in their antibacterial activities, possibly via a synergistic effect with other components. This has been delineated in detail for the EOs of *Salvia officinalis* L. (sage, Marino *et al*. 2001), certain *Thymus vulgaris* (thyme, Paster *et al*. 1995; Marino *et al*. 1999) and *Origanum vulgare* (oregano, Paster *et al*. 1995) species.

It must further be pointed out that the chemical composition of the EOs depends greatly on their harvesting period and cultivation site (Arras and Grella 1992; Marotti *et al*. 1994; McGimpsey *et al*. 1994; Cosentino *et al*. 1999; Marino *et al*. 1999; Juliano *et al*. 2000; Faleiro *et al*. 2002; Chorianopoulos *et al*. 2004, 2006a, 2007). This can be rationalized considering the biosynthetic pathway of their major components. For example, in the case of *Origanum*, *Satureja* and *Thymus* species, *p*-cymene and *γ*-terpinene constitute the precursors of their phenol monoterpenes carvacrol and thymol (**Fig. 1**; Muller-Riebau *et al*. 1997; Cosentino *et al*. 1999; Jerkovic *et al*. 2001; Ultee *et al*. 2002; Chorianopoulos *et al*. 2006a). Thus, research findings on EOs of Greek *Origanum*, *Satureja* and *Thymus* plants have indicated that the sum of these monoterpenes always represents the bulk of their EOs, regardless of their cultivation site (Kokkini *et al*. 1997; Chorianopoulos *et al*. 2004, 2006a, 2007) and/or harvesting time (**Fig. 2**; Jerkovic *et al*. 2001; Chorianopoulos *et al*. 2006a). Similar findings were obtained for EOs of *Thymus vulgaris* from Italy (Marino *et al*. 1999) and Greek *Satureja thymbra* and *Satureja parnassica* (Chorianopoulos *et al*. 2006a). In **Fig. 2** we present the seasonal variation of these compounds for the EO of *Satureja thymbra* (Chorianopoulos *et al*. 2006a), indicating that during their premature vegetative stage, *γ*-terpinene and *p*-cymene constitute the major components of the EO. As the flowering period approaches, a simultaneous gradual diminishment of monoterpene precursors and the prevalence of their phenolic metabolites are observed (**Fig. 2**). Thus, during the full flowering period, carvacrol prevails as the major component, while the end of the flowering stage delineates a sharp decrease of carvacrol levels and the predomi-

Table 1 Major components of selected *Lamiaceae* EOs that display significant antibacterial properties.

EO	Major components	Composition % (w/v)	References
Origanum vulgare (Oregano)	Carvacrol	0-89	Lawrence 1984; Prudent *et al*. 1995; Sivropoulou *et al*. 1996; Kokkini *et al*. 1997; Russo *et al*. 1998; Daferera *et al*. 2000; Demetzos and Perdetzoglou 2001; Marino *et al*. 2001; Chorianopoulos *et al*. 2004, 2007
	Thymol	0-64	
	γ-Terpinene	2-52	
	p-Cymene	0-52	
Origanum dictamus	Carvacrol	52-55	Chorianopoulos *et al*. 2004, 2007
	γ-Terpinene	7-14	
	p-Cymene	9-13	
	Linalool	4	
Thymus vulgaris (Thyme)	Thymol	10-64	McGimpsey *et al*. 1994; Cosentino *et al*. 1999; Marino *et al*. 1999; Daferera *et al*. 2000; Juliano *et al*. 2000
	p-Cymene	10-56	
	γ-Terpinene	2-31	
	Carvacrol	2-11	
Thymus longicaulis	Carvacrol	16-61	Chorianopoulos *et al*. 2004, 2007
	Geraniol	3-42	
	Thymol	4-32	
	γ-Terpinene	4-13	
	p-Cymene	0-8	
	Geranyl acetate	0-10	
	Borneol	0-8	
	Linalool	0-7	
	β-Caryophyllene	0-6	
Satureja thymbra L.	Thymol	13-41	Chorianopoulos *et al*. 2004, 2006a
	Carvacrol	4-39	
	γ-Terpinene	11-25	
	p-Cymene	9-18	
	β-Caryophyllene	4-8	
Satureja spinosa	Carvacrol	27-44	Chorianopoulos *et al*. 2004
	Thymol	15-24	
	γ-Terpinene	6-11	
	p-Cymene	6-9	
	β-Caryophyllene	5-9	
Salvia officinalis L. (Sage)	*α*-thujone	20-42	Marino *et al*. 2001
	Camphor	6-15	
	1, 8-Cineole	6-14	
	β-Pinene	2-10	
	α-Pinene	4-5	
Rosmarinus officinalis (Rosemary)	1, 8-Cineole	3-89	Daferera *et al*. 2000; Pintore *et al*. 2002; Daferera *et al*. 2003
	α-Pinene	2-25	
	Bornyl acetate	0-17	
	Camphor	2-14	

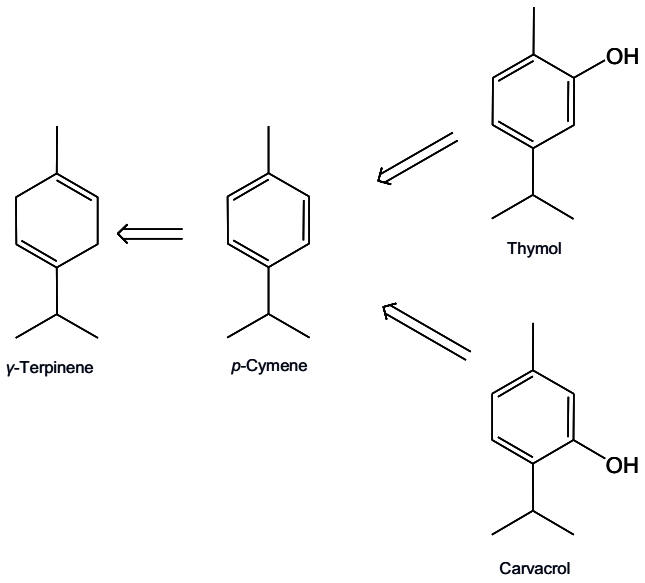

Fig. 1 Biosynthesis of monoterpenic phenols (carvacrol and thymol).

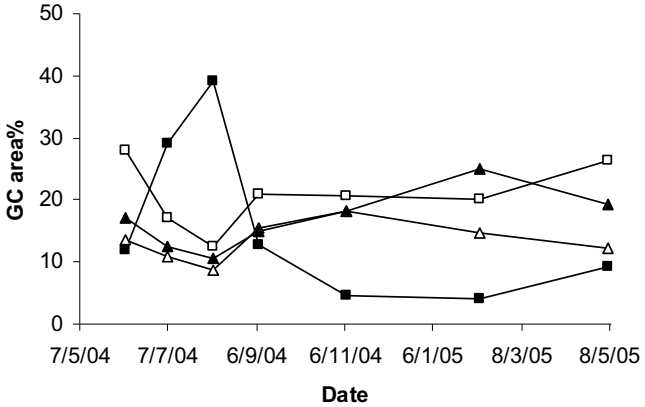

Fig. 2 Seasonal variation of the major constituents (■: Carvacrol, □: Thymol, ▲: *γ*-Terpinene and Δ*:* *p*-Cymene) of *Satureja thymbra* plants. Values at (X) axis cover period of 12 months - 7/6/2004 to 7/5/2005 - (Figure taken from Chorianopoulos *et al*. 2006a).

nance of thymol as major component of the essential oils. Few months later, as the premature vegetative stage approached, the level of monoterpene precursors was restored (**Fig. 2**). The aforementioned data indicate that the four compounds are biologically and functionally associated, supporting the theory that thymol and carvacrol are biosynthesized from *p*-cymene and *γ*-terpinene (**Fig. 1**; Kokkini *et al*. 1997; Chorianopoulos *et al*. 2006a). In this regard, it is also evident that EOs obtained during (or immediately after) the flowering season of a plant, exhibit the most significant antimicrobial activities (McGimpsey *et al*. 1994; Marino *et al*. 1999; Chorianopoulos *et al*. 2004, 2006a, 2007). Finally, the EO composition obtained from different

parts of the same plant may also vary significantly. For example, EO obtained from coriander seeds (*Coriandum sativum* L.) has quite different chemical composition as compared with the EO of *Coriandrum sativum* (cilantro), which is produced from the immature leaves of the same plant (Delaquis *et al*. 2002).

IN VITRO ANTIBACTERIAL ACTIVITY

Methods used to assay the antimicrobial activities of the EOs

The antimicrobial activities of plant derived compounds against diverse types of microbes, including food-borne pathogens, is well documented in the literature (Davidson and Branen 1993; Nychas 1995; Nychas *et al*. 2003). These results are not directly comparable, since various distinct and divergent data have been reported for the same antimicrobial compound and/or mixture (Mann and Markham 1998; Manu *et al*. 1998; Skandamis 2001; Skandamis *et al*. 2001; Chorianopoulos 2007). Moreover, it is not always clear whether the cited method is capable of assaying the bacteriostatic (or bactericidal activity) or simply measures the combined result of both activities.

These literature assay methods measure: (i) the inhibition zone of bacterial growth around a paper disk containing the compound (or mixture) tested, on various non-specific substrates (Farag *et al*. 1989; Aureli *et al*. 1992; Kim *et al*. 1995a, 1995b; Senatore *et al*. 2000; Wilkinson *et al*. 2003; Chorianopoulos *et al*. 2004; Proestos *et al*. 2005; Chorianopoulos *et al*. 2007); (ii) minimum inhibitory concentration (MIC) that is necessary to inhibit the bacterial growth (Carson *et al*. 1995a; Wan *et al*. 1998b; Cosentino *et al*. 1999; Hammer *et al*. 1999; Lambert and Pearson 2000; Lambert *et al*. 2001; Chorianopoulos *et al*. 2006); (iii) inhibition of bacterial growth on an agar medium when the tested compound (or mixture) is diffused in agar (Deans and Ritsie 1987; Smith-Palmer *et al*. 1998; Wan *et al*. 1998; Dorman and Deans 2000); (iv) optical density changes of a growth medium (non selective broth) in which inoculum and antimicrobial compound(s) were added (Shelef *et al*. 1984; Kim *et al*. 1995a; Sivropoulou *et al*. 1996; Lambert and Pearson 2000; Lambert *et al*. 2001; Chorianopoulos *et al*. 2006b); (v) changes in the impedance of a non-specific growth medium (broth), with or without the addition of EO (Smith-Palmer *et al*. 1998; Chorianopoulos *et al*. 2004, 2006a, 2006b); and (vi) comparative bacteriostatic activities of the antimicrobial compounds (e.g. EOs) in an agar-diffusion, versus a serial dilution assay (Farag *et al*. 1989; Stecchini *et al*. 1993; Hammer *et al*. 1999; Juven *et al*. 1994; Pandit and Shelef 1994; Quattara *et al*. 1997; Wilkinson *et al*. 2003).

The utilization of the above methods for the antimicrobial activity measurement of an EO is usually affected by: (i) the sample composition (plant species, geographical location and collection period), (ii) microorganism (strain, conditions of growth, inoculum etc), and (iii) method used to grow and enumerate the surviving bacteria. In this regard, many of the literature data have been based on subjective observations as in the disc diffusion method or the various rapid techniques such as the measurement of the optical density (turbidimetry). More specifically, in the disc diffusion technique the inhibition area depends on either the ability of the EO to be uniformly diffused through the agar medium or the oil vapours released on the bacteria (Nychas *et al*. 2003). The antimicrobial assay is also influenced by the presence of multiple active components, which at low concentrations display antagonistic, additive or synergist activities. Thus, the rate of active components partition between lipid and aqueous phase influences the antimicrobial activity of an EO producing different results when tested in a complex (e.g. food) or in a simple system (Stechinni *et al*. 1993, 1998). For example, the activity of mint (*Mentha piperita*) and cilantro EOs were considerably diminished when used in products with high level of fat, such as pâté – which generally contains 30-45 % fat – (Tassou *et al*. 1995) or a ham coating that contains canola oil (*Brassica napus* L., Gill *et al*. 2002). On the other hand, the immobilisation of cilantro EO in a gelatine gel improved its antibacterial activity against *L. monocytogenes* in ham (Gill *et al*. 2002).

With respect to the Viable Counts (VC) technique, turbidimetry is a rapid, non-destructive, inexpensive and relatively easy to automate method exhibiting the serious disadvantage of low sensitivity. On the other hand, turbidimetry detects only the upper parts of the growth curves and often requires correction procedures and calibration methods in order to correlate turbidimetric results with the VC's (Koch 1981; Bloomfield 1991; Cuppers and Smelt 1993; McClure *et al*. 1993; Dalgaard and Koutsoumanis 2001; Skandamis *et al*. 2001). The absorbance differences are evident only when the population level reaches 10^6-10^7 cfu/ml and clearly depend on the size of the bacterial cells in connection with the different growth stage of the tested organism. The correlation between absorbance changes and viable counts numbers are available only for identically treated samples, since different calibration curves must be obtained for every differently treated sample (Dalgaard and Koutsoumanis 2001). The latter is necessary because the addition of the EO prior to inoculation increases the initial absorbance in relation to the control. Furthermore, it has been established that the presence of an EO leads to the reduction of the bacterial cell size (Alviano *et al*. 2005; Helal *et al*. 2006). The latter in connection with the physiological (damaged, injured or healthy) and the oxidation stage of the EO, as well as the inadequate dissolution of the compound(s) tested also affect the absorbance measurement in the growth media (Nychas *et al*. 2003).

Unlike the plate count technique, impedance measures microbial metabolism in a real time mode. Thus, the impedimetric method is widely recognized as a promising alternative rapid method, not only for screening the biocide activity of novel antimicrobial agents against food spoilage and pathogenic bacteria but also for the estimation of growth kinetic parameters (Tranter *et al*. 1993; Ayres *et al*. 1993; Johansen *et al*. 1995; Tassou and Nychas 1995a, 1995b, 1995c; Tassou *et al*. 1995; MacRae *et al*. 1997; Koutsoumanis *et al*. 1997; Tassou *et al*. 1997; Ayres *et al*. 1998; Koutsoumanis *et al*. 1998; Lachowicz *et al*. 1998; Chorianopoulos *et al*. 2006b). This can be accomplished by using a medium that offers a sharp detectable impedimetric change in accordance with the bacterial population growth, converting thus the low conductivity nutrients to highly charged products. It must be noted however that − for reasons of comparison − a correlation between the standard plating procedure and the corresponding impedimetric data must be established (Dumont and Slabyj 1993; Koutsoumanis *et al*. 1998).

Finally, VC − the traditional microbiological method − should remain the gold standard procedure in order to determine the restrictions within each of the aforementioned methodologies. This method has the major advantage of requiring low capital investment. On the other hand, is a laborious, material intensive method that requires a long elapse time and often exhibits low reproducibility.

Terms for antibacterial activity tests

Terms used to describe the outcome of the EOs antibacterial activities tests include the:

A) MIC, which is defined as: (i) the lowest concentration essential to maintain the inoculum viability (Carson *et al*. 1995a); (ii) the lowest concentration required for complete inhibition of test organism up for to 48 h incubation (Wan *et al*. 1998; Canillac and Mourey 2001); (iii) the lowest concentration that inhibits the visible growth of test organism (Karapinar and Aktug 1987; Onawummi 1989; Hammer *et al*. 1999; Delaquis *et al*. 2002); (iv) the lowest concentration that results in a significant decrease of inoculum viability (Cosentino *et al*. 1999); and (v) concentration above which no growth is observed relative to the control

test (Lambert and Pearson 2000),

B) Minimum Bactericidal Concentration (MBC), which corresponds to the concentration capable to kill >99.9% of the initial inoculum (Carson *et al* 1995b; Cosentino *et al*. 1999; Canillac and Mourey 2001),

C) Non-Inhibitory Concentration (NIC), that refers to the concentration above which the inhibitor begin to display a negative effect on growth (Lambert and Pearson 2000), and

D) Bacteriostatic Concentration, which was defined as the lowest concentration that bacteria fail to grow in broth systems but may be cultured when either broth is plated onto agar and/or the bacteria are not cultivable when broth is plated onto agar with the bactericidal concentration (Smith-Palmer *et al*. 1998).

Minimum inhibitory concentration (MIC)

It is already mentioned that must be established a balance between the sensory acceptability and the antimicrobial efficacy. In this regard, there is an increasing demand for the accurate measurement of the EOs' Minimum Inhibitory Concentration (MIC). This can be accomplished by either *in vitro* or/and *in vivo* studies. The *in vitro* evaluation techniques (e.g. diffusion, dilutions, impedance, and optical density methods), along with their limitations have already been delineated in detail (Koutsoumanis *et al*. 1998, 1999; Tassou *et al*. 2000; Dalgaard and Koutsoumanis 2001; Lambert *et al*. 2001; Skandamis *et al*. 2001; Chorianopoulos *et al*. 2006b) indicating that the dilution method provides accurate quantitative results (Manou *et al*. 1998) that are not comparable with the corresponding results obtained by the other methodologies (Tassou *et al*. 2000; Lambert *et al*. 2001; Skandamis *et al*. 2001).

Novel efficient technique for the MIC determination is based on the broth micro dilution of the tested agents (Carson *et al*. 1995b). In this regard, the most frequently used methods concern either the enumeration of populations by viable counts or the optical density measurements (Burt 2004). The first method is time-consuming and labor-intensive, while the second is automated but has the disadvantage of not taking into account various crucial parameters such as the possible oxidation of the EO, the physiological state of cells etc. These limitations may possibly alter the results obtained (Nychas *et al*. 2003). Other widely used methods for the MIC determination are the agar dilution technique − developed by Mann and Markham (1998) − and a novel micro dilution technique that uses resazurin (a redox indicator) as the visual indicator for the MIC measurement. The latter is more sensitive as compared with the dilution technique but banks on visual means (Burt 2004), which many times are attitudinal. A similar technique − developed by Burt and Reinders (2003) as a modification of Salvat *et al*. 2001 method − uses a patented color indicator (based on resazurin). This is an automated method which measures the end-point by fluorescence instead of visual means (Burt 2004). In conclusion, it is evident that in complex systems the traditional methods of MIC assessment are time-consuming, resource-intensive and often introduce subjectivity into the evaluation procedure.

On the contrary, a modification of the method developed by Lambert and Pearson (2000) to assay the inhibi-

Table 2 MICs of *Lamiaceae* EOs or their components tested *in vitro* against food borne pathogens (MICs from the references have been converted to % v/v).

EO or component	Bacterial species	MIC range (% v/v)	References
Origanum spp.	*Escherichia coli*	0.05-0.12	Prudent *et al*. 1995; Hammer *et al*. 1999; Burt and Reinders 2003
	Salmonella ser. Typhimurium	0.12	Hammer *et al*. 1999
	Stapylococcus aureus	0.05-0.12	Prudent *et al*. 1995; Hammer *et al*. 1999
Thymus spp.	*Escherichia coli*	0.045-0.125	Farag *et al*. 1989; Smith-Palmer *et al*. 1998; Cosentino *et al*. 1999; Hammer *et al*. 1999; Burt and Reinders 2003
	Listeria monocytogenes	0.016-0.045	Firouzi *et al*. 1989; Smith-Palmer *et al*. 1998; Cosentino *et al*. 1999
	Salmonella ser. Typhimurium	0.045->2	Cosentino *et al*. 1999; Hammer *et al*. 1999
	Stapylococcus aureus	0.02-0.25	Farag *et al*. 1989; Smith-Palmer *et al*. 1998; Cosentino *et al*. 1999; Hammer *et al*. 1999
Satureja spp.	*Bacillus cereus*	0.049-0.058	Chorianopoulos *et al*. 2006b
	Escherichia coli	0.054-0.066	Chorianopoulos *et al*. 2006b
	Listeria monocytogenes	0.035-0.08	Chorianopoulos *et al*. 2006a, 2006b
	Salmonella ser. Enteritidis	0.056-0.094	Chorianopoulos *et al*. 2006a, 2006b
	Stapylococcus aureus	0.035-0.043	Chorianopoulos *et al*. 2006b
Salvia spp.	*Escherichia coli*	0.35-0.5	Farag *et al*. 1989; Smith-Palmer *et al*. 1998; Hammer *et al*. 1999
	Listeria monocytogenes	0.02	Smith-Palmer *et al*. 1998
	Salmonella ser. Typhimurium	0.1-0.2	Shelef *et al*. 1984; Hammer *et al*. 1999
	Stapylococcus aureus	0.075-0.1	Shelef *et al*. 1984; Farag *et al*. 1989; Smith-Palmer *et al*. 1998; Hammer *et al*. 1999
Rosmarinus spp.	*Bacillus cereus*	0.02	Chaibi *et al*. 1997
	Escherichia coli	0.45->1	Farag *et al*. 1989; Smith-Palmer *et al*. 1998; Hammer *et al*. 1999; Pintore *et al*. 2002
	Listeria monocytogenes	0.02	Smith-Palmer *et al*. 1998
	Salmonella ser. Typhimurium	>2	Hammer *et al*. 1999
	Stapylococcus aureus	0.04-1	Farag *et al*. 1989; Smith-Palmer *et al*. 1998; Hammer *et al*. 1999; Pintore *et al*. 2002
Carvacrol	*Bacillus cereus*	0.019-0.09	Cosentino *et al*. 1999
	Escherichia coli	0.023-0.5	Kim *et al*. 1995a; Cosentino *et al*. 1999
	Listeria monocytogenes	0.038-0.5	Kim *et al*. 1995a; Cosentino *et al*. 1999; Pol and Smid 1999
	Salmonella ser. Typhimurium	0.023-0.025	Kim *et al*. 1995a; Cosentino *et al*. 1999
	Stapylococcus aureus	0.018-0.045	Cosentino *et al*. 1999; Lambert *et al*. 2001
Thymol	*Bacillus cereus*	0.045	Cosentino *et al*. 1999
	Escherichia coli	0.023-0.045	Cosentino *et al*. 1999
	Listeria monocytogenes	0.045	Cosentino *et al*. 1999
	Salmonella ser. Typhimurium	0.005	Cosentino *et al*. 1999
	Stapylococcus aureus	0.014-0.023	Cosentino *et al*. 1999; Lambert *et al*. 2001
α-Terpineol	*Bacillus cereus*	0.09	Cosentino *et al*. 1999
	Escherichia coli	0.045->0.09	Cosentino *et al*. 1999
	Listeria monocytogenes	>0.09	Cosentino *et al*. 1999
	Salmonella ser. Typhimurium	0.023	Cosentino *et al*. 1999
	Stapylococcus aureus	0.09	Cosentino *et al*. 1999

tion of pure compounds (e.g. thymol), permits the quick and efficient measurement of MIC in complex systems and mixtures (Lambert *et al*. 2001). More specifically, the method evaluates the effectiveness of various concentrations of the tested material through optical density measurements, leading to the MIC determination that is based on growth or no growth information (Lambert *et al*. 2001). In a recent study (Chorianopoulos *et al*. 2006b), a novel aspect of this method was introduced by the replacement of the optical density measurements with conductance measurements. The latter does not depend on either the active state of cells (including their shape and size) and/or the possible EO oxidation stage (Nychas *et al*. 2003). These were accomplished by the use of Malthus apparatus which permits the cells metabolism measurement, thereby displaying the EO inhibition as a delay in bacteria metabolism as compared to the control. Moreover, the accuracy of MIC determinations is ensured through the mathematical process of data. Indeed, the results obtained by using the impedance measurements produce better curve fittings in comparison with those obtained by optical density measurements, especially at concentrations near MIC. This may be rationalized considering that the inactivated cells induce only absorbance (and not conductance) changes. Thus, the utilization of impedance measurements in MIC determination has the advantage of the maximum sensitivity near the growth boundaries (where the bacterial metabolism is very slow).

Table 2 summarizes all the relative literature data of MIC assays for *Lamiaceae* EOs, obtained by the aforementioned methodologies.

IN SITU ANTIBACTERIAL ACTIVITY

EO Legislation

Currently, the use of natural antibacterials derived from plant species in food preparations is not yet a well established practice in order to receive wide application by food industries. However, EOs are being incorporated in various food preparations such as salad dressings, sauces, fermented sausages, various ethnic foods etc. The recent interest for consumption of more 'natural' foods, in connection with the negative consumer perceptions against the artificial preservatives, have produced a positive trend towards the use of natural antimicrobials derived from plant-herbs and spices (Beuchat and Golden 1989). To date, most of research efforts on food preservatives have been focused on the development of chemically defined substances. Thus, more research is required towards the development of natural compound mixtures (e.g. EOs) as food preservatives and the exploitation of their potential toxicological effects, which do not correlate with their known corresponding toxicological data, when used by cosmetics industry (**Table 3**). In this regard, these compounds have to be recognized by the food industry as safe (GRAS) food additives. In addition, various regulatory authorities (e.g. Commission of the European Union) should amend and maintain list(s) of the EOs as recognized additives with antibacterial and/or antioxidant properties. They must also define their purity criteria and appropriate daily intake quantities. Furthermore, they should compose and update a catalogue with active ingredients and evaluation protocols, in order to produce a list of authorized additives for food preservation. Finally, they have to propose a new amendment demanding the indication of a full ingredient label which will ensure the optimal consumer information in respect to the foodstuff composition. Thus, necessary information will be provided to consumers who − for health or ethical reasons − have (or want) to avoid certain ingredients. Another problem to be resolved is their carry-over additives, since they may contain ingredients that act as allergens, when are in misuse or overdose. In this case, the indication of name and category is sufficient to enable susceptible consumers to avoid the consumption of such products.

Therefore, the clear binding and labeling rules are expected to ensure that consumers will have available the complete information of product characteristics such as composition, storage and use allowing its proper choose.

Table 3 Lethal dose (LD_{50}) of *Lamiaceae* EOs determined in rats.

Plant/herb	LD_{50}[1] (g/Kg)
Ocimum basilicum	A
Lavandula angustifolia	B/C
Origanum marjorana	B
Origanum vulgare	A
Rosemarinus officinalis	C
Mentha viridis	B
Salvia officinalis	B
Thymus vulgaris	B

[1] A: <2.0 g/Kg, B: 2-5 g/Kg, C: >5 g/Kg.
Data from Skandamis (2001), modified accordingly.

Assessment of EOs' antibacterial activities in food systems

Although a limited number of food preservatives based on EOs are commercially available the relative research activity in the field was poor until the early 1990s, accounting for the publication of only a few relative papers (Board and Gould 1991). An overview of all published literature reports concerning the antibacterial effects of *Lamiaceae* EOs (or their components) in foodstuffs is presented in **Table 4**. It must be noted however, that all potent antibacterial EOs assayed during *in vitro* studies have to be used in higher concentrations in order to produce similar effects on foodstuffs (Shelef 1983; Davidson 1997; Smid and Gorris 1999). This can be rationalized considering that factors such as the food composition and/or the microbial competition significantly influence the microbial growth in foodstuffs (Pin *et al.* 1999). For example, the ratio between *in vitro* and *in situ* studies has been calculated to be approximately 2–fold in semi-skimmed milk (Karatzas *et al*. 2001), 10–fold in pork liver sausage (Pandit and Shelef 1994), 50–fold in soup (Ultee and Smid 2001) and 25–fold to 100–fold in soft cheese (Mendoza-Yepes *et al*. 1997). It is evident however, that all these studies have recorded the effects of foodstuffs on microbial resistance to EOs but none has quantified them or has explained sufficiently their mechanism (Burt 2004). Suggestions explaining this activity difference may concern: i) the availability of nutrients in large quantities at food preparations, as compared to the laboratory media, which may enable the fast repair of damaged cells by the respective bacteria (Gill *et al*. 2002), and ii) the influence of bacteria's sensitivity by both intrinsic (fat/protein/water content, antioxidants, preservatives, pH, salt and other additives) and extrinsic (temperature, packaging in vacuum/gas/air, characteristics of microorganisms) food properties (Shelef 1983; Tassou *et al*. 1995). In general, it is well established that the susceptibility of bacteria to the antimicrobial effect of EOs is increasing when parameters such as pH, storage temperature and amount of oxygen within the packaging are decreasing (Tassou *et al*. 1995, 1996; Skandamis and Nychas 2000; Tsigarida *et al*. 2000). In particular, at lower pH values the hydrophobicity of an EO increases leading to its easier dissolution in the cell membrane lipids of the target bacteria (Juven *et al*. 1994).

It is also evident that the presence of large quantities of fat and/or protein in foodstuffs protects their bacteria from the action of the EOs (Aureli *et al*. 1992; Pandit and Shelef 1994; Tassou *et al*. 1995). Moreover, foods contain lower amount of water − in respect the laboratory media − hampering the approach of antibacterial agents to the bacterial cell target sites (Smith-Palmer *et al*. 2001). For example, the presence of mint oil in high fat products pate and fish roe salad produces only a limited antibacterial effect against *L. monocytogenes* and *Salm*. ser. Enteritidis, whereas when the same EO is used in cucumber and yoghurt salads (both constitute low fat and pH products) was much more effective (Tassou *et al*. 1995).

Table 4 Antibacterial activity of *Lamiaceae* EOs –or their components– in various foodstuffs (applied concentrations from the references have been converted to % v/v or to % v/w).

Foodstuff	EO or component	Applied conc. (% v/v or % v/w)	Bacterial species	Effectiveness[1]	References
Beef fillets	*Origanum* spp.	0.8	*L. monocytogenes*	B/C	Tsigarida *et al*. 2000
Beef muscle slices	*Origanum* spp.	1	*L. monocytogenes*, *Pseudomonas* spp.	C	Oussalah *et al*. 2004
Fresh beef	*Origanum* spp.	AP	Natural flora	C	Skandamis and Nychas 2002
Beef	*Rosmarinus* spp.	AP	Natural flora	C	Nerin *et al*. 2006
Minced beef	*Origanum* spp.	0.05-1	Natural flora	C/D	Skandamis and Nychas 2001
Minced pork	*Thymus* spp.	0.08	*L. monocytogenes*, *Pseudomonas* spp.	C/D	Aureli *et al*. 1992
Minced pork	*Origanum* spp.	0.01-0.02	*C. botulinum* spores	D	Ismaiel and Pierson 1990
Pork liver sausage	*Rosmarinus* spp.	1-5	*L. monocytogenes*	B/C	Pandit and Shelef 1994
Chicken noodles	*Salvia* spp.	0.02-0.05	*B. cereus*, *Staph. aureus*, *Salm.* ser. Typhimurium	D	Shelef *et al*. 1984
Fresh chicken breast	*Origanum* spp.	0.1-1	Natural flora	C	Chouliara *et al*. 2007
Pate	*Mentha* spp.	0.5-2	*L. monocytogenes*, *Salm.* ser. Enteritidis	D	Tassou *et al*. 1995
Salmon fillets	*Origanum* spp.	0.05	*Ph. phosphoreum*	D	Mejlholm and Dalgaard 2002
Cod fillets	*Origanum* spp.	0.05	*Ph. phosphoreum*	C	Mejlholm and Dalgaard 2002
Asian sea bass	*Origanum* spp., *Thymus* spp.	0.05	Natural flora	C	Harpaz *et al*. 2003
Sea bream	*Origanum* spp.	1	*Staph. aureus*, *Salm.* ser. Enteritidis	C	Tassou *et al*. 1996
Red grouper fillet	Carvacrol	0.5-3	*Salm.* ser. Typhimurium	A/B	Kim *et al*. 1995b
Cooked shrimps	*Thymus* spp.	0.75-1.5	*Ps. putida*	C	Quattara *et al*. 2001
Taramasalad	*Origanum* spp.	0.5-2	*Salm.* ser. Enteritidis	B	Koutsoumanis *et al*. 1999
Taramasalad	*Mentha* spp.	0.5-2	*Salm.* ser. Enteritidis, *L. monocytogenes*	D	Tassou *et al*. 1995
Taramasalad	*Origanum* spp.	1-2	*E. coli*	A/B	Skandamis *et al*. 2002
Semi skimmed milk	Carvacrol	0.03-0.05	*L. monocytogenes*	D	Karatzas *et al*. 2001
Soft cheese	DMC	0.025-0.25	*L. monocytogenes*	C/D	Mendoza-Yepes *et al*. 1997
Tzatziki	*Mentha* spp.	0.5-2	*Salm.* ser. Enteritidis, *L. monocytogenes*	B/C	Tassou *et al*. 1995
Tzatziki	*Origanum* spp.	1-2	*E. coli*	A/B	Skandamis *et al*. 2002
Carrots	*Thymus* spp.	0.01-1	*E. coli*	C	Singth *et al*. 2002
Carrot broth	Thymol, carvacrol	0.005-0.02	*B. cereus*	C/D	Valero and Frances 2006
Carrot broth	Thymol, carvacrol	0.4-2	*B. cereus*	B/C	Valero and Giner 2006
Lettuce	*Thymus* spp.	0.01-1	*E. coli*	C/D	Singth *et al*. 2002
Lettuce	BMC	0.1-1	Natural flora	C	Wan *et al*. 1998
Eggplant salad	*Origanum* spp.	0.7-2	*E. coli*	B/C	Skandamis and Nychas 2000
Rice	Carvacrol	0.15-0.75	*B. cereus*	B/C	Ultee *et al*. 2000
Rice	*Salvia* spp.	0.02-0.05	*B. cereus*, *Staph. aureus*, *Salm.* ser. Typhimurium	D	Shelef *et al*. 1984
Honeydew melon	Carvacrol	0.015	Natural flora	C/D	Roller and Seedhar 2002
Table grapes	Thymol	AP	Fungi	B	Valero *et al*. 2006
Kiwifruit	Carvacrol	0.015	Natural flora	C	Roller and Seedhar 2002

[1] Characterization of the EO – or component – effectiveness (extension of lag phase and/or reduction in the final population) where, A: intense, B: mediocre, C: slight and D: negligible.

The reaction between carvacrol − a phenolic component of various *Lamiaceae* EOs − and proteins is considered as the limiting factor of their antibacterial activity against *Bacillus cereus* in dairy products (Pol *et al*. 2001). Furthermore, the presence of carbohydrates in foods does not appear to protect the bacteria from the EOs action, in respect to the protective role of fats and proteins (Shelef *et al*. 1984). On the contrary, large quantities of water and/or salt facilitate the action of EOs (Shelef *et al*. 1984; Tassou *et al*. 1995; Skandamis and Nychas 2000).

Another parameter limiting the antibacterial activity of an EO is the physical structure of a food. Relative research reports concerning the performance of oregano EO against *Salm*. ser. Typhimurium, in broth and gelatine gel, have revealed that the gel matrix dramatically reduced the inhibitory effect of the EO, presumably because the diffusion was limited by the structure of the gel matrix (Skandamis *et al*. 2000). In general, the MIC value of an EO on a bacterial isolate is slightly lower in broth, as compared with the corresponding value determined in agar (Hammer *et al*. 1999). Research concerning the growth characteristics of *L. monocytogenes* and *Yersinia enterocolitica* in oil-in-water emulsions indicated that − depending on the mean droplet size of the emulsion − the bacteria may grow in films, colonies or as planktonic cells (Brocklehurst *et al*. 1995). It is also well established that the colonial growth restricts the diffusion of oxygen (Wimpenny and Lewis 1977), while the cells that are situated within a colony are somehow shielded by the outer cells from the emulsion substrates. Thus, the size of the oil droplets of a food emulsion is possible to promote the bacterial growth within colonies by protecting them from the EO action.

In meat and meat products (**Table 4**) the EOs exhibit more pronounced antibacterial activities, as compared to other antibacterial agents used in meat preparations. Carvacrol, oregano and thyme EOs have already been recognized as effective agents, since they have found to inhibit pathogens and autochthonous spoilage flora in meat products, by causing a marked initial reduction to many cells (Aureli *et al*. 1992; Stecchini *et al*. 1993; Tsigarida *et al*. 2000; Skandamis and Nychas 2001). On the contrary, in the same systems mint and sage oils were screened as being much less effective (Shelef *et al*. 1984; Tassou *et al*. 1995). It is further evident that the high fat content reduces markedly the activity of EOs in meat products (Tassou *et al*. 1995). In this regard, literature reports have indicated that the encapsulated rosemary oil is much more effective against *L. monocytogenes* in pork liver sausage as compared with pure rosemary EO (Pandit and Shelef 1994). Finally, the activity of the oregano EO against *Clostridium botulinum* spores was studied in a vacuum-packed and pasteurised minced (ground) pork product (Ismaiel and Pierson 1990).

Similar results were obtained in fisheries (**Table 4**) indicating that the high fat content reduced the effectiveness of antibacterial EOs. For example, oregano oil was assayed as more effective against the spoilage organism *Photobacterium phosphoreum* on cod fillets, as compared with salmon which is a fattier fish (Mejlholm and Dalgaard 2002). The

same EO displayed superior antibacterial activity on fisheries in comparison with the mint EO, even in fatty fish dishes (Tassou *et al*. 1995; Koutsoumanis *et al*. 1999).

Similar experiments on dairy products (**Table 4**) indicated that the EO of mint is effective antibacterial against *Salm*. ser. Enteritidis in low fat yoghurts (Tassou *et al*. 1995). In vegetables (**Table 4**), the antimicrobial activity of the EOs was benefited − as in the corresponding meat products − by the food's storage temperature and/or pH decrease (Skandamis and Nychas 2000). It must be noted however, that in general the vegetables display a low fat content enhancing thus the antibacterial activities of the EOs. Consequently, all EOs (and their components) tested on vegetables were assayed as effective antibacterial agents against the natural spoilage flora and food borne pathogens (Wan *et al*. 1998; Singh *et al*. 2002). More specifically, oregano oil inhibited the growth of *E. coli* O157:H7 in eggplant salads by reducing considerably its final populations (Skandamis and Nychas 2000). In rice (**Table 4**), the EO of sage was ineffective against *B. cereus*, whereas carvacrol was assayed as very effective displaying the capability to extend the lag phase and reduce considerably its final population (Shelef *et al*. 1984; Ultee *et al*. 2000). Finally, in fruits (**Table 4**), carvacrol was screened as an effective antibacterial agent that reduces the viable counts of the natural flora on kiwifruit (*Actinidia deliciosa*). The same EO was less effective when tested on honeydew melon (*Cucumis melo*, Roller and Seedhar 2002).

Active packaging (AP)

Packaging converts foodstuffs to a more convenient form and simultaneously protects them against micro organisms, biological and chemical changes, assuring a longer shelf life for the packaged foods (Tsigarida *et al*. 2000). As a result, packaging has become an indispensable element of food manufacturing process. In order to meet the food industry's growing demand, during the past decades a vigorous research activity was initiated towards the development of efficient food packaging techniques. Among the various packaging technologies developed by (and for) the food industry, modified atmosphere packaging (MAP) is responsible for the evolution − during the past two decades − of fresh and minimally processed food preparations, especially for meat and meat products (Skandamis and Nychas 2002).

In such packaging systems the initial atmosphere is generated either by permitting the air to be enclosed or by injecting a desired initial gas mixture. Then, this blend is altered by changing the following multiple variables: (i) permeation of oxygen, carbon dioxide, and water vapours through the package material, (ii) transmission of oxygen, carbon dioxide and water vapours through the seal and defective structural areas, iii) temperature of the package material which may lead to small changes in permeation, iv) surface area of the package material and v) thickness of the package material (Tsigarida and Nychas 2001). Such changes influence/affect the contribution of different members of microbial association, consequencing the extension of the initial food's shelf life. It is noteworthy however, that despite the extended shelf life of refrigerated products − when they are stored under vacuum /modified atmosphere packaging conditions − there is an increased concern about the growth/survival of microaerophilic psychrotrophic pathogens (Garcia de Fernando *et al*. 1995). Thus, additional hurdle(s) should be used in order to ensure the safety of such products. Smart, interactive and active packagings (Labuza 1996; Han 2000; Katz 2000; Skandamis and Nychas 2002; Kerry *et al*. 2006; Nerin *et al*. 2006; Winther and Nielsen 2006) are terms that have been used to describe some innovative concept of package structures. Various packaging types refer to changes in packaging condition in order to extend the shelf life or improve its safety and/or the sensory characteristics, without affect the quality of the food. Since most food packaging systems consist of the packaging material, the preserved food and the headspace in the package, antimicrobial agents may either be initially in-

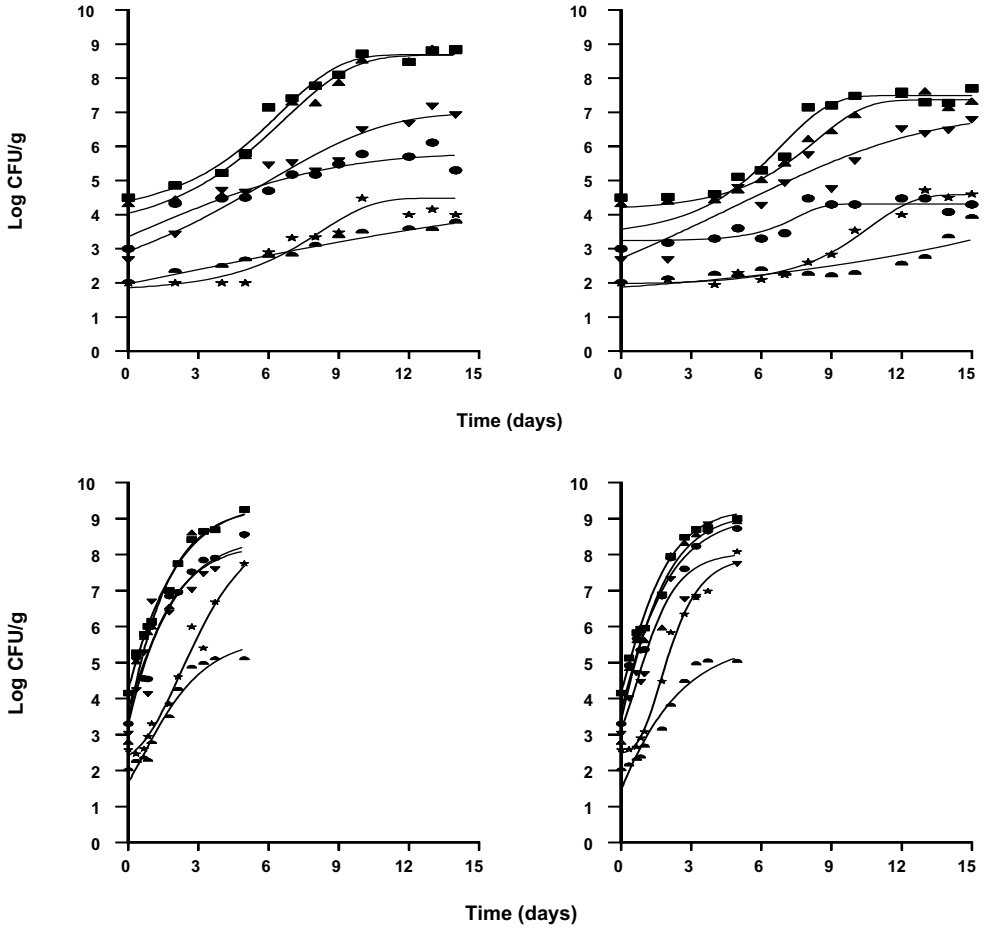

Fig. 3 Effect of volatile compounds of oregano EO (Active Packaging) in microbial association of meat stored under 40% CO_2 / 30% O_2 / 30% N_2 MAP conditions at 5°C (top diagrams) and 15°C (bottom diagrams) where, Total Viable Count: ■, Pseudomands: ▲, *Br. thermosphacta*: ▼, Lactic acid bacteria: ●, Enterobacteriaceae: ★ and Yeasts: ◓ (Figure taken from Skandamis and Nychas 2002).

corporated into the packaging materials and migrate into the food through diffusion and/or partition or be released through evaporation in the headspace. The latter may be accomplished through the use of antibacterial EOs which are volatile and regarded as ‘‘natural’’ alternatives of chemical preservatives (Skandamis and Nychas 2002). In addition, their use in foods meets the current demand of consumers for mildly processed or natural products (Nychas 1995). In this regard – according to *Mintel's Global New Products Database* – during the first half of 2005 in Europe the food processing industry was released a total of 564 products labelled as either without preservatives or additives or billed as "all natural". During the same period of 2004, only 438 products had been released, reflecting a growth rate of 28%. The vast majority of the product releases were in the no preservative or no additive sub-category, with some also being labelled as organic. Many of the "All natural" products were made up of one ingredient, such as cheeses, meats or vegetables. Within the overall grouping, chilled foods prevailed by making up the 36% of the category. It must be noted that the Mintel's database records what processors state on the labels of the food products and as Europe are considered data from Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Russia, Spain, Sweden, Switzerland, Turkey and the UK.

For the time being, the practical applications of the EOs in food preservation are limited because of their strong aroma and/or decrease of effectiveness caused by their interaction with food ingredients and structure. In any case, their application in active packaging is of great importance (Juven *et al*. 1994; Skandamis and Nychas 2002; Chorianopoulos 2007). In **Fig. 3** the effect of volatile compounds of oregano EO is presented (Active Packaging) in microbial association of meat that is stored under 40% CO_2: 30% O_2: 30% N_2 MAP conditions at 5 and 15°C (Skandamis and Nychas 2002).

Biofilms

The use of EOs as antimicrobial agents is well known in the literature (Nychas *et al*. 2003; Burt 2004). Limited information is available however, on the comparative evaluation of EOs and/or their by-products (e.g. hydrosols) as disinfectants against bacterial biofilms. For example, polytoxinol a topical essential oil-based formulation displays potent antibacterial activity against biofilm-positive strains of coagulate-negative staphylococci (Al-Shuneigat *et al*. 2005). In addition, the effectiveness of various EO components has been demonstrated against biofilm strains of *E. coli* and *Pseudomonas* spp. (Niu and Gilbert 2004), while a recent report of Lebert *et al*. (2007) indicated that the EO of *Satureja thymbra* is effective against spoilage and pathogen bacteria on monoculture and mixed culture biofilms that are associated with traditional fermented sausages.

A recent study of Chorianopoulos (2007) showed that chemical sanitizers such as lactic acid, HCl, ethanol and NaOH failed to eliminate efficiently the biofilms from stainless steel surfaces. On the contrary, the EO of *S. thymbra* − and/or its hydrosol − when tested as natural sanitizers on the same surfaces, were found to possess very potent disinfectant activities against bacterial species grown as monoculture or as mixed culture biofilms (Chorianopoulos 2007). In most experiments they caused the maximum possible log-reduction of bacterial population of the biofilms. It must be noted however, that despite the biofilm damage caused by these natural sanitizers, the corresponding data of conductance measurements have revealed that small biofilm residues were still remained on surfaces (Chorianopoulos 2007). This indicates the usefulness of the impedance method as a tool to quantify the remaining biofilm (Giaouris *et al*. 2005) which may be resulted by the incomplete sanitation.

Despite the strong antimicrobial activity of EOs on biofilms, their practical application is hampered by some limitations which refer to their strong smell, when used at effective doses (Davidson 1997) and the difficulty to flush them satisfactorily from surfaces. In this regard, their hydrosols constitute aqueous solutions which can easily be rinsed out from surfaces and possess potent antimicrobial activities (Sagdic 2003; Sagdic and Ozcan 2003). Additional advantages of hydrosols concern their i) nature as not useful by-products of EOs production by steam distillation, and ii) light odor as compared to the initial EOs. Thus, more research effort has to be devoted towards the application of hydrosols in surface cleaning by the food processing industry, since they represent a promising tool in food safety procedures with obvious financial interest (Chorianopoulos 2007).

Mode of action

Although the antimicrobial effects of EOs are well established, their mechanism of action is poorly investigated (Nychas and Tassou 2000). Literature abounds with investigations on the bacteriocins and weak acids mode of action while there are only scarce reports on the insight of the EOs (or their active components) action. In general, it is accepted that the EOs mode of action depends on the concentration of their active components (Prindle and Wright 1977). More specifically, their low concentration affects the activity of enzymes associated with the energy production, while their presence in larger amounts results in the precipitation of proteins. It must be noted however that this argument is under dispute. For example, Judis (1963) has questioned whether the alleged damage caused to cell membrane is directly related with the amount of active antibacterial compounds that act directly on cells or the effect proceeds via the initial cause of small damages which are followed by the cell breakdown.

To date, there are many different mechanisms proposed in the literature to delineate the antimicrobial activities of the EOs (or their active components). For example, it has been reported that the inhibitory action of EOs is implemented through the impairment of various enzyme systems, including those involved in energy production and structural component synthesis. Recent findings on the antimicrobial action of EOs and their components indicated that they cause deleterious effects on cellular membranes, e.g. on their permeability and structural–functional damages to the plasma membranes. This membrane impairment is mainly reflected on the dissipation of the two components of the proton motive force, the pH gradient (ΔpH) and the electrical potential ($\Delta\psi$) (Sikkema *et al*. 1995; Davidson 1997; Ultee *et al*. 1999, 2000, 2002). In particular, carvacrol – the active component of many *Lamiaceae* EOs – is reported to act through the destabilization of the cytoplasmic membrane by disintegrating the outer membrane but also as a ‘proton exchanger’ that results in a further reduction of the pH gradient across the cytoplasmic membrane (Helander *et al*. 1998; Lambert *et al*. 2001; Ultee *et al*. 2002). This proton motive force collapse and the depletion of the ATP pool leads eventually to the cell death (Ultee *et al*. 2002). These detrimental effects of antimicrobial compounds on proton motive force are strongly correlated with the leakage of specific ions (Bakker and Mangerich 1981; Kroll and Booth 1981). Indeed, the action of various preservatives including EOs, phenols and bacteriocins on the permeability barrier of cytoplasmic membrane results in the leakage of various substances, such as ions, ATP, nucleic acids and amino acids, e.g. glutamate, etc. (Tranter *et al*. 1993; Gonzalez *et al*. 1996; Tahara *et al*. 1996; Cox *et al*. 1998; Helander *et al*. 1998; Ultee *et al*. 1999; Tassou *et al*. 2000). In this regard, it is reported that the EOs of tea and mint, as well as carvacrol are capable of causing leakage of cellular material, e.g. material absorbing at 260 nm and K^+ (Cox *et al*. 1998; Gustafson *et al*. 1998; Ultee *et al*. 1999). Further cell damage may also be related to nutrient uptake, nucleic acid synthesis and ATPase activity etc. Several reports have demons-

trated that most EOs (at approx. 100 mg/l) impair the respiratory activity of different bacteria or yeasts (e.g. *Saccharomyces cerevisiae*) (Conner *et al*. 1984a, 1984b; Denyer and Hugo 1991; Tassou *et al*. 2000).

Unlike many antibiotics, EOs are capable of gaining access to the periplasm of Gram-negative bacteria, through the porin proteins of their outer membrane (Helander *et al*. 1998), since the cell membrane permeability depends on: i) the hydrophobicity of the solution which have to cross the membrane, and ii) the membrane composition (Helander *et al*. 1998; Sikkema *et al*. 1995; Ultee *et al*. 2002). Temperature is another parameter that also affects their activity, since at low temperature their solubility decreases hampering the membrane penetration (Wanda *et al*. 1976). Furthermore, the partition coefficient of an EO on cell membranes constitutes a crucial determinant of its inhibitory effectiveness, since is an indication whether the cell wall plays an important role in the relative resistance of whole cells lysis. The latter explains the quantitative differences on EOs activities reported in the literature against bacteria (degree of sensitivity), especially when bacteria with different Gram staining response were examined.

In the case of trace elements, their presence in the EOs is well established in the literature (Bozhanov *et al*. 2007). It is evident however, that the use of EOs in food preparations may consequence the trace elements absorbance. It has been reported that clove (*Syzygium aromaticum*) and oregano oils can acquire a dark pigmentation upon absorbance of iron (Bauer *et al* 2001). The solubility of the latter – which is involved in many bacteria as enzyme co-factor that permit their oxygenation – is negatively affected by the antimicrobial activity of the natural products. They influence the growth rate of aerobic or facultative aerobic bacteria, as compared to their growth without the presence of antimicrobial compounds. Finally, the reaction of ferrous ion with phenolic compounds can indirectly cause damage to the cells through oxidative stress (Friedman and Smith 1984; Nagaraj 2001).

Another possible mode of action might include the interactions (formation of complexes) between the antimicrobial constituents (e.g. phenolic constituents) and the bacterial membrane components (proteins) involved in cell membrane biosynthesis. Thus, highly reactive aldehyde groups of various natural compounds (e.g. citral, salicyldehyde, etc.) form Schiff bases that modify (or prevent) cell wall biosynthesis (Friedman 1996; Patte 1996; Friedman 1999). The previously described molecular mechanisms of antimicrobial activity against pathogens may be inhibited or enhanced, depending on the complexity of the microbial ecology and food environment in which the antimicrobial is added.

Antimicrobial agents, including antibiotics and/or related chemical or medicinal substances, have substantially reduced the threat from various infectious diseases. These compounds have greatly contributed to elongating the life expectancy during the end of the 20th century, but their benefits are currently under question because of the emergence and spread of microbes that have gained resistance to these compounds (Nychas *et al.* 2003). Since many of these bacteria may also contaminate foodstuffs, the next issue is connected to their resistance development against the "natural antimicrobials". In this regard, there is no adequate information on the resistance mechanisms of microorganisms against naturally occurring antimicrobial compounds. The ability of phenolic and phytoalexin components of the EOs to affect many cell types was extensively presented, concluding that they mainly act by causing membrane perturbations which lead to cell malfunction. This kind of activity is desirable since it is difficult for microbes to evolve resistant strains (Weinstein and Albersheim 1983). In this regard, it has been reported that EO phenolics and phytoalexins are causing rather static than out right toxic effects (Tokutake *et al*. 1992); cell membranes that leak or function poorly would not necessarily be convicted but would most probably cause a deceleration of certain metabolic processes such as cell division (Darvill and Albersheim 1984; Kubo *et al*. 1985). In general, Gram-positives are more sensitive than Gram-negative bacteria to the antimicrobial compounds found in spices (Dabbah *et al*. 1970; Shelef 1983; Farag *et al*. 1989; Tassou *et al*. 1995). However, a broad variation in the rate (or extent) of inhibition is also evident among the Gram-negative bacteria. For example, *E. coli* is less resistant than *Pseudomonas fluorescents* or *Serratia marcescens* when tested with sage, rosemary, cumin, caraway, clove and thyme EOs (Farag *et al*. 1989). Inhibition of growth ranged from 88% for *Aerobacter aerogenes* to 100% for *Alcaligenes faecalis* as test organisms (Dabbah *et al*. 1970). On the other hand, *Salmonella* ser. Typhimurium was found to be more sensitive than *Pseudomonas aeruginosa* to oregano and thyme EOs (Paster *et al*. 1990).

CONCLUDING REMARKS

During the food chain (e.g. production, processing, and distribution at retail level), preservation is responsible for the maintenance of raw material quality, physico-chemical properties and functionality, while simultaneously assure the product safety displaying low spoilage potential. According to Gould (1995), the main means used to maintain the microbiological quality and safety of foods should have the following influence on microorganisms: (i) prevent their access to foods, (ii) inactivate them, if nevertheless they gain access, and (iii) slow down, suppress or prevent the growth of those that have not been inactivated. These results may be achieved through a well designed process that varies in accordance to the preserved foodstuff. In order to fulfill the aforementioned objectives, food industry has developed various combined preservation means, such as mild heat treatment in conjunction with low concentration of preservatives. In addition, various alternative physical treatments that include the use of ultra high pressure (UHP) or pulsed electric fields (PEF) are now been investigated in order to replace the classical heat treatment. The detailed exploitation of the potential application of ''natural'' preservatives presented herein, in combination with physical treatments (i.e., mild-heat, UHP, PEF), is expected to afford the development of novel, mild preservation regimes, tailored to the organoleptic quality needs of each individual product. In this regard, several reports have already shown that UHP ''denature'' microbial cell wall proteins facilitating greatly the access of ''natural'' preservatives to cell wall and membrane (Pagan and Mackey 2000).

In order to extend our knowledge on the effectiveness of plant-derived antimicrobials and explore their application in combination with other food preservation systems, research must be focused on the following issues:

(i) The elucidation of the physiological and/or molecular cell mechanism (signal transduction, stress proteins induced, energy cost, activation of specific pathways etc) that microorganisms respond when they are treated with plant derived antimicrobials.

(ii) The delineation of food matrix effects on the antimicrobial efficiency of a plant derived antimicrobial in combination with other hurdles, by evaluating/controlling the spatial distribution of ingredients and the physico-chemical properties of the foodstuff.

(iii) The exploitation of food matrix effects on the respective organisms and the elucidation of the surface adhesion role on the microbial physiology, with (or without) the presence of natural preservatives.

(iv) The exploitation of emerging technologies which act synergistically for food safety and their shelf life extension, in conjunction with the natural antimicrobials.

(v) The development of mathematical models that permit the accurate prediction of the shelf life or the true physico-chemical conditions pertinent to either survival or inactivation of pathogenic bacteria in food products.

(vi) The validation of the procedures and mathematical models by their end users in the food industry and the development of the corresponding software to disseminate the

results, and

(vii) The understanding of consumer attitude and quality perception.

The combined application of such models and methods will offer a pioneering/novel alternative to the conventional evaluation (assessment) of the safety and quality of specific food products.

Finally, more emphasis must be focused on the assay of pathogenic microorganisms prevalence in connection with the use of natural antimicrobials during various production stages at the production plant and supermarket levels. The inclusion of several factors (e.g. food matrix, physiological status of microorganisms under stress responses leading to adaptation or survival) into mathematical models describing microbial growth and death would represent a significant advance in comparison with the empirical, descriptive models of microbial growth of limited predictive capability, currently in use by the food industries (Zwietering *et al*. 1994; Cuppers *et al*. 1997; Koutsoumanis *et al*. 1998; Skandamis and Nychas 2000; Skandamis *et al*. 2002).

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