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Review

Essential oils: their antibacterial properties and potential applications in foods—a review

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Abstract

In vitro studies have demonstrated antibacterial activity of essential oils (EOs) against *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Shigella dysenteria*, *Bacillus cereus* and *Staphylococcus aureus* at levels between 0.2 and 10 μl ml^{-1}. Gram-negative organisms are slightly less susceptible than gram-positive bacteria. A number of EO components has been identified as effective antibacterials, e.g. carvacrol, thymol, eugenol, perillaldehyde, cinnamaldehyde and cinnamic acid, having minimum inhibitory concentrations (MICs) of 0.05–5 μl ml^{-1} in vitro. A higher concentration is needed to achieve the same effect in foods. Studies with fresh meat, meat products, fish, milk, dairy products, vegetables, fruit and cooked rice have shown that the concentration needed to achieve a significant antibacterial effect is around 0.5–20 μl g^{-1} in foods and about 0.1–10 μl ml^{-1} in solutions for washing fruit and vegetables. EOs comprise a large number of components and it is likely that their mode of action involves several targets in the bacterial cell. The hydrophobicity of EOs enables them to partition in the lipids of the cell membrane and mitochondria, rendering them permeable and leading to leakage of cell contents. Physical conditions that improve the action of EOs are low pH, low temperature and low oxygen levels. Synergism has been observed between carvacrol and its precursor *p*-cymene and between cinnamaldehyde and eugenol. Synergy between EO components and mild preservation methods has also been observed. Some EO components are legally registered flavourings in the EU and the USA. Undesirable organoleptic effects can be limited by careful selection of EOs according to the type of food.

Keywords: Essential oils; Antibacterial; Preservatives; Food borne pathogens

1. Introduction

In spite of modern improvements in slaughter hygiene and food production techniques, food safety is an increasingly important public health issue (WHO, 2002a). It has been estimated that as many as 30% of people in industrialised countries suffer from a food borne disease each year and in 2000 at least two million people died from diarrhoeal disease worldwide (WHO, 2002a). There is therefore still a need for new methods of reducing or eliminating food borne pathogens, possibly in combination with existing methods (the hurdle principle; Leistner, 1978). At the same time, Western society appears to be experiencing a trend of 'green' consumerism (Tuley de Silva, 1996; Smid and Gorris, 1999), desiring fewer synthetic food additives and products with a smaller

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impact on the environment. Furthermore, the World Health Organization has recently called for a worldwide reduction in the consumption of salt in order to reduce the incidence of cardio-vascular disease (WHO, 2002b). If the level of salt in processed foods is reduced, it is possible that other additives will be needed to maintain the safety of foods. There is therefore scope for new methods of making food safe which have a natural or 'green' image. One such possibility is the use of essential oils as antibacterial additives.

Essential oils (EOs) (also called volatile or ethereal oils; Guenther, 1948) are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, enfleurage or extraction but the method of steam distillation is most commonly used for commercial production of EOs (Van de Braak and Leijten, 1999). The term 'essential oil' is thought to derive from the name coined in the 16th century by the Swiss reformer of medicine, Paracelsus von Hohenheim; he named the effective component of a drug *Quinta essentia* (Guenther, 1948). An estimated 3000 EOs are known, of which about 300 are commercially important—destined chiefly for the flavours and fragrances market (Van de Braak and Leijten, 1999). It has long been recognised that some EOs have antimicrobial properties (Guenther, 1948; Boyle, 1955) and these have been reviewed in the past (Shelef, 1983; Nychas, 1995) as have the antimicrobial properties of spices (Shelef, 1983) but the relatively recent enhancement of interest in 'green' consumerism has lead to a renewal of scientific interest in these substances (Nychas, 1995; Tuley de Silva, 1996). Besides antibacterial properties (Deans and Ritchie, 1987; Carson et al., 1995a; Mourey and Canillac, 2002), EOs or their components have been shown to exhibit antiviral (Bishop, 1995), antimycotic (Azzouz and Bullerman, 1982; Akgül and Kivanç, 1988; Jayashree and Subramanyam, 1999; Mari et al., 2003), antitoxigenic (Akgül et al., 1991; Ultee and Smid, 2001; Juglal et al., 2002), antiparasitic (Pandey et al., 2000; Pessoa et al., 2002), and insecticidal (Konstantopoulou et al., 1992; Karpouhtsis et al., 1998) properties. These characteristics are possibly related to the function of these compounds in plants (Guenther, 1948; Mahmoud and Croteau, 2002).

The purpose of this paper is to provide an overview of the published data on the antibacterial activity of those EOs and their components that could be considered suitable for application in or on foods, and to describe their possible modes of action. The current knowledge on potential antagonists and synergists is presented; legal and safety aspects are discussed and areas for future research are proposed. Although some data are presented on spoilage flora, this paper will focus chiefly on the antibacterial effect of EOs on food borne pathogens and, in particular, those for which food animals are the major reservoir.

1.1. Historical use of essential oils

Although spices have been used for their perfume, flavour and preservative properties since antiquity (Bauer et al., 2001), of the known EOs, only oil of turpentine was mentioned by Greek and Roman historians (Guenther, 1948). Distillation as a method of producing EOs was first used in the East (Egypt, India and Persia) (Guenther, 1948) more than 2000 years ago and was improved in the 9th century by the Arabs (Bauer et al., 2001). The first authentic written account of distillation of essential oil is ascribed to Villanova (ca. 1235–1311), a Catalan physician (Guenther, 1948). By the 13th century EOs were being made by pharmacies and their pharmacological effects were described in pharmacopoeias (Bauer et al., 2001) but their use does not appear to have been widespread in Europe until the 16th century, from which time they were traded in the City of London (Crosthwaite, 1998). Publishing separately in that century on the distillation and use of EOs, two Strassburg physicians, Brunschwig and Reiff, mention only a relatively small number of oils between them; turpentine, juniper wood, rosemary, spike (lavender), clove, mace, nutmeg, anise and cinnamon (Guenther, 1948). According to the French physician, Du Chesne (Quercetanus), in the 17th century the preparation of EOs was well known and pharmacies generally stocked 15–20 different oils (Guenther, 1948). The use of tea tree oil for medical purposes has been documented since the colonisation of Australia at the end of the 18th century, although it is likely to have been used by the native Australians before that (Carson and Riley, 1993). The first experimental measurement of the bactericidal properties of the

vapours of EO is said to have been carried out by De la Croix in 1881 (Boyle, 1955). However, in the course of the 19th and 20th centuries the use of EOs in medicine gradually became secondary to their use for flavour and aroma (Guenther, 1948).

1.2. Current use of EOs

The greatest use of EOs in the European Union (EU) is in food (as flavourings), perfumes (fragrances and aftershaves) and pharmaceuticals (for their functional properties) (Bauer and Garbe, 1985; Van Welie, 1997; Van de Braak and Leijten, 1999). The well-known use of EO in aromatherapy constitutes little more than 2% of the total market (Van de Braak and Leijten, 1999). Individual components of EOs are also used as food flavourings, either extracted from plant material or synthetically manufactured (Oosterhaven et al., 1995).

The antibacterial properties of essential oils and their components are exploited in such diverse commercial products as dental root canal sealers (Manabe et al., 1987), antiseptics (Bauer and Garbe, 1985; Cox et al., 2000) and feed supplements for lactating sows and weaned piglets (Van Krimpen and Binnendijk, 2001; Ilsley et al., 2002). A few preservatives containing EOs are already commercially available. 'DMC Base Natural' is a food preservative produced by DOMCA S.A., Alhendín, Granada, Spain and comprises 50% essential oils from rosemary, sage and citrus and 50% glycerol (Mendoza-Yepes et al., 1997). 'Protecta One' and 'Protecta Two' are blended herb extracts produced by Bavaria Corp. Apopka, FL, USA and are classed as generally recognized as safe (GRAS) food additives in the US. Although the precise contents are not made known by the manufacturer, the extracts probably contain one or more EOs and are dispersed in solutions of sodium citrate and sodium chloride, respectively (Cutter, 2000). Further physiological effects of EOs are made use of in widely differing products such as commercial potato sprout suppressants (Hartmans et al., 1995) and insect repellents (Carson and Riley, 1993).

2. Composition of EOs

Steam distillation is the most commonly used method for producing EOs on a commercial basis. Extraction by means of liquid carbon dioxide under low temperature and high pressure produces a more natural organoleptic profile but is much more expensive (Moyler, 1998). The difference in organoleptic profile indicates a difference in the composition of oils obtained by solvent extraction as opposed to distillation and this may also influence antimicrobial properties. This would appear to be confirmed by the fact that herb EOs extracted by hexane have been shown to exhibit greater antimicrobial activity than the corresponding steam distilled EOs (Packiyasothy and Kyle, 2002). EOs are volatile and therefore need to be stored in airtight containers in the dark in order to prevent compositional changes.

Numerous publications have presented data on the composition of the various EOs. The major components of the economically interesting EOs are summarised by Bauer et al. (2001). Detailed compositional analysis is achieved by gas chromatography and mass spectrometry of the EO or its headspace (Salzer, 1977; Scheffer and Baerheim Svendsen, 1981; Wilkins and Madsen, 1991; Daferera et al., 2000; Juliano et al., 2000; Jerkovic et al., 2001; Delaquis et al., 2002). EOs can comprise more than sixty individual components (Senatore, 1996; Russo et al., 1998). Major components can constitute up to 85% of the EO, whereas other components are present only as a trace (Senatore, 1996; Bauer et al., 2001). The phenolic components are chiefly responsible for the antibacterial properties of EOs (Cosentino et al., 1999). The major components of a number of EOs with antibacterial properties are presented in Table 1 and the structural formulae of a number of antibacterial components are presented in Fig. 1. These components have either been shown to have antibacterial activity or the data on their mode of action is discussed in this paper. There is some evidence that minor components have a critical part to play in antibacterial activity, possibly by producing a synergistic effect between other components. This has been found to be the case for sage (Marino et al., 2001), certain species of *Thymus* (Lattaoui and Tantaoui-Elaraki, 1994; Paster et al., 1995; Marino et al., 1999) and oregano (Paster et al., 1995).

The composition of EOs from a particular species of plant can differ between harvesting seasons and between geographical sources (Arras and Grella, 1992; Marotti et al., 1994; McGimpsey et al., 1994; Cosentino et al., 1999; Marino et al., 1999; Juliano et al.,

Table 1
Major components of selected[a] EOs that exhibit antibacterial properties

Common name of EO	Latin name of plant source	Major components	Approximate % composition[b]	References
Cilantro	*Coriandrum sativum* (immature leaves)	Linalool	26%	(Delaquis et al., 2002)
		E-2-decanal	20%	
Coriander	*Coriandrum sativum* (seeds)	Linalool	70%	(Delaquis et al., 2002)
		E-2-decanal	–	
Cinnamon	*Cinnamomum zeylandicum*	Trans-cinnamaldehyde	65%	(Lens-Lisbonne et al., 1987)
Oregano	*Origanum vulgare*	Carvacrol	Trace-80%	(Lawrence, 1984; Prudent et al., 1995;
		Thymol	Trace-64%	Charai et al., 1996; Sivropoulou et al., 1996;
		γ-Terpinene	2–52%	Kokkini et al., 1997; Russo et al., 1998;
		p-Cymene	Trace-52%	Daferera et al., 2000; Demetzos and Perdetzoglou, 2001; Marino et al., 2001)
Rosemary	*Rosmarinus officinalis*	α-pinene	2–25%	(Daferera et al., 2000, 2003; Pintore et al., 2002)
		Bornyl acetate	0–17%	
		Camphor	2–14%	
		1,8-cineole	3–89%	
Sage	*Salvia officinalis* L.	Camphor	6–15%	(Marino et al., 2001)
		α-Pinene	4–5%	
		β-pinene	2–10%	
		1,8-cineole	6–14%	
		α-tujone	20–42%	
Clove (bud)	*Syzygium aromaticum*	Eugenol	75–85%	(Bauer et al., 2001)
		Eugenyl acetate	8–15%	
Thyme	*Thymus vulgaris*	Thymol	10–64%	(Lens-Lisbonne et al., 1987;
		Carvacrol	2–11%	McGimpsey et al., 1994;
		γ-Terpinene	2–31%	Cosentino et al., 1999; Marino et al., 1999;
		p-Cymene	10–56%	Daferera et al., 2000; Juliano et al., 2000)

[a] EOs which have been shown to exert antibacterial properties in vitro or in food models and for which the composition could be found in the literature.
[b] Percentages of total volatiles rounded up to the nearest whole number.

2000; Faleiro et al., 2002). This can be explained, at least in part, by the formation of antibacterial substances from their precursors. *p*-Cymene (1-methyl-4-(1-methylethyl)-benzene) and γ-terpinene (1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene) are the precursors of carvacrol (2-methyl-5-(1-methylethyl)-phenol) and thymol (5-methyl-2-(1-methylethyl)phenol) in species of *Origanum* and *Thymus* (Cosentino et al., 1999; Jerkovic et al., 2001; Ultee et al., 2002). The sum of the amounts of these four compounds present in Greek oregano plants has been found to be almost equal in specimens derived from different geographical regions (Kokkini et al., 1997) and to remain stable in plants harvested during different seasons (Jerkovic et al., 2001). The same is true of *Thymus vulgaris* from Italy (Marino et al., 1999). This indicates that the four compounds are biologically and functionally closely associated and supports the theory that thymol is formed via *p*-cymene from γ-terpinene in *T. vulgaris* (Kokkini et al., 1997). Generally, EOs produced from herbs harvested during or immediately after flowering possess the strongest antimicrobial activity (McGimpsey et al., 1994; Marino et al., 1999). Enantiomers of EO components have been shown to exhibit antimicrobial activity to different extents (Lis-Balchin et al., 1999). The composition of EOs from different parts of the same plant can also differ widely. For example, EO obtained from the seeds of coriander (*Coriandrum sativum* L.) has a quite different composition to EO of cilantro, which is obtained from the immature leaves of the same plant (Delaquis et al., 2002).

3. In vitro tests of antibacterial activity

Tests of antimicrobial activity can be classified as diffusion, dilution or bioautographic methods (Rios et al., 1988). The principles and practice of these test

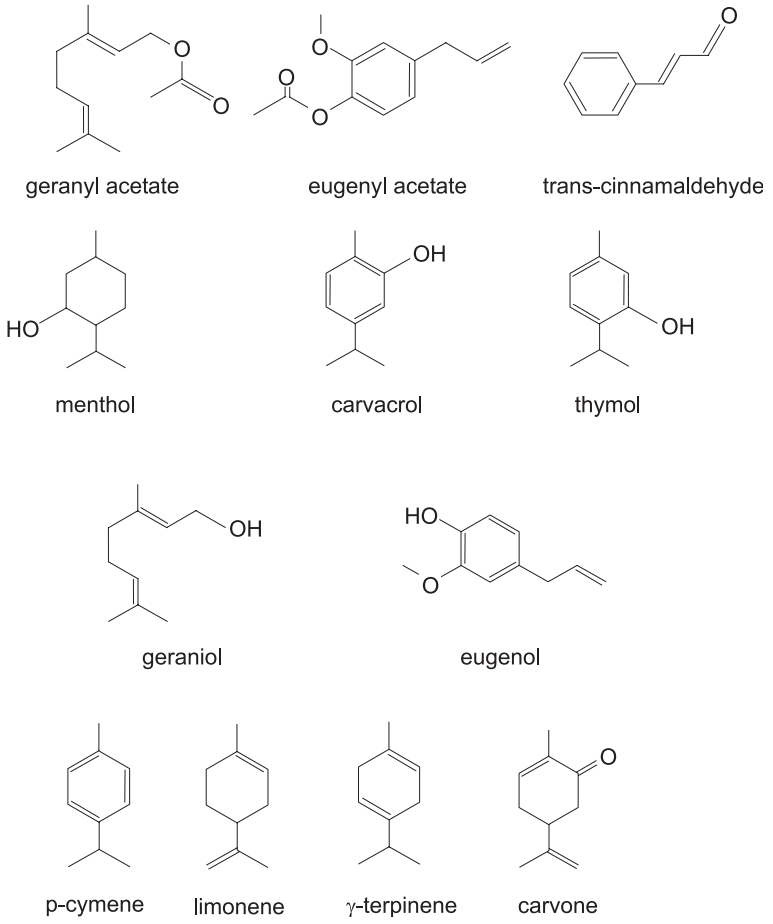

Fig. 1. Structural formulae of selected components of EOs.

methods are explained in the literature (Barry, 1976; Davidson and Parish, 1989; Hodges and Hanlon, 1991) but it appears that no standardised test has been developed for evaluating the antibacterial activity of possible preservatives against food-related microorganisms, although the need for such has been indicated (Davidson and Parish, 1989). The NCCLS method for antibacterial susceptibility testing, which is principally aimed at the testing of antibiotics has been modified for testing EOs (Hammer et al., 1999; NCCLS, 2000). Researchers adapt experimental methods to better represent possible future applications in their particular field. However, since the outcome of a test can be affected by factors such as the method used to extract the EO from plant material, the volume of inoculum, growth phase, culture medium used, pH of the media and incubation time and temperature (Rios et al., 1988), comparison of published data is complicated (Janssen et al., 1987; Friedman et al., 2002). A number of reviewers have surveyed the methods used for antibacterial activity studies carried out with EOs (Koedam, 1977a,b; Shelef, 1983; Janssen et al., 1987; Rios et al., 1988; Nychas, 1995). In papers published since, the number of variations on culture medium, size of inoculum, choice of emulsifier and basic test method has further increased.

The minimum inhibitory concentration (MIC) is cited by most researchers as a measure of the antibacterial performance of EOs. The definition of the MIC differs between publications and this is another obstacle to comparison between studies. In some cases, the minimum bactericidal concentration

(MBC) or the bacteriostatic concentration is stated, both terms agreeing closely with the MIC. A list of the most frequently used terms in antibacterial activity testing of EOs is presented in Table 2. In addition, the term 'minimum cidal concentration' has been used but is not defined (Hammer et al., 1999). The terms 'minimum lethal dilution (or concentration)' (Janssen, 1989; Janssen et al., 1987) and 'minimum inhibitory dilution' (Janssen, 1989) appear to have fallen out of use, at least in food microbiology literature.

A summary of the techniques used to test the antibacterial activity of EOs is presented in Table 3. Screening of EOs for antibacterial activity is often done by the disk diffusion method, in which a paper disk soaked with EO is laid on top of an inoculated agar plate. This is generally used as a preliminary check for antibacterial activity prior to more detailed studies. Factors such as the volume of EO placed on the paper disks, the thickness of the agar layer and whether a solvent is used vary considerably between studies. This means that this method is useful for selection between EOs but comparison of published data is not feasible. The agar well test in which the EO is deposited into wells cut into the agar can be used as a screening method when large numbers of EOs and/or large numbers of bacterial isolates are to be screened (Deans et al., 1993; Dorman and Deans, 2000). In order to make bacterial growth easier to visualise, triphenyl tetrazolium chloride may be added to the growth medium (Elgayyar et al., 2001; Mourey and Canillac, 2002).

The strength of the antibacterial activity can be determined by dilution of EO in agar or broth. The published studies using dilution in agar have used different solvents to incorporate the EO in the medium (Prudent et al., 1995; Pintore et al., 2002), different volumes of inoculum (1–100 μl) (Juven et al., 1994; Prudent et al., 1995), which is sometimes dotted (Pintore et al., 2002) and sometimes streaked (Farag et al., 1989) onto the agar surface. Despite these variations, the MICs of EOs determined by agar dilution generally appear to be in approximately the same order of magnitude (Farag et al., 1989; Prudent et al., 1995; Pintore et al., 2002). In broth dilution studies a number of different techniques exist for determining the end-point—the most used methods are that of optical density (OD) (turbidity) measurement and the enumeration of colonies by viable count. The former method has the advantage of being automated; the latter is labour intensive. The measurements of conductance/conductivity and end-point determination by visual monitoring have been less often used. A new microdilution method for determining the MIC of oil-based compounds uses the redox indicator resazurin as a visual indicator of the MIC. The results compare favourably with those obtained by viable count and OD measurement and the method is more sensitive than the agar dilution method (Mann and Markham, 1998). A patented colour indicator based on resazurin has been used to determine the MICs for methanolic extracts of plant materials (Salvat et al., 2001) and EOs

Table 2
Terms used in antibacterial activity testing

Term	Definition, with reference to concentration of EO	Reference
Minimum inhibitory concentration (MIC)	Lowest concentration resulting in maintenance or reduction of inoculum viability	(Carson et al., 1995a)
	Lowest concentration required for complete inhibition of test organism up to 48 h incubation	(Wan et al., 1998; Canillac and Mourey, 2001)
	Lowest concentration inhibiting visible growth of test organism	(Karapinar and Aktug, 1987; Onawunmi, 1989; Hammer et al., 1999; Delaquis et al., 2002)
	Lowest concentration resulting in a significant decrease in inoculum viability (>90%)	(Cosentino et al., 1999)
Minimum bactericidal concentration (MBC)	Concentration where 99.9% or more of the initial inoculum is killed	(Carson et al., 1995b; Cosentino et al., 1999; Canillac and Mourey, 2001)
	Lowest concentration at which no growth is observed after subculturing into fresh broth	(Onawunmi, 1989)
Bacteriostatic concentration	Lowest concentration at which bacteria fail to grow in broth, but are cultured when broth is plated onto agar	(Smith-Palmer et al., 1998)
Bactericidal concentration	Lowest concentration at which bacteria fail to grow in broth, and are not cultured when broth is plated onto agar	(Smith-Palmer et al., 1998)

Table 3
Test methods used to measure the antibacterial activity of EOs and their constituents

Purpose	Test method		References
Screening for antibacterial activity	Disk diffusion		(Farag et al., 1989; Aureli et al., 1992; Kim et al., 1995a,b; Sivropoulou et al., 1996; Firouzi et al., 1998; Renzini et al., 1999; Senatore et al., 2000; Elgayyar et al., 2001; Skandamis et al., 2001; Cimanga et al., 2002; Faleiro et al., 2002; Packiyasothy and Kyle, 2002; Burt and Reinders, 2003; Wilkinson et al., 2003)
	Agar wells		(Deans and Ritchie, 1987; Smith-Palmer et al., 1998; Wan et al., 1998; Dorman and Deans, 2000; Ruberto et al., 2000)
Determination of strength of antibacterial properties	Agar dilution method		(Karapinar and Aktug, 1987; Farag et al., 1989; Paster et al., 1990; Ting and Deibel, 1991; Stecchini et al., 1993; Juven et al., 1994; Pandit and Shelef, 1994; Prudent et al., 1995; Ouattara et al., 1997; Hammer et al., 1999; Pintore et al., 2002; Wilkinson et al., 2003; Moleyar and Narasimham, 1992; Negi et al., 1999)
	Broth dilution	Visible growth	(Onawunmi, 1989; Cosentino et al., 1999; Elgayyar et al., 2001; Delaquis et al., 2002; Mourey and Canillac, 2002; Bassole et al., 2003)
		Optical density/turbidity	(Shelef et al., 1984; Ismaiel and Pierson, 1990; Kim et al., 1995a; Sivropoulou et al., 1995; Sivropoulou et al., 1996; Chaibi et al., 1997; Ultee et al., 1998; Pol and Smid, 1999; Lambert et al., 2001; Skandamis et al., 2001; Ultee and Smid, 2001)
		Absorbance	(Smith-Palmer et al., 1998; Mejlholm and Dalgaard, 2002)
		Colorimetric	(Gill et al., 2002; Burt and Reinders, 2003)
		Conductance/conductivity/impedance	(Tassou et al., 1995; Wan et al., 1998; Marino et al., 1999; Tassou et al., 2000; Marino et al., 2001)
		Viable count	(Beuchat, 1976; Shelef et al., 1984; Tassou et al., 1995; Sivropoulou et al., 1996; Hammer et al., 1999; Pol and Smid, 1999; Koidis et al., 2000; Skandamis et al., 2000; Canillac and Mourey, 2001; Periago and Moezelaar, 2001; Periago et al., 2001; Friedman et al., 2002)
Determination of rapidity and duration of antibacterial activity	Time-kill analysis/Survival curves		(Beuchat, 1976; Shelef et al., 1984; Ting and Deibel, 1991; Aureli et al., 1992; Stecchini et al., 1993; Tassou et al., 1995; Sivropoulou et al., 1996; Ultee et al., 1998; Wan et al., 1998; Pol and Smid, 1999; Periago and Moezelaar, 2001; Skandamis et al., 2001; Ultee and Smid, 2001; Mejlholm and Dalgaard, 2002; Pintore et al., 2002; Burt and Reinders, 2003; Cressy et al., 2003)
Observation of physical effects of antibacterial activity	Scanning electron microscopy		(Lambert et al., 2001; Skandamis et al., 2001; Burt and Reinders, 2003)

(Burt and Reinders, 2003) and the method can be automated by measuring the end point by fluorescence instead of visual means (Lancaster and Fields, 1996). Triphenyl tetrazolium chloride has been used for visual end point determination in the study of tea tree oil in broth but, although it is an indicator of bacterial growth, the colour change did not fully correlate with the MIC (Carson et al., 1995b).

The rapidity of a bactericidal effect or the duration of a bacteriostatic effect can be determined by time-kill analysis (survival curve plot) whereby the number of viable cells remaining in broth after the addition of EO is plotted against time. The most frequently used methods for this are measurement of OD and viable count after plating out onto agar. Damage to the bacterial cell wall and loss of cell contents can be

studied by scanning electron microscopy (SEM) (Lambert et al., 2001; Skandamis et al., 2001; Burt and Reinders, 2003). Careful preparation of the samples for SEM is necessary to ensure that the observed difference between control and treated cells are due to the effect of the EO and not to the preparation method.

Several studies have used the measurement of OD or conductance to perform further calculations rather

Table 4
Selected[a] MICs of essential oils tested in vitro against food borne pathogens

Plant from which EO is derived	Species of bacteria	MIC, approximate range (μl ml^{-1})[b]	Reference(s)
Rosemary	*Escherichia coli*	4.5–>10	(Farag et al., 1989; Smith-Palmer et al., 1998; Hammer et al., 1999; Pintore et al., 2002)
	Salmonella typhimurium	>20	(Hammer et al., 1999)
	Bacillus cereus	0.2	(Chaibi et al., 1997)
	Staphylococcus aureus	0.4–10	(Farag et al., 1989; Smith-Palmer et al., 1998; Hammer et al., 1999; Pintore et al., 2002)
	Listeria monocytogenes	0.2	(Smith-Palmer et al., 1998)
Oregano	*E. coli*	0.5–1.2	(Prudent et al., 1995; Hammer et al., 1999; Burt and Reinders, 2003)
	S. typhimurium	1.2	(Hammer et al., 1999)
	Staph. aureus	0.5–1.2	(Prudent et al., 1995; Hammer et al., 1999)
Lemongrass	*E. coli*	0.6	(Hammer et al., 1999)
	S. typhimurium	2.5	(Hammer et al., 1999)
	Staph. aureus	0.6	(Hammer et al., 1999)
Sage	*E. coli*	3.5–5	(Farag et al., 1989; Smith-Palmer et al., 1998; Hammer et al., 1999)
	S. typhimurium	10–20	(Shelef et al., 1984; Hammer et al., 1999)
	Staph. aureus	0.75–10	(Shelef et al., 1984; Farag et al., 1989; Smith-Palmer et al., 1998; Hammer et al., 1999)
	L. monocytogenes	0.2	(Smith-Palmer et al., 1998)
Clove	*E. coli*	0.4–2.5	(Farag et al., 1989; Smith-Palmer et al., 1998; Hammer et al., 1999)
	S. typhimurium	>20	(Hammer et al., 1999)
	Staph. aureus	0.4–2.5	(Farag et al., 1989; Smith-Palmer et al., 1998; Hammer et al., 1999)
	L. monocytogenes	0.3	(Smith-Palmer et al., 1998)
Thyme	*E. coli*	0.45–1.25	(Farag et al., 1989; Smith-Palmer et al., 1998; Cosentino et al., 1999; Hammer et al., 1999; Burt and Reinders, 2003)
	S. typhimurium	0.450–>20	(Cosentino et al., 1999; Hammer et al., 1999)
	Staph. aureus	0.2–2.5	(Farag et al., 1989; Smith-Palmer et al., 1998; Cosentino et al., 1999; Hammer et al., 1999)
	L. monocytogenes	0.156–0.45	(Firouzi et al., 1998; Smith-Palmer et al., 1998; Cosentino et al., 1999)
Turmeric	*E. coli*	>0.2	(Negi et al., 1999)
	B. cereus	0.2	(Negi et al., 1999)
Tea bush (*Lippia* spp.)	*E. coli*	2.5–>80	(Bassole et al., 2003)
	Shigella dysenteria	5–>80	(Bassole et al., 2003)
	Staph. aureus	0.6–40	(Bassole et al., 2003)
	B. cereus	5–10	(Bassole et al., 2003)

[a] EOs derived from plants used as herbs, spices or infusions in cooking were selected and MICs for a selection of important food borne pathogens cited.

[b] In the references MICs have been reported in the units ppm, mg ml^{-1}, % (v/v), μl l^{-1} and μg ml^{-1}. For ease of comparison these have been converted to μl ml^{-1}, whereby it was assumed that EOs have the same density as water. In some references the MIC was termed minimum bactericidal or bacteriostatic concentration (Smith-Palmer et al., 1998; Cosentino et al., 1999; Burt and Reinders, 2003).

than directly stating the MIC. The OD of the test suspension and control may be used to calculate an inhibition index (Chaibi et al., 1997). Measurements of conductance can be used to calculate the period elapsing before growth can be detected, the detection time (DT), after treatment of cells with EO (Marino et al., 1999; Tassou et al., 2000; Marino et al., 2001). Comparison of the maximum specific growth rate (μ_{max}) of bacteria based on data from viable counts or absorbance measurements has also been done in a number of studies (Ultee et al., 1998; Skandamis et al., 2000; Mejlholm and Dalgaard, 2002). A new method of calculating the MIC from OD measurements has been found suitable for testing combinations of antibacterial substances (Lambert and Pearson, 2000; Lambert et al., 2001). In one study the percentage of EO resulting in a 50% decrease in the viable count was determined from plots of percentage kill against concentration (Friedman et al., 2002). The diversity of ways of reporting the antibacterial activity of EOs limits comparison between studies and could lead to duplication of work.

One feature of test methods that varies considerably is whether or not an emulsifier or solvent is used to dissolve the EO or to stabilise it in water-based culture media. Several substances have been used for this purpose: ethanol (Beuchat, 1976; Deans and Ritchie, 1987; Karapinar and Aktug, 1987; Aureli et al., 1992; Moleyar and Narasimham, 1992; Juven et al., 1994; Lattaoui and Tantaoui-Elaraki, 1994; Pandit and She-

Table 5
Selected[a] MICs of essential oil components tested in vitro against food borne pathogens

Essential oil component	Species of bacteria	MIC, approximate range (μl ml^{-1})[b]	References
α-Terpineol	*Escherichia coli*	0.450–>0.9	(Cosentino et al., 1999)
	Salmonella typhimurium	0.225	(Cosentino et al., 1999)
	Staphylococcus aureus	0.9	(Cosentino et al., 1999)
	Listeria monocytogenes	>0.9	(Cosentino et al., 1999)
	Bacillus cereus	0.9	(Cosentino et al., 1999)
Carvacrol	*E. coli*	0.225–5	(Kim et al., 1995a; Cosentino et al., 1999)
	S. typhimurium	0.225–0.25	(Kim et al., 1995a; Cosentino et al., 1999)
	Staph. aureus	0.175–0.450	(Cosentino et al., 1999; Lambert et al., 2001)
	L. monocytogenes	0.375–5	(Kim et al., 1995a; Cosentino et al., 1999; Pol and Smid, 1999)
	B. cereus	0.1875–0.9	(Cosentino et al., 1999; Pol and Smid, 1999)
Citral	*E. coli*	0.5	(Onawunmi, 1989; Kim et al., 1995a)
	S. typhimurium	0.5	(Kim et al., 1995a)
	Staph. aureus	0.5	(Onawunmi, 1989)
	L. monocytogenes	0.5	(Kim et al., 1995a)
Eugenol	*E. coli*	1.0	(Kim et al., 1995a)
	S. typhimurium	0.5	(Kim et al., 1995a)
	L. monocytogenes	>1.0	(Kim et al., 1995a)
Geraniol	*E. coli*	0.5	(Kim et al., 1995a)
	S. typhimurium	0.5	(Kim et al., 1995a)
	L. monocytogenes	1.0	(Kim et al., 1995a)
Perillaldehyde	*E. coli*	0.5	(Kim et al., 1995a)
	S. typhimurium	0.5	(Kim et al., 1995a)
	L. monocytogenes	1.0	(Kim et al., 1995a)
Thymol	*E. coli*	0.225–0.45	(Cosentino et al., 1999)
	S. typhimurium	0.056	(Cosentino et al., 1999)
	Staph. aureus	0.140–0.225	(Cosentino et al., 1999; Lambert et al., 2001)
	L. monocytogenes	0.450	(Cosentino et al., 1999)
	B. cereus	0.450	(Cosentino et al., 1999)

[a] EOs components present in plants used in cooking were selected and MICs for a selection of important food borne pathogens cited.
[b] In the references MICs have been reported in the units mg ml^{-1}, % (v/v), μl l^{-1}, μg ml^{-1} and mmol l^{-1}. For ease of comparison these have been converted to μl ml^{-1}, whereby it was assumed that EOs have the same density as water. In one reference, the MIC was termed minimum bactericidal concentration (Cosentino et al., 1999).

lef, 1994; Sivropoulou et al., 1995, 1996; Ouattara et al., 1997; Marino et al., 1999; Pol and Smid, 1999; Marino et al., 2001; Packiyasothy and Kyle, 2002), methanol (Onawunmi, 1989), Tween-20 (Kim et al., 1995b; Mann and Markham, 1998; Hammer et al., 1999), Tween-80 (Paster et al., 1990; Juven et al., 1994; Carson and Riley, 1995; Cosentino et al., 1999; Mourey and Canillac, 2002; Bassole et al., 2003; Wilkinson et al., 2003), acetone in combination with Tween-80 (Prudent et al., 1995), polyethylene glycol (Pintore et al., 2002), propylene glycol (Negi et al., 1999), *n*-hexane (Senatore et al., 2000), dimethyl sulfoxide (Firouzi et al., 1998) and agar (Mann and Markham, 1998; Delaquis et al., 2002; Gill et al., 2002; Burt and Reinders, 2003). However, a number of researchers found it unnecessary to use an additive (Smith-Palmer et al., 1998; Wan et al., 1998; Cosentino et al., 1999; Renzini et al., 1999; Dorman and Deans, 2000; Tassou et al., 2000; Canillac and Mourey, 2001; Elgayyar et al., 2001; Lambert et al., 2001; Cimanga et al., 2002; Mejlholm and Dalgaard, 2002). One study employed vigorous shaking in phosphate saline buffer to suspend EOs (Friedman et al., 2002). The performance of the most frequently used solvents, ethanol and Tween-80, has been compared with that of agar for the stabilisation of oregano and clove oils. The use of agar (0.2%) was found to produce as homogenous a dispersion as a true solution in absolute ethanol (Remmal et al., 1993b). Furthermore, the MICs of oregano and clove EOs were significantly lower in the presence of agar than in the presence of Tween-80 or ethanol. It was concluded that solvents and detergents could decrease the antibacterial effect of EOs (Remmal et al., 1993a,b). This is supported by the fact that Tween-80 has been recommended as a neutraliser of phenolic disinfectants (Cremieux et al., 1981) and this has been confirmed in work on the action of thyme oil against *Salmonella typhimurium* (Juven et al., 1994). Tween-80 has also been shown to protect *Listeria monocytogenes* from the antibacterial activity of an EO component during freeze–thaw cycles (Cressy et al., 2003). A further disadvantage of the use of Tween-80 to dissolve EOs is the fact that the turbidity of the resulting dispersion can hamper visual observations and OD measurements (Carson et al., 1995b).

A selection of MICs for EOs and EO components tested in vitro against food borne pathogens is presented in Tables 4 and 5. Considering the diversity of test methods, bacterial isolates (clinical or reference) and origins of the EOs used, the range of MICs appears considerably narrow in most cases.

4. Tests of antibacterial activity of EOs in food systems

Although, as mentioned previously, a small number of food preservatives containing EOs is commercially available, until the early 1990s very few studies of the activity of EOs in foods had been published (Board and Gould, 1991). Since then a fair number of trials have been carried out with EOs in foods. An overview of the literature reporting studies on the antibacterial effect of EOs or their components in foods is presented in Table 6. Reports of studies using diluted foods or food slurries (Pol et al., 2001; Smith-Palmer et al., 2001) and studies using dried herbs or spices or their extracts (Tassou et al., 1996; Hao et al., 1998a,b) have not been included in the table.

However well EOs perform in antibacterial assays in vitro, it has generally been found that a greater concentration of EO is needed to achieve the same effect in foods (Shelef, 1983; Smid and Gorris, 1999). The ratio has been recorded to be approximately twofold 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- to 100-fold in soft cheese (Mendoza-Yepes et al., 1997). An exception to this phenomenon is *Aeromonas hydrophila*; no greater proportion of EO was needed to inhibit this species on cooked pork and on lettuce in comparison to tests in vitro (Stecchini et al., 1993; Wan et al., 1998). Several studies have recorded the effect of foodstuffs on microbial resistance to EOs but none appears to have quantified it or to have explained the mechanism, although suggestions have been made as to the possible causes. The greater availability of nutrients in foods compared to laboratory media may enable bacteria to repair damaged cells faster (Gill et al., 2002). Not only are the intrinsic properties of the food (fat/protein/water content, antioxidants, preservatives, pH, salt and other additives) relevant in this respect—the extrinsic determinants (temperature, packaging in vacuum/gas/air, characteristics of microorganisms) can also influence

bacterial sensitivity (Shelef, 1983; Tassou et al., 1995). Generally, the susceptibility of bacteria to the antimicrobial effect of EOs also appears to increase with a decrease in the pH of the food, the storage temperature and the amount of oxygen within the packaging (Tassou et al., 1995, 1996; Skandamis and Nychas, 2000; Tsigarida et al., 2000). At low pH the hydrophobicity of an EO increases, enabling it to more easily dissolve in the lipids of the cell membrane of target bacteria (Juven et al., 1994).

It is generally supposed that the high levels of fat and/or protein in foodstuffs protect the bacteria from the action of the EO in some way (Aureli et al., 1992; Pandit and Shelef, 1994; Tassou et al., 1995). For example, if the EO dissolves in the lipid phase of the food there will be relatively less available to act on bacteria present in the aqueous phase (Mejlholm and Dalgaard, 2002). Another suggestion is that the lower water content of food compared to laboratory media may hamper the progress of antibacterial agents to the target site in the bacterial cell (Smith-Palmer et al., 2001). Mint oil in the high fat products pâté and fish roe salad exhibited little antibacterial effect against *L. monocytogenes* and *S. enteritidis*, whereas in cucumber and yoghurt salad (low fat) the same EO was much more effective (Tassou et al., 1995). Although the improved effectiveness in cucumber and yoghurt salad may be partly attributed to the low pH (4.3 as opposed to pH 6.8 in pâté), fish roe salad also has a low pH (4.9). This would seem to indicate that fat percentage might exert a greater influence on the antibacterial effect of EOs than the pH.

A reaction between carvacrol, a phenolic component of various EOs, and proteins has been put forward as a limiting factor in the antibacterial activity against *Bacillus cereus* in milk (Pol et al., 2001). Protein content has also been put forward as a factor inhibiting the action of clove oil on *Salmonella enteritidis* in diluted low-fat cheese (Smith-Palmer et al., 2001). Carbohydrates in foods do not appear to protect bacteria from the action of EOs as much as fat and protein do (Shelef et al., 1984). A high water and/or salt level facilitates the action of EOs (Shelef et al., 1984; Wendakoon and Sakaguchi, 1993; Tassou et al., 1995; Skandamis and Nychas, 2000).

The physical structure of a food may limit the antibacterial activity of EO. A study of the relative performance of oregano oil against *S. typhimurium* in broth and in gelatine gel revealed that the gel matrix dramatically reduced the inhibitory effect of the oil. This was presumed to be due to the limitation of diffusion by the structure of the gel matrix (Skandamis et al., 2000). MICs for a particular EO on a particular bacterial isolate have been shown to be generally slightly lower in broth than in agar (Hammer et al., 1999). Research into the growth characteristics of *L. monocytogenes* and *Yersinia enterocolitica* in oil-in-water emulsions has shown that, depending on the mean droplet size of the emulsion, the bacteria can grow in films, in colonies or as planktonic cells (Brocklehurst et al., 1995). It is known that colonial growth restricts diffusion of oxygen (Wimpenny and Lewis, 1977) and cells situated within a colony may be shielded to a certain extent by the outer cells from substrates in the emulsion. If the oil droplets in a food emulsion are of the appropriate size, it could be possible for bacteria growing within colonies to be protected from the action of EOs in this way.

4.1. Meat and meat products

With reference to Table 6, certain oils stand out as better antibacterials than others for meat applications. Eugenol and coriander, clove, oregano and thyme oils were found to be effective at levels of 5–20 μl g^{-1} in inhibiting *L. monocytogenes*, *A. hydrophila* and autochthonous spoilage flora in meat products, sometimes causing a marked initial reduction in the number of recoverable cells (Aureli et al., 1992; Stecchini et al., 1993; Hao et al., 1998a,b; Tsigarida et al., 2000; Skandamis and Nychas, 2001) whilst mustard, cilantro, mint and sage oils were less effective or ineffective (Shelef et al., 1984; Tassou et al., 1995; Gill et al., 2002; Lemay et al., 2002). A high fat content appears to markedly reduce the action of EOs in meat products. For example, mint and cilantro EOs were not effective in products with a high level of fat, such as pâté (which generally contains 30–45% fat) and a coating for ham containing canola oil (Tassou et al., 1995; Gill et al., 2002). Immobilising cilantro EO in a gelatine gel, however, improved the antibacterial activity against *L. monocytogenes* in ham (Gill et al., 2002).

One study found that encapsulated rosemary oil was much more effective than standard rosemary EO against *L. monocytogenes* in pork liver sausage,

Table 6
Overview of studies testing the antibacterial activity of essential oils or their components in foods

Food group	Food	EO or component	Concentration applied	Concentration applied ($\mu l\ g^{-1}$ or $\mu l\ ml^{-1}$)[a]	Bacterial species	Notes on experimental set-up[b]	Observations		References
							Extension of lag phase of growth	Reduction in final population[c]	
Meat	Minced mutton	Clove oil	0.5–1%	5–10	*L. monocytogenes*	Two storage temperatures	Yes	+	(Vrinda Menon and Garg, 2001)
	Roast beef sirloin, sliced	Eugenol	0.1 ml spread over surface of 25 g slice		*L. monocytogenes*	Two levels of inoculum and two storage temperatures	Yes	++	(Hao et al., 1998b)
					A. hydrophila	Two levels of inoculum and two storage temperatures	Yes	++	(Hao et al., 1998b)
	Cooked chicken breast, pieces	Eugenol	0.1 ml spread over surface of 25 g piece of chicken		*L. monocytogenes*	Two levels of inoculum and two storage temperatures	Yes	++	(Hao et al., 1998a)
					A. hydrophila	Two levels of inoculum and two storage temperatures	Yes	+++	(Hao et al., 1998a)
	Pork liver sausage	Rosemary oil, encapsulated rosemary oil	1% and 5%, respectively	10 and 50, respectively	*L. monocytogenes*		Yes	Rosemary oil: + Encapsulated oil: +++	(Pandit and Shelef, 1994)
	Chicken noodles, beef	Sage oil	200–500 ppm	0.2–0.5	*B. cereus, Staph. aureus, S. typhimurium*		No	Chicken noodles: 0 Beef: 0	(Shelef et al., 1984)
	Minced beef	Oregano oil	0.05–1%	0.5–10	Natural flora	Three packaging regimes tested: air, carbon dioxide and modified atmosphere (40% CO_2, 30% N_2, 30% O_2)	n.d.[d]	+	(Skandamis and Nychas, 2001)
	Cooked pork	Coriander oil	1250 $\mu g/cm^2$		*A. hydrophila*	Two storage temperatures and two inoculation levels tested.	Yes	++ to +++	(Stecchini et al., 1993)
		Clove oil	500 $\mu g/cm^2$		*A. hydrophila*	Two storage temperatures and two inoculation levels tested	Yes	++ to +++	(Stecchini et al., 1993)

	Beef fillets	Oregano oil	0.8% v/w	8	*L. monocytogenes* amongst natural flora	Modified atmosphere packaging (40% CO_2, 30% N_2, 30% O_2) and packaging in air	In MAP but not in air	MAP: +++ Air: +	(Tsigarida et al., 2000)
	Pâté	Mint oil	0.5–2.0% v/w	5–20	*L. monocytogenes*	Two storage temperatures	No	0	(Tassou et al., 1995)
					S. enteritidis	Two storage temperatures	No	0	(Tassou et al., 1995)
	Minced pork	Thyme oil	0.02 ml mixed with 25g meat	0.8	*L. monocytogenes*		Yes	+	(Aureli et al., 1992)
	Vacuum packed ham	Cilantro oil	0.1–6% v/v in coating	1–60	*L. monocytogenes*	Coating: gelatine gel or canola oil	No	0	(Gill et al., 2002)
	Vacuum packed minced pork product	Oregano oil	100–200 ppm	0.1–0.2	*C. botulinum* spores	Two levels of inoculation	No	0	(Ismaiel and Pierson, 1990)
	Cooked chicken sausage	Mustard oil	0.1% w/w	1	*E. coli*		Yes	0	(Lemay et al., 2002)
Fish	Cooked shrimps	Thyme oil, Cinnamaldehyde	0.75–1.5% and 0.15–0.3% in coating, respectively	7.5–15 and 1.5–3 in coating, respectively	*Pseudomonas putida*		Very slight	++	(Ouattara et al., 2001)
	Red grouper fillet, cubed	Carvacrol, citral, geraniol	0.5–3.0% w/v in dipping solution	5–30 in dipping solution	*S. typhimurium*		Carvacrol at 30 µl ml^{-1} killed all cells	Carvacrol: ++ Citral and geraniol: +	(Kim et al., 1995b)
	Asian sea bass, whole	Thyme oil or oregano oil	0.05% v/v (sic) sealed in packaging with whole fish	0.5	Natural flora		Both on surface and in flesh	Surface: ++ Flesh: +	(Harpaz et al., 2003)
	Cod fillets	Oregano oil	0.05% v/w	0.5	*Photobacterium phosphoreum*		Yes	+	(Mejlholm and Dalgaard, 2002)
	Salmon fillets	Oregano oil	0.05% v/w	0.5	*P. phosphoreum*		No	0	(Mejlholm and Dalgaard, 2002)
	Taramasalad (cod's roe salad)	Oregano oil	0.5–2.0% v/w	5–20	*S. enteritidis*	Various pHs and storage temperatures tested	Yes	+++	(Koutsoumanis et al., 1999)
	Taramosalata (fish roe salad)	Mint oil	0.5–2.0% v/w	5–20	*S. enteritidis*	Two storage temperatures	No	0	(Tassou et al., 1995)
					L. monocytogenes	Two storage temperatures	No	0	(Tassou et al., 1995)
Dairy	Mozzarella cheese	Clove oil	0.5–1%	5–10	*L. monocytogenes*		Yes	+	(Vrinda Menon and Garg, 2001)
	Semi skimmed milk	Carvacrol	2–3 mmol l^{-1}	0.3–0.45	*L. monocytogenes*	Two temperatures	n.d.	+	(Karatzas et al., 2001)

(continued on next page)

Table 6 (*continued*)

Food group	Food	EO or component	Concentration applied	Concentration applied ($\mu l\ g^{-1}$ or $\mu l\ ml^{-1}$)[a]	Bacterial species	Notes on experimental set-up[b]	Observations		References
							Extension of lag phase of growth	Reduction in final population[c]	
Dairy	Soft cheese	'DMC Base Natural' preservative comprising 50% EOs of rosemary, sage and citrus	250–2500 ppm	0.25–2.5	*L. monocytogenes pool* (10 strains)		From 1.0 $\mu l\ g^{-1}$	+	(Mendoza-Yepes et al., 1997)
					E. coli O157:H7		No	0	(Mendoza-Yepes et al., 1997)
	Yoghurt	Clove, cinnamon, cardamom, peppermint oils	0.005–0.5% in milk before fermentation	0.05–5	*Streptococcus thermophilus*		n.d.	Mint oil: + Cardamom, clove: ++ Cinnamon: +++	(Bayoumi, 1992)
					Lactobacillus bulgaricus		n.d.	Mint oil: 0 Cardamom, clove: + Cinnamon: +++	(Bayoumi, 1992)
	Tzatziki (yogurt and cucumber salad)	Mint oil	0.5–2.0% v/w	5–20	*S. enteritidis*	Two storage temperatures	From 1.5% v/w	++	(Tassou et al., 1995)
					L. monocytogenes	Two storage temperatures	Increase in growth	– –	(Tassou et al., 1995)
Vegetables	Lettuce, Romaine	Thyme oil	0.1–10 ml l^{-1} in rinsing solution	0.1–10	*E. coli* O157:H7	EO added to washing water	n.d.	+	(Singh et al., 2002)
	Carrots	Thyme oil	0.1–10 ml l^{-1} in rinsing solution	0.1–10	*E. coli* O157:H7	EO added to washing water	n.d.	+ to ++	(Singh et al., 2002)
	Lettuce, Iceberg green	Basil methyl chavicol (BMC)	0.1–1.0% v/v	1–10	Natural flora	BMC added to washing water	n.d.	++	(Wan et al., 1998)

	Eggplant salad	Oregano oil	0.7–2.1% v/w	7–21	*E. coli* O157:H7	Four storage temperatures and three different pHs	Yes	++	(Skandamis and Nychas, 2000)
	Alfalfa seeds	Cinnamaldehyde, thymol	200, 600 mg l^{-1} air		*Salmonella* spp., 6 serotypes	Fumigation at 50 or 70°C	n.d.	50 °C: + 70 °C: 0	(Weissinger et al., 2001)
Rice	Boiled rice	Carvacrol	0.15–0.75 mg g^{-1}	0.15–0.75	*B. cereus*		Yes—dose dependent effect	++	(Ultee et al., 2000b)
	Boiled rice	Sage oil	200–500 ppm	0.2–0.5	*B. cereus Staph. aureus S. typhimurium*		No	0	(Shelef et al., 1984)
Fruit	Kiwifruit	Carvacrol	1 mM in dipping solution	0.15 μl ml^{-1} in dipping solution	Natural flora		n.d.	+++	(Roller and Seedhar, 2002)
		Cinnamic acid	1 mM in dipping solution	0.15 μl ml^{-1} in dipping solution	Natural flora	Two storage temperatures tested	n.d.	+++	(Roller and Seedhar, 2002)
	Honeydew melon	Carvacrol	1 mM in dipping solution	0.15 μl ml^{-1} in dipping solution	Natural flora		Yes	0	(Roller and Seedhar, 2002)
		Cinnamic acid	1 mM in dipping solution	0.15 μl ml^{-1} in dipping solution	Natural flora	Two storage temperatures tested	Yes	+	(Roller and Seedhar, 2002)

In papers where the combined effect of an EO or an EO component has been studied in combination with another preservation method, only the results for EO tested alone are cited.

[a] For ease of comparison, the concentration of EO or EO component applied has been converted into μl g^{-1} or μl ml^{-1} food, whereby it was assumed that EOs have the same density as water. Where no conversion could be calculated due to the experimental method this column is left blank.

[b] Products were mostly stored under refrigeration, but temperatures used range from 2 to 30 °C.

[c] The following classification has been used: +++ Large reduction compared to control (>3 log cfu g^{-1} or ml^{-1} fewer). ++ Medium reduction compared to control (1.5–3.0 log cfu g^{-1} or ml^{-1} fewer). + Slight reduction compared to control (up to 1.5 log cfu g^{-1} or ml^{-1} fewer). 0 Negligible effect compared to control. − Slight stimulation of growth compared to control (up to 1.5 log cfu g^{-1} or ml^{-1} more). −− Medium stimulation of growth (>1.5 log cfu g^{-1} or ml^{-1} more). These classifications apply to the end point of the experiment, which varies between references from 15 min to 33 days.

[d] n.d. = not done.

although whether the effect was due to the encapsulation or the greater percentage level used was not further elucidated (Pandit and Shelef, 1994).

The activity of oregano EO against *Clostridium botulinum* spores has been studied in a vacuum packed and pasteurised minced (ground) pork product. Concentrations of up to 0.4 μl g^{-1} oregano EO were found not to significantly influence the number of spores or to delay growth. However, in the presence of low levels of sodium nitrite which delayed growth of bacteria and swelling of cans when applied alone, the same concentration of oregano EO enhanced the delay. The delay of growth was dependent on the number of inoculated spores; at 300 spores g^{-1} the reduction was greater than at 3000 spores g^{-1} (Ismaiel and Pierson, 1990).

4.2. Fish dishes

In fish, just as in meat products, a high fat content appears to reduce the effectiveness of antibacterial EOs. For example, oregano oil at 0.5 μl g^{-1} is more effective against the spoilage organism *Photobacterium phosphoreum* on cod fillets than on salmon, which is a fatty fish (Mejlholm and Dalgaard, 2002).

Oregano oil is more effective in/on fish than mint oil, even in fatty fish dishes; this was confirmed in two experiments with fish roe salad using the two EOs at the same concentration (5–20 μl g^{-1}) (Tassou et al., 1995; Koutsoumanis et al., 1999). The spreading of EO on the surface of whole fish or using EO in a coating for shrimps appears effective in inhibiting the respective natural spoilage flora (Ouattara et al., 2001; Harpaz et al., 2003).

4.3. Dairy products

Mint oil at 5–20 μl g^{-1} is effective against *S. enteritidis* in low fat yoghurt and cucumber salad (Tassou et al., 1995). Mint oil inhibits the growth of yoghurt starter culture species at 0.05–5 μl g^{-1} but cinnamon, cardamom and clove oils are much more effective (Bayoumi, 1992).

4.4. Vegetables

It appears that, in vegetable dishes just as for meat products, the antimicrobial activity of EOs is benefited by a decrease in storage temperature and/or a decrease in the pH of the food (Skandamis and Nychas, 2000). Vegetables generally have a low fat content, which may contribute to the successful results obtained with EOs.

All EOs and their components that have been tested on vegetables appear effective against the natural spoilage flora and food borne pathogens at levels of 0.1–10 μl g^{-1} in washing water (Wan et al., 1998; Singh et al., 2002). Cinnamaldehyde and thymol are effective against six *Salmonella* serotypes on alfalfa seeds when applied in hot air at 50 °C as fumigation. Increasing the temperature to 70 °C reduced the effectiveness of the treatment (Weissinger et al., 2001). This may be due to the volatility of the antibacterial compounds.

Oregano oil at 7–21 μl g^{-1} was effective at inhibiting *Escherichia coli* O157:H7 and reducing final populations in eggplant salad compared to the untreated control. Although the salad recipe appears to have a high fat content, the percentage of fat was not stated (Skandamis and Nychas, 2000).

4.5. Rice

Sage oil at 0.2–0.5 μl g^{-1} when used against *B. cereus* in rice was ineffective, whereas carvacrol at 0.15–0.75 μl g^{-1} was very effective at extending the lag phase and reducing the final population compared to a control (Shelef et al., 1984; Ultee et al., 2000b).

4.6. Fruit

Carvacrol and cinnamaldehyde were very effective at reducing the viable count of the natural flora on kiwifruit when used at 0.15 μl ml^{-1} in dipping solution, but less effective on honeydew melon. It is possible that this difference has to do with the difference in pH between the fruits; the pH of kiwifruit was 3.2–3.6 and of the melon 5.4–5.5 (Roller and Seedhar, 2002). As mentioned before, the lower the pH, the more effective EOs and their components generally are.

4.7. Food models

EOs of clove, cinnamon, bay and thyme were tested against *L. monocytogenes* and *S. enteritidis* in

soft cheese diluted 1:10 in buffer. The former species was less easily inhibited in diluted full-fat cheese than in the low-fat version, indicating the protective action of fat. The level of fat in the cheese protected the bacterial cells to a different extent depending on which oil was used; clove oil was in fact more effective against *S. enteritidis* in full-fat than in low-fat cheese slurry (Smith-Palmer et al., 2001).

In view of the published data on EOs in foods, the following approximate general ranking (in order of decreasing antibacterial activity) can be made: oregano/clove/coriander/cinnamon>thyme>mint>rosemary>mustard>cilantro/sage. An approximate general ranking of the EO components is as follows (in order of decreasing antibacterial activity): eugenol>carvacrol/cinnamic acid>basil methyl chavicol>cinnamaldehyde>citral/geraniol.

5. Mode of antibacterial action

Although the antimicrobial properties of essential oils and their components have been reviewed in the past (Koedam, 1977a,b; Shelef, 1983; Nychas, 1995), the mechanism of action has not been studied in great detail (Lambert et al., 2001). Considering the large number of different groups of chemical compounds present in EOs, it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell (Skandamis et al., 2001; Carson et al., 2002). The locations or mechanisms in the bacterial cell thought to be sites of action for EO components are indicated in Fig. 2. Not all of these mechanisms are separate targets; some are affected as a consequence of another mechanism being targeted.

An important characteristic of EOs and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable (Knobloch et al., 1986; Sikkema et al., 1994). Leakage of ions and other cell contents can then occur (Oosterhaven et al., 1995; Gustafson et al., 1998; Helander et al., 1998; Cox et al., 2000; Lambert et al., 2001; Skandamis et al., 2001; Carson et al., 2002; Ultee et al., 2002). Although a certain amount of leakage from bacterial cells may be tolerated without loss of viability, extensive loss of cell contents or the exit of critical molecules and ions will lead to death (Denyer and Hugo, 1991a). There is some evidence from studies with tea tree oil and *E. coli* that cell death may occur before lysis (Gustafson et al., 1998).

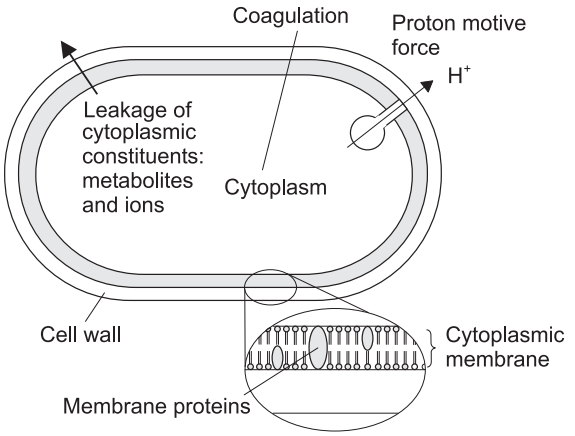

Fig. 2. Locations and mechanisms in the bacterial cell thought to be sites of action for EO components: degradation of the cell wall (Thoroski et al., 1989; Helander et al., 1998); damage to cytoplasmic membrane (Knobloch et al., 1989; Sikkema et al., 1994; Oosterhaven et al., 1995; Ultee et al., 2000a, 2002); damage to membrane proteins (Juven et al., 1994; Ultee et al., 1999); leakage of cell contents (Oosterhaven et al., 1995; Gustafson et al., 1998; Helander et al., 1998; Cox et al., 2000; Lambert et al., 2001); coagulation of cytoplasm (Gustafson et al., 1998) and depletion of the proton motive force (Ultee and Smid, 2001; Ultee et al., 1999).

Generally, the EOs possessing the strongest antibacterial properties against food borne pathogens contain a high percentage of phenolic compounds such as carvacrol, eugenol (2-methoxy-4-(2-propenyl)phenol) and thymol (Farag et al., 1989; Thoroski et al., 1989; Cosentino et al., 1999; Dorman and Deans, 2000; Juliano et al., 2000; Lambert et al., 2001). It seems reasonable that their mechanism of action would therefore be similar to other phenolics; this is generally considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force (PMF), electron flow, active transport and coagulation of cell contents (Denyer and Hugo, 1991b; Sikkema et al., 1995; Davidson, 1997).

The chemical structure of the individual EO components affects their precise mode of action and antibacterial activity (Dorman and Deans, 2000). The importance of the presence of the

hydroxyl group in phenolic compounds such as carvacrol and thymol has been confirmed (Knobloch et al., 1986; Dorman and Deans, 2000; Ultee et al., 2002). The relative position of the hydroxyl group on the phenolic ring does not appear strongly to influence the degree of antibacterial activity; the action of thymol against *B. cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* appears to be comparable to that of carvacrol, for example (Lambert et al., 2001; Ultee et al., 2002). However, in one study carvacrol and thymol were found to act differently against gram-positive and gram-negative species (Dorman and Deans, 2000). The significance of the phenolic ring itself (destabilised electrons) is demonstrated by the lack of activity of menthol compared to carvacrol (Ultee et al., 2002). In one study the addition of an acetate moiety to the molecule appeared to increase the antibacterial activity; geranyl acetate was more active against a range of gram-positive and negative species than geraniol (Dorman and Deans, 2000). As far as non-phenolic components of EOs are concerned, the type of alkyl group has been found to influence activity (alkenyl>alkyl). For example, limonene (1-methyl-4-(1-methylethenyl)-cyclohexene) is more active than *p*-cymene (Dorman and Deans, 2000).

Components of EO also appear to act on cell proteins embedded in the cytoplasmic membrane (Knobloch et al., 1989). Enzymes such as ATPases are known to be located in the cytoplasmic membrane and to be bordered by lipid molecules. Two possible mechanisms have been suggested whereby cyclic hydrocarbons could act on these. Lipophilic hydrocarbon molecules could accumulate in the lipid bilayer and distort the lipid–protein interaction; alternatively, direct interaction of the lipophilic compounds with hydrophobic parts of the protein is possible (Juven et al., 1994; Sikkema et al., 1995). Some EOs have been found to stimulate the growth of pseudomycelia (a series of cells adhering end-to-end as a result of incomplete separation of newly formed cells) in certain yeasts. This could be an indication that EOs act on the enzymes involved in the energy regulation or synthesis of structural components (Conner and Beuchat, 1984). Cinnamon oil and its components have been shown to inhibit amino acid decarboxylases in *Enterobacter aerogenes*. The mechanism of action was thought to be the binding of proteins (Wendakoon and Sakaguchi, 1995). Indications that EO components may act on proteins were also obtained from studies using milk containing different protein levels (Pol et al., 2001).

5.1. Carvacrol and thymol

The mode of action of carvacrol, one of the major components of oregano and thyme oils, appears to have received the most attention from researchers. Thymol is structurally very similar to carvacrol, having the hydroxyl group at a different location on the phenolic ring. Both substances appear to make the cell membrane permeable (Lambert et al., 2001).

Carvacrol and thymol are able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane to ATP. The presence of magnesium chloride has been shown to have no influence on this action, suggesting a mechanism other than chelation of cations in the outer membrane (Helander et al., 1998).

Studies with *B. cereus* have shown that carvacrol interacts with the cell membrane, where it dissolves in the phospholipid bilayer and is assumed to align between the fatty acid chains (Ultee et al., 2000a). This distortion of the physical structure would cause expansion and destabilisation of the membrane, increasing membrane fluidity, which in turn would increase passive permeability (Ultee et al., 2002). Measurement of the average phase transition temperature of the bacterial lipids confirmed that membranes instantaneously became more fluid in the presence of carvacrol (Ultee et al., 2000a). The passage of *B. cereus* cell metabolites across the cell membrane on exposure to carvacrol has also been investigated. Intracellular and extracellular ATP measurements revealed that the level of ATP within the cell decreased whilst there was no proportional increase outside the cell. It is therefore presumed that the rate of ATP synthesis was reduced or that the rate of ATP hydrolysis was increased. Measurements of the membrane potential ($\Delta\varphi$) of exponentially growing cells revealed a sharp decrease on the addition of carvacrol and indicated a weakening of the proton motive force. The pH gradient across the cell membrane was weakened by the presence of carvacrol

and was completely dissipated in the presence of 1 mM or more. Furthermore, intracellular levels of potassium ions dropped whilst extracellular amounts increased proportionately, the total amount remaining constant (Ultee et al., 1999). It was concluded that carvacrol forms channels through the membrane by pushing apart the fatty acid chains of the phospholipids, allowing ions to leave the cytoplasm (Ultee, 2000). Oregano EO, containing carvacrol as a major component, causes leakage of phosphate ions from *Staph. aureus* and *P. aeruginosa* (Lambert et al., 2001).

Aside from the inhibition of the growth of vegetative bacterial cells, the inhibition of toxin production is also of interest to food microbiologists. Carvacrol is able to inhibit the production of diarrhoeal toxin by *B. cereus* in broth and in soup. Two theories are offered for the mode of action of toxin limitation: If toxin excretion is an active process, there may be insufficient ATP or PMF to export it from the cell. Alternatively, the lower specific growth rate may mean that the cells use all the available energy to sustain viability, leaving little over for toxin production (Ultee and Smid, 2001).

Juven et al. (1994) examined the working of thymol against *S. typhimurium* and *Staph. aureus* and hypothesised that thymol binds to membrane proteins hydrophobically and by means of hydrogen bonding, thereby changing the permeability characteristics of the membrane. Thymol was found to be more inhibitive at pH 5.5 than 6.5. At low pH the thymol molecule would be undissociated and therefore more hydrophobic, and so may bind better to the hydrophobic areas of proteins and dissolve better in the lipid phase (Juven et al., 1994).

5.2. Eugenol

Eugenol is a major component (approximately 85%) of clove oil (Farag et al., 1989). Sub-lethal concentrations of eugenol have been found to inhibit production of amylase and proteases by *B. cereus*. Cell wall deterioration and a high degree of cell lysis were also noted (Thoroski et al., 1989). The hydroxyl group on eugenol is thought to bind to proteins, preventing enzyme action in *E. aerogenes* (Wendakoon and Sakaguchi, 1995).

5.3. p-Cymene

The biological precursor of carvacrol, *p*-cymene is hydrophobic and causes swelling of the cytoplasmic membrane to a greater extent than does carvacrol (Ultee et al., 2002). *p*-Cymene is not an effective antibacterial when used alone (Juven et al., 1994; Dorman and Deans, 2000; Juliano et al., 2000; Ultee et al., 2000a), but when combined with carvacrol, synergism has been observed against *B. cereus* in vitro and in rice (Ultee et al., 2000b). The greater efficiency of *p*-cymene at being incorporated in the lipid bilayer of *B. cereus* very likely facilitates transport of carvacrol across the cytoplasmic membrane (Ultee et al., 2002).

5.4. Carvone

When tested in a liposome model system at concentrations above the MIC, carvone (2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one) dissipated the pH gradient and membrane potential of cells. The specific growth rate of *E. coli*, *Streptococcus thermophilus* and *L. lactis* decreased with increasing concentrations of carvone, which suggests that it acts by disturbing the metabolic energy status of cells (Oosterhaven et al., 1995). In contrast, another study found that carvone was ineffective on the outer membrane of *E. coli* and *S. typhimurium* and did not affect the intracellular ATP pool (Helander et al., 1998).

5.5. Cinnamaldehyde

Although cinnamaldehyde (3-phenyl-2-propenal) is known to be inhibitive to growth of *E. coli* O157:H7 and *S. typhimurium* at similar concentrations to carvacrol and thymol, it did not disintegrate the outer membrane or deplete the intracellular ATP pool (Helander et al., 1998). The carbonyl group is thought to bind to proteins, preventing the action of amino acid decarboxylases in *E. aerogenes* (Wendakoon and Sakaguchi, 1995).

5.6. Terpinene

γ-Terpinene did not antagonize growth of *S. typhimurium* (Juven et al., 1994), whereas α-terpinene

inhibited 11 of the 25 bacterial species screened (Dorman and Deans, 2000).

6. Susceptibility of gram-negative and gram-positive organisms

Most studies investigating the action of whole EOs against food spoilage organisms and food borne pathogens agree that, generally, EOs are slightly more active against gram-positive than gram-negative bacteria (Shelef, 1983; Shelef et al., 1984; Farag et al., 1989; Mendoza-Yepes et al., 1997; Ouattara et al., 1997; Smith-Palmer et al., 1998; Marino et al., 1999, 2001; Negi et al., 1999; Juliano et al., 2000; Ruberto et al., 2000; Senatore et al., 2000; Canillac and Mourey, 2001; Demetzos and Perdetzoglou, 2001; Lambert et al., 2001; Marino et al., 2001; Cimanga et al., 2002; Delaquis et al., 2002; Pintore et al., 2002; Harpaz et al., 2003). That gram-negative organisms are less susceptible to the action of antibacterials is perhaps to be expected, since they possess an outer membrane surrounding the cell wall (Ratledge and Wilkinson, 1988), which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (Vaara, 1992). However, not all studies on EOs have concluded that gram-positives are more susceptible (Wilkinson et al., 2003). *A. hydrophila* (gram-negative) appears in fact to be one of the most sensitive species (Deans and Ritchie, 1987; Stecchini et al., 1993; Hao et al., 1998a,b; Wan et al., 1998). In one study mint (*Mentha piperita*) EO achieved a greater reduction in the viable count of *S. enteritidis* than for *L. monocytogenes* when added to the Greek appetisers taramosalata and tzatziki (Tassou et al., 1995). In another study no obvious difference between gram-positives and gram-negatives was measured in the susceptibility after 24 h, but the inhibitory effect was more often extended to 48 h with gram-negative than with gram-positive organisms (Ouattara et al., 1997). A study testing 50 commercially available EOs against 25 genera found no evidence for a difference in sensitivity between gram-negative and gram-positive organisms (Deans and Ritchie, 1987). However, a later study using the same test method and the same bacterial isolates but apparently using freshly distilled EOs, revealed that gram-positive bacteria were indeed more susceptible to two of the EOs tested and equally sensitive to four other EOs than were gram-negative species (Dorman and Deans, 2000). It was postulated that individual components of EOs exhibit different degrees of activity against gram-positives and gram-negatives (Dorman and Deans, 2000) and it is known that the chemical composition of EOs from a particular plant species can vary according to the geographical origin and harvesting period (vide supra). It is therefore possible that variation in composition between batches of EOs is sufficient to cause variability in the degree of susceptibility of gram-negative and gram-positive bacteria.

Of the gram-negative bacteria, Pseudomonads, and in particular *P. aeruginosa*, appear to be least sensitive to the action of EOs (Knobloch et al., 1986; Deans and Ritchie, 1987; Paster et al., 1990; Cosentino et al., 1999; Lis-Balchin et al., 1999; Dorman and Deans, 2000; Ruberto et al., 2000; Senatore et al., 2000; Tsigarida et al., 2000; Pintore et al., 2002; Wilkinson et al., 2003).

7. Synergism and antagonism between components of EOs

The inherent activity of an oil can be expected to relate to the chemical configuration of the components, the proportions in which they are present and to interactions between them (Dorman and Deans, 2000; Marino et al., 2001; Delaquis et al., 2002). An additive effect is observed when the combined effect is equal to the sum of the individual effects. Antagonism is observed when the effect of one or both compounds is less when they are applied together than when individually applied. Synergism is observed when the effect of the combined substances is greater than the sum of the individual effects (Davidson and Parish, 1989). Some studies have concluded that whole EOs have a greater antibacterial activity than the major components mixed (Gill et al., 2002; Mourey and Canillac, 2002), which suggests that the minor components are critical to the activity and may have a synergistic effect or potentiating influence.

The two structurally similar major components of oregano EO, carvacrol and thymol, were found to give an additive effect when tested against *Staph. aureus* and *P. aeruginosa* (Lambert et al., 2001).

As discussed above, synergism between carvacrol and its biological precursor *p*-cymene has been noted when acting on *B. cereus* vegetative cells. It appears that *p*-cymene, a very weak antibacterial, swells bacterial cell membranes to a greater extent than carvacrol does. By this mechanism *p*-cymene probably enables carvacrol to be more easily transported into the cell so that a synergistic effect is achieved when the two are used together (Ultee et al., 2000a).

Fractions of cilantro, coriander, dill and eucalyptus EOs (each containing several components), when mixed in various combinations, resulted in additive, synergistic or antagonistic effects (Delaquis et al., 2002). A mixture of cinnamaldehyde and eugenol at 250 and 500 μg ml^{-1} respectively inhibited growth of *Staphylococcus* sp., *Micrococcus* sp. *Bacillus* sp. and *Enterobacter* sp. for more than 30 days completely, whereas the substrates applied individually did not inhibit growth (Moleyar and Narasimham, 1992).

8. Synergism and antagonism between EO components and food preservatives or preservation methods

A number of potential synergists have been suggested for use with EOs: low pH, low water activity, chelators, low oxygen tension, mild heat and raised pressure, although not all of these have been researched in foodstuffs (Gould, 1996). This section will summarise studies on the combined effect of EOs or their components with the food additives sodium chloride, sodium nitrite and nisin and with preservation techniques of mild heat treatment, high hydrostatic pressure and anaerobic packaging.

Sodium chloride has been shown to work as a synergist and an antagonist under different circumstances with EOs and/or their components. Synergism between NaCl and mint oil against *S. enteritidis* and *L. monocytogenes* has been recorded in taramosalata (Tassou et al., 1995). The combined use of 2–3% NaCl and 0.5% clove powder (containing eugenol and eugenyl acetate) in mackerel muscle extract has been found to totally prevent growth and histamine production by *E. aerogenes*. The suggested mechanism for this is that eugenol increases the permeability of the cells after which NaCl inhibits growth by its action on intracellular enzymes (Wendakoon and Sakaguchi, 1993). Antagonistic effects of salt were found with carvacrol and *p*-cymene against *B. cereus* in rice: carvacrol and *p*-cymene worked synergistically, but this effect was reduced when salt was added (1.25 g l^{-1} rice) (Ultee et al., 2000b). In the same study, soy sauce was shown to exhibit synergy with carvacrol. However, this synergy was also cancelled out by the presence of salt (Ultee et al., 2000b). Salt at 4% w/v in agar did not improve the antibacterial activity of cinnamaldehyde against gram-positive and gram-negative bacteria (Moleyar and Narasimham, 1992).

Combinations of oregano EO with sodium nitrite have been examined for their effect on growth and toxin production by *C. botulinum* (a combination of types A, B and E). Oregano oil acted synergistically with nitrite to inhibit growth in broth, whereas oregano oil applied alone at up to 400 ppm had no significant inhibitive effect on growth. The proposed mechanism of synergism depends on oregano EO reducing the number of spores which germinate and sodium nitrite inhibiting the outgrowth of spores. Both substances affect vegetative growth (Ismaiel and Pierson, 1990).

The simultaneous application of nisin (0.15 μg ml^{-1}) and carvacrol or thymol (0.3 mmol l^{-1} or 45 μg ml^{-1}) caused a larger decline in viable counts for strains of *B. cereus* than was observed when the antimicrobials were individually applied. The maximum reduction of viability was achieved in cells that had experienced prior exposure to mild heat treatment at 45 °C (5 min for exponentially growing cells and 40 min for stationary phase cells) (Periago et al., 2001). Carvacrol was found not to increase the sensitivity of vegetative *B. cereus* cells to pulsed-electric-field (PEF) treatment nor did it sensitise spores to nisin or PEF (Pol and Smid, 1999). At pH 7 the synergistic action of nisin and carvacrol was significantly greater at 30 °C than at 8 °C, which would appear to indicate temperature-induced changes in the permeability of the cytoplasmic membrane (Periago and Moezelaar, 2001). The mechanism of synergy is not known. Previously, it was hypothesised that carvacrol may increase the number, size or duration of existence of the pores created by nisin in the cell membrane (Pol and Smid, 1999). Later it became clear that this was not so—the mechanism may lie in the enhanced dissipation of the membrane potential and

a reduction in the pH gradient and intracellular ATP (Pol et al., 2002).

The combined effect of carvone (5 mmol l^{-1}) and mild heat treatment (45 °C, 30 min) on exponentially growing cells of *L. monocytogenes* grown at 8 °C has been studied. Separately, the two treatments demonstrated no loss in viability but a decrease of 1.3 log units in viable cell numbers was recorded when they were combined. Cells grown at 35 or 45 °C were not susceptible to the same combined treatment. The authors hypothesised that the phospholipid composition of the cytoplasmic membrane of cells grown at 8 °C has a higher degree of unsaturation in order to maintain fluidity and function at low temperatures. This high degree of unsaturation causes the membranes of these cells to be more fluid at 45 °C than the membranes of cells grown at that temperature. This increased fluidity would enable carvone to dissolve more easily into the lipid bilayer of cells grown at 8 °C than into the bilayer of cells grown at 45 °C. Membranes of cells grown at 45 °C are less fluid because there is a 'normal' ratio of saturated to unsaturated fatty acids in their phospholipids and carvone is therefore less effective against them (Karatzas et al., 2000).

Thymol and carvacrol have been shown to have a synergistic effect with high hydrostatic pressure (HHP). The viable numbers of mid-exponential phase *L. monocytogenes* cells were reduced more by combined treatment with 300 MPa HHP and 3 mmol l^{-1} thymol or carvacrol than by the separate treatments. Since HHP is believed to cause damage to the cell membrane, it is suggested that this common target is the root of the observed synergism (Karatzas et al., 2001).

The antibacterial activity of EOs is influenced by the degree to which oxygen is available. This could be due to the fact that when little oxygen is present, fewer oxidative changes can take place in the EOs and/or that cells obtaining energy via anaerobic metabolism are more sensitive to the toxic action of EOs (Paster et al., 1990). The antibacterial activity of oregano and thyme EOs was greatly enhanced against *S. typhimurium* and *Staph. aureus* at low oxygen levels (Paster et al., 1990). The use of vacuum packing in combination with oregano EO may have a synergistic effect on the inhibition of *L. monocytogenes* and spoilage flora on beef fillets; 0.8% v/w oregano EO achieved a 2–3 $\log_{10}$ initial reduction in the microbial flora but was found to be even more effective in samples packed under vacuum in low-permeability film when compared to aerobically stored samples and samples packaged under vacuum in highly permeable film (Tsigarida et al., 2000). Similarly, the lethal effect of clove and coriander EOs on *A. hydrophila* on pork loin steak stored at 2 and 10 °C was more pronounced in vacuum packed pork than on samples stored in air (Stecchini et al., 1993). Oregano EO was found to delay microbial growth and to suppress final counts of spoilage microorganisms in minced beef under modified atmosphere packaging (MAP, 40% CO_2, 30% N_2 and 30% O_2) when, in contrast, no pronounced inhibition was evident in beef packed under air (Skandamis and Nychas, 2001).

9. Legal aspects of the use of EOs and their components in foods

A number of EO components have been registered by the European Commission for use as flavourings in foodstuffs. The flavourings registered are considered to present no risk to the health of the consumer and include amongst others carvacrol, carvone, cinnamaldehyde, citral, *p*-cymene, eugenol, limonene, menthol and thymol. Estragole and methyl eugenol were deleted from the list in 2001 due to their being genotoxic (Commission Decision of 23 January, 2002). New flavourings may only be evaluated for registration after toxicological and metabolic studies have been carried out (Commission Decision of 23 Feb., 1999; Commission Regulation (EC) No. 1565/2000; Commission Regulation (EC) No. 622/2002; Regulation (EC) No. 2232/96), which could entail a considerable financial outlay.

The EU registered flavourings listed above also appear on the 'Everything Added to Food in the US' (EAFUS) list (http://www.cfsan.fda.gov/~dms/eafus.html, date consulted: 26 February 2003), which means that the United States Food and Drug Administration (FDA) has classified the substances as generally recognised as safe (GRAS) or as approved food additives. Estragole, specifically prohibited as flavouring in the EU, is on the EAFUS list.

In other countries and if added to food for a purpose other than flavouring, these compounds may be treated as new food additives. Approval as a food additive would probably involve expensive safety and metabolic studies, the cost of which may be prohibitive. From a

legislative point of view it would in those countries be economically more feasible to use a whole spice or herb or a whole EO as an ingredient than to use individual EO components (Smid and Gorris, 1999).

10. Safety data

In spite of the fact that a considerable number of EO components are GRAS and/or approved food flavourings, some research data indicate irritation and toxicity. For example, eugenol, menthol and thymol, when applied in root canal treatments, have been known to cause irritation of mouth tissues. The results of a cytotoxicity study on these compounds suggest that gum irritation may be related to membrane lysis and surface activity and that tissue penetration may be related at least partly to membrane affinity and lipid solubility (Manabe et al., 1987). Cinnamaldehyde, carvacrol, carvone and thymol appear to have no significant or marginal effects in vivo whilst in vitro they exhibit mild to moderate toxic effects at the cellular level. Genotoxicity data appear not to raise concern in view of the present levels of use (Stammati et al., 1999).

Some EOs and their components have been known to cause allergic contact dermatitis in people who use them frequently. Preventive measures may be needed to ensure the well-being of workers if these substances were to be used on a larger scale (Carson and Riley, 2001; Bleasel et al., 2002).

Some oils used in the fields of medicine, paramedicine and aromatherapy have been shown to exhibit spasmolytic or spasmogenic properties, although these are difficult to associate with a particular component (Lis-Balchin et al., 1996; Madeira et al., 2002). Enantiomers of α-pinene have been shown to have very different spasmogenic effects (Lis-Balchin et al., 1999).

It is recommended that more safety studies be carried out before EOs are more widely used or at greater concentrations in foods that at present.

11. Organoleptic aspects

If EOs were to be more widely applied as antibacterials in foods, the organoleptic impact would be important. Foods generally associated with herbs, spices or seasonings would be the least affected by this phenomenon and information on the flavour impact of oregano EO in meat and fish supports this. The flavour of beef fillets treated with 0.8% v/w oregano oil was found to be acceptable after storage at 5 °C and cooking (Tsigarida et al., 2000). The flavour, odour and colour of minced beef containing 1% v/w oregano oil improved during storage under modified atmosphere packaging and vacuum at 5 °C and was almost undetectable after cooking (Skandamis and Nychas, 2001). Oregano oil (0.05% v/w) on cod fillets produced a ‘distinctive but pleasant’ flavour, which decreased gradually during storage at 2 °C (Mejlholm and Dalgaard, 2002). Thyme and oregano oils spread on whole Asian sea bass at 0.05% (v/v) (sic) also imparted a herbal odour, which during storage up to 33 days at 0–2 °C became more pronounced (Harpaz et al., 2003). The addition of thyme oil at up to 0.9% (v/w) in a coating for cooked shrimps had no ill effects on the flavour or appearance. However, 1.8% thyme oil in the coating significantly decreased the acceptability of the shrimps (Ouattara et al., 2001). Individual EO components, many of them being approved food flavourings, also impart a certain flavour to foods. On fish, carvacrol is said to produce a ‘warmly pungent’ aroma; citral is ‘lemon-like’ and geraniol ‘rose-like’ (Kim et al., 1995b). Treatment of fresh kiwifruit and honeydew melon with 1 mM carvacrol or cinnamic acid has been found to delay spoilage without causing adverse organoleptic changes (Roller and Seedhar, 2002).

12. Future perspectives

Arguably the most interesting area of application for EOs is the inhibition of growth and reduction in numbers of the more serious food borne pathogens such as *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes*. The delay of spoilage and improvement of organoleptic qualities in vacuum packed meat or fish may also be interesting from a commercial point of view. In view of their organoleptic properties, EOs could most readily be incorporated in manufactured foods that are traditionally associated with herbs (savoury dishes such as meat and fish dishes, cheese, vegetable dishes, soups and sauces) or with spices (drinks and desserts containing fruit and/or dairy

products). It may be possible to use EOs in foods not previously associated with a herby or spicy flavour if the presence of one or more synergists can produce the desired antibacterial effect at a concentration which does not produce undesirable changes in the flavour or aroma.

The use of EOs in consumer goods is expected to increase in the future due to the rise of 'green consumerism', which stimulates the use and development of products derived from plants (Tuley de Silva, 1996). This applies to the food and cosmetic sectors but also to medicinal products (Bassett et al., 1990). If EOs were to be required in much greater volumes than at present, bioengineering of their synthesis in plants could provide greater yields (McCaskill and Croteau, 1999; Mahmoud and Croteau, 2002). International standardisation of the composition of commercially available EOs would be essential for reliable applications (Carson and Riley, 2001).

13. Areas for future research

The action of EO components on proteins embedded in the cytoplasmic membrane and on phospholipids in the membrane is not yet fully identified and is a focal area for future research. Further elucidation of these mechanisms would provide insights that may prove useful for technological applications.

The antibacterial activity against bacterial cells in the stationary phase is a particularly appropriate subject for study (Rees et al., 1995). The extent to which bacteria can adapt to the presence of EOs in foods is also important for further evaluation; *B. cereus* has been shown to become less sensitive to carvacrol after being grown in the presence of non-lethal concentrations. The decrease in sensitivity was achieved by changing the fatty acid and phospholipid head-group composition in the membrane, which reduced fluidity and passive permeability of the cell membrane (Ultee et al., 2000a).

Interactions between EOs and their components and other food ingredients and food additives need to be investigated. Clove and oregano oils can acquire a dark pigmentation when in contact with iron (Bauer et al., 2001); this may impose limitations on their application. Synergistic effects could be exploited so as to maximise the antibacterial activity of EOs and to minimise the concentrations required to achieve a particular antibacterial effect. Antagonism between EO and food ingredients is undesirable and research is needed so it can be avoided in practical applications.

The stability of EOs during food processing will also need to be studied. The heat stability of cinnamaldehyde has been investigated; it was found to decompose to benzaldehyde at temperatures approaching 60 °C when heated alone. When combined with eugenol or cinnamon leaf oil, however, cinnamaldehyde was stable even after 30 min at 200 °C (Friedman et al., 2000).

Possible secondary or indirect consequences of the use of EOs would need to be explored: would the addition of EOs have any disadvantageous effects on the safety of the food, such as influencing the stress tolerance of pathogens? *L. monocytogenes* has been shown to become more tolerant of mild heat (56 °C) after being stressed by the presence of ethanol, hydrogen peroxide or low pH (Lou and Yousef, 1996). Could a similar phenomenon occur with EOs?

In the past there has been little standardisation of test methods for testing antibacterials for use in food. This is a field where a selection of standard methods would accelerate the study of promising antibacterial components and their synergistic or antagonistic action with each other and with food ingredients.

14. Conclusion

A number of EOs and several of their individual components exhibit antibacterial activity against food borne pathogens in vitro and, to a lesser extent, in foods. The phenolic components are most active and appear to act principally as membrane permeabilisers. Gram-positive organisms are generally more sensitive to EOs than gram-negative organisms. Undesirable organoleptic effects can be limited by careful selection of EO according to the type of food. Synergism and antagonism between components of EOs and food constituents require more study before these substances can be reliably used in commercial applications. If the active substances are to be added to foods in greater concentrations than is currently normal practice for flavourings, further safety studies may be necessary.

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