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Review

Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials

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Abstract

This review examines the potency of natural antimicrobial agents from plants, outlining the ranges of microbial susceptibility and factors affecting antimicrobial action. Methods used for estimation of inhibitory activity are evaluated and currently understood mechanisms of their action are described. The potential value of these agents as secondary preservatives is considered as well as the effectiveness and use of similar aromatic and phenolic compounds in wood smoke for the safe extension of perishable food shelf-life.
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Keywords: Natural antimicrobials; Food safety; Shelf-life; Essential oils; Plant phenolics; Smoke

Contents

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1. Introduction

Many food products are perishable by nature and require protection from spoilage during their preparation, storage and distribution to give them desired shelf-life. Because food products are now often sold in areas of the world far remote from their production sites, the need for extended safe shelf-life for these products has also expanded. Improvements in the cold distribution chain have made international trade of perishable foods possible, but refrigeration alone cannot assure the quality and safety of all perishable foods. Although the value of traditional food preservatives has been recognized, their safety has been questioned (Branen, 1983).

Most perishable food products are stored at low temperature and sometimes they are packaged under modified atmosphere (MAP) in order to extend their shelf-life. However, these steps do not eliminate undesirable microorganisms from these products. Alternative preservation techniques such as pulsed light, high pressure, pulsed electric and magnetic fields, irradiation and natural antimicrobial ingredients are being used or investigated for their application to food products (Ward et al., 1998; Calderon-Miranda et al., 1999a, b; Eliot-Godereaux et al., 2001; Fernanda San Martin et al., 2001; Mainville et al., 2001; Bendicho et al., 2002; Butz and Tauscher, 2002; Cserhalmi et al., 2002; Lado and Yousef, 2002; Spilimbergo et al., 2002; Wuytack et al., 2002).

Hurdle technology, which involves simultaneous multiple preservation approaches, has generally met with success in controlling pathogens and maintaining food quality during storage (Leistner, 2000), yet food safety issues remain. While heat treatment is the most effective hurdle used against pathogenic microorganisms, aseptic packaging is necessary, but not always practical, to maintain the effectiveness of the heat process during food storage and distribution. A wide range of food grade chemicals has been added during food manufacture to extend shelf-life by stabilizing chemical change or by preventing or inhibiting microbial growth. Traditional and natural antimicrobial agents with potential or current value for use in foods as "secondary preservatives" were recently reviewed (Branen, 1983; Naidu, 2000) and the regulatory status of many of these in the US was outlined (Davidson and Harrison, 2002).

Because of greater consumer awareness and concern regarding synthetic chemical additives, foods preserved with natural additives have become popular. This has led researchers and food processors to look for natural food additives with a broad spectrum of antimicrobial activity (Marino et al., 2001). Both plant essential oils as well as similar compounds in wood smoke have shown promise as natural antimicrobials. Some have been reported to have antimicrobial activity against a wide range of spoilage and pathogenic bacteria (Kivanc et al., 1991) and often have, in common, active phenolic groups. A promising recent development involves incorporating antimicrobials into packaging materials, rather than the food itself. This concentrates the antimicrobial at the surface of the product which is where noxious organisms grow and reduces interference from food constituents (Han, 2000, 2003). Whatever the delivery system, selection of an antimicrobial should be based on the sensory and chemical compatibility of the antimicrobial with the target food, its stability given the type of primary preservation system used, its effectiveness against the expected undesirable microorganisms, its safety and its cost effectiveness (Wanger and Moberg, 1989).

2. Antimicrobial activity of plant essential oils

Antimicrobial compounds present in foods can extend shelf-life of unprocessed or processed foods by reducing microbial growth rate or viability (Beuchat and Golden, 1989). Originally added to change or improve taste, spices and herbs can also enhance shelf-life because of their antimicrobial nature. Some of these same substances are also known to contribute to the self-defence of plants against infectious organisms (Deans and Ritchie, 1987; Kim et al., 2001).

Essential (volatile) plant oils occur in edible, medicinal and herbal plants, which minimizes questions regarding their safe use in food products. Essential oils and their constituents have been widely used as flavouring agents in foods since the earliest recorded history and it is well established that many have wide spectra of antimicrobial action (Kim et al., 1995a; Packiyasothy and Kyle, 2002; Alzoreky and Nakahara, 2002). The composition, structure as well as functional groups of the oils play an important role in determining their antimicrobial activity. Usually compounds with phenolic groups are most effective (Deans et al., 1995; Dorman and Deans, 2000). Among these, the oils of clove, oregano, rosemary, thyme, sage and vanillin have been found to be most consistently effective against microorganisms. They are generally more inhibitory against Gram-positive than against Gram-negative bacteria (Zaika, 1988; Mangena and Muyima, 1999; Marino et al., 2001). While this is true of many essential oils, there are some which are effective against both groups (oregano, clove, cinnamon and citral; Kim et al., 1995a; Sivropoulou et al., 1996; Skandamis et al., 2002). There are also some non-phenolic constituents of oils which are more effective (allyl isothiocyanate, AIT; Ward et al., 1998) or quite effective against Gram-negative bacteria (garlic oil; Yin and Cheng, 2003). In addition, AIT is also effective

against many Gram-positive fungi (Nielsen and Rios, 2000).

Plant essential oils are usually mixtures of several components. The oils with high levels of eugenol (allspice, clove bud and leaf, bay, and cinnamon leaf), cinnamamic aldehyde (cinnamon bark, cassia oil) and citral are usually strong antimicrobials (Lis-Balchin et al., 1998b; Davidson and Naidu 2000). Activity of sage and rosemary is due to borneol and other phenolics in the terpene fraction. The volatile terpenes carvacrol, *p*-cymene and thymol are probably responsible for the antimicrobial activity of oregano, thyme and savory. In sage, the terpene thejone and in rosemary a group of terpenes (borneol, camphore, 1,8 cineole, α-pinene, camphone, verbenonone and bornyl acetate) is responsible (Davidson and Naidu, 2000).

For combating infectious or parasitic agents, plants synthesize secondary metabolites which may be present constitutively (Cowan, 1999; Rauha et al., 2003) or generated from inactive precursors in response to stress (Sofos et al., 1998). Preformed substances (pro- or inhibitins) in plant tissue include phenolic compounds, flavonols, flavonoids, glycosides, alkaloids, and even polyacetylenes. Post-inhibitins are stored as inactive precursors which are activated by hydrolases or oxidases, usually in the plant tissue. Examples are onion sulphoxides and mustard glucosinolates. In onion and garlic, the precursor alliin is converted by alliinase to yield allicin (which is antimicrobial) plus pyruvate and ammonia. In mustard and horseradish, precursor glucosinolates are converted by the enzyme myrosinase to yield a variety of isothiocyanates (as well as thiocyanate, nitriles and glucose) including the allyl form which is strongly antimicrobial (Delaquis and Mazza, 1995).

Spices (woody stemmed plants) which have strong antimicrobial activity include allspice, cinnamon, clove, mustard and vanillin. Among herbs (green stemmed plants), the following are most antimicrobial: basil, oregano, rosemary, sage and thyme. Generally, it is the phenolic components in the essential oils which exhibit the antimicrobial activity (Deans et al., 1995; Kim et al., 1995a, b). Exceptions include: mustard where allyl and related isothiocyanates are responsible; and allicin in garlic and onion. These are both non-phenolic, aliphatic compounds. Phenolic compounds in olive oil (oleuropein) and tea-tree oil (terpenes), which are not classified as either spices or herbs, also show antimicrobial activity. Spices and herbs which show limited antimicrobial activity include: anise, bay (laurel), black pepper, cardamom, cayene (red pepper), celery seed, chili powder, coriander, cumin, curry powder, dill, fenugreek, ginger, juniper oil, mace, marjoram, mint, nutmeg, orris root, paprika, sesame, spearmint, tarragon, and white pepper (Davidson and Naidu, 2000).

2.1. Methods for determining antimicrobial potency

Most methods used to assess antimicrobial activity of spices, herbs and their essential oils are adaptations of those outlined by Zaika (1988), but the impedence detection and spiral gradient endpoint tests are new. Most researchers currently use agar or broth dilution series (Davidson and Naidu, 2000; Rauha et al., 2000) or both for comparative purposes because antimicrobial performance in the two systems can vary (Hammer et al., 1999). Although tube macrodilution and diffusion from inhibitor-impregnated paper discs on agar surfaces are still used (Galindo-Cuspinera et al., 2003), there is heavy reliance on microwell plate systems containing inhibitors and target organisms in broth. Inhibitor effectiveness is measured by differences in rates of change of optical density from controls and bacterial numbers in non-turbid wells are monitored by plating on agar with enumeration after incubation. However, systems based on absorbance in broth lack sensitivity below $5 \log_{10}$ bacteria ml^{-1}. This has largely been overcome by application of impedence measurement using inoculated broth. The Bactometer (Vitek Systems, bioMérieux, Marcy l'Etoile, France) is most often used to measure changes in conductivity of test broths that occur following production of bacterial metabolites. A time to detection is measured and can be used to estimate changes in the length of the lag phase well before development of turbidity occurs. These systems can also be used to distinguish between bacteriostatic and bactericidal effects (Oh and Marshall, 1992; Marino et al., 1999, 2001; Wan et al., 1998; Skandamis et al., 1999).

Solubility of many phenolic-based compounds in aqueous environments is poor. Workers have used dimethyl sulphoxide (Firouzi et al., 1998; Iscan et al., 2002; Vardar-Unlu et al., 2003), 2.5–6% ethanol (Walsh et al., 2003; Wen et al., 2003), Tween 20 or 80 and even sorbitan monolaurate (Mann and Markham, 1998) to facilitate antimicrobial dispersion in test media. While controls to monitor for possible solvent effects on bacterial viability should be included when solvents are used, there is always the possibility for interactive effects (e.g. quenching) that cannot be evaluated with a solvent-only control (Lambert et al., 2001). The hydrophobicity of phenolics limits the value of agar disc/diffusion tests for estimating antimicrobial potency accurately. Since essential oils are characterized as being volatile, methods that test the antimicrobial activity of such agents in their vapour phase have been outlined (Zaika, 1988), and modified (Weissinger et al., 2001; Ward et al., 1998). In the latter study agar discs were inoculated with test bacteria and placed on a glass slide inserted into a glass jar along with horseradish essential oil that had been placed on a watch glass. The jar was sealed and placed at the desired temperature for exposure to essential oil

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vapour. Viability of exposed cells on the agar surface was later assessed. The system worked well and allowed for quantification of the inhibitor in the vapour phase. Filter paper impregnated with AIT was used by Weissinger et al. (2001) to expose segregated alfalfa seed to vapour while both were enclosed in a glass jar.

Although agar gradient plates are no longer in common use, Razavi-Rohani and Griffiths (1994) used a solution of glycerol monolaurate (a fatty acid ester) to form a concentric antimicrobial gradient tracked on the surface of uninoculated plates with a spiral plating device (Spiral Biotech, Norwood MA). Once the gradient had equilibrated overnight, plates were surface inoculated with test organisms and the spiral gradient end point test was used to calculate minimum inhibitory concentrations (MICs, the lowest concentration at which the inoculum level is maintained or reduced). Results from this type of experiment can now be read using the manufacturer's software. This system may be applicable for less volatile and more hydrophilic natural antimicrobials. Eugenol was too hydrophobic and suitable agar gradients were not obtained with this system (Holley, unpublished). Another system which shows promise is the Bioscreen Microbiological Growth Analyser used by Lambert et al. (2001) to assess oregano essential oil and its major components, thymol and carvacrol, for activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The device allows for the simultaneous evaluation of multiple antimicrobial concentrations and calculates an MIC based on mathematical processing. Predicted fractional inhibition can also be calculated. A very useful development has been the adoption of microwell titre plates containing broth to which 0.15% (w/v) agar is added to suspend partially soluble antimicrobials in the colloidal agar matrix (Mann and Markham, 1998). This method continues to be used (Gill et al., 2002; Wen et al., 2003) and as originally described the broths contained resazurin dye as a redox indicator to give a visual signal reflecting bacteria growth. A prerequisite is that each test strain must be calibrated for its ability to reduce the resazurin dye before tests are conducted since rates may vary. The optimum test cell concentration is about one $\log_{10}$ lower than the level necessary to reduce resazurin to a pink/purple colour. Microwell titre plates are attractive for this type of study because only small reaction volumes ($\leqslant 300\,\mu l$/test) are needed, replicate tests are easily prepared using multi-channel pipettors, and multi-well plates lend themselves to adoption of protocols where two or more antimicrobials can be used simultaneously in gradients to examine reactants for interactive (synergistic or antagonistic) effects. The checker board assay typifies the approach used (Wen et al., 2003).

Walsh et al. (2003) used conventional broth dilutions monitored for changes in absorbance at 540 nm as well as MICs determined by placing 1 μl spots of bacterial culture ($6\log_{10}$ cfu ml^{-1}) on agar plates containing natural antimicrobial inhibitors (eugenol and thymol) using a multi-channel (Denley) inoculator. After incubation, tests were compared to control plates without inhibitors. This technique can be recommended for screening natural antimicrobials.

Iscan et al. (2002) used a combination of microdilution, agar diffusion and a bioautography bioassay to examine mint essential oil for activity against plant and human pathogens. For the bioassay, samples were subjected to thin layer chromatography (TLC) on parallel plates. One plate was chemically developed and the other was coated in agar, inoculated with the test organism and incubated at 37 °C for 24 h. The latter plate was sprayed with 1% tetrazolium violet, incubated 1 h at 37 °C and inhibition zones visualized against the coloured background.

In another modified agar method, Bagamboula et al. (2003) added dried, course ground herbal and spice plant material directly to agar which was then solidified, surface inoculated with a single organism and incubated. This method is also useful for screening agents. However, there is concern that essential oils may only be partially released during grinding, leaving some trapped in cell compartments and unavailable for reaction with target organisms.

There is clearly still a need for the adoption of standardized protocols for the evaluation of natural antimicrobials during in vitro tests to avoid the generation of contradictory results (Cowan, 1999). However, it is unlikely that a single standard method will have general appeal. This is because studies often have different purposes and objectives, and frequently employ different experimental designs. These translate into different needs with respect to incubation temperature (4–37 °C), pH (4–8) and length of exposure (hours or weeks). There are a host of other factors, which influence experimental outcomes during potency testing of natural antimicrobials. These include: variability in composition or content of active agents that result from agronomic history, varietal differences, and maturity of the plant material studied (Zaika, 1988; Gill et al., 2002); physical and chemical characteristics of the antimicrobial itself (hydrophobicity, volatility, compatibility in the test system); presence of protein, starch or lipid that may complex with and neutralize antimicrobial activity or partition the agent away from its target (Hao et al., 1998a; Davidson and Naidu, 2000); and the inoculum size, genus of microorganism, species and even strain susceptibility as well as previous culture history (Gill et al., 2002; Bagamboula et al., 2003; Wen et al., 2003). Differences in sensitivity of target cells have been shown to result from differences in the physiological state of tested cultures with stationary phase cells sometimes being more resistant (Karatazas et al., 1999). It is probable that prior stress of cells by exposure to

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unfavourable conditions may change susceptibility of cells to natural antimicrobials, but this is an area still to be investigated (Gill et al., 2002). Incomplete control over the factors identified above will lead to generation of inconsistent results. Perhaps the greatest source of variation in study results arises from the use of unstandardized natural antimicrobials of different potency and composition.

2.2. Antibacterial activity

Plant essential oils have been shown to have activity against *Aeromonas hydrophila*, *Listeria monocytogenes*, *Clostridium botulinum*, *Enterococcus faecalis*, *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., *Enterobacteriaceae*, *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Pseudomonas fluorescens, Bacillus cereus*, *Shigella* spp., *Salmonella enterica* Typhimurium and Enteritidis, and *Escherichia coli* as well as yeasts and moulds (*Saccharomyces cervisiae*, *Aspergillus flavus*, *Aspergillus parasiticus*) (Karapinar and Aktug, 1987; Beuchat and Golden, 1989; Moleyar and Narasimham, 1992; Hao et al., 1998a, b; Smith-Palmer et al., 1998; Marino et al., 2001; Bagamboula et al., 2003).

Little information is available on interaction among constituents in essential oils and the effects they have on antimicrobial activity. Phenolic components are responsible for antimicrobial action and other constituents are believed to have little activity. Dependability of essential oils as antimicrobials could be improved if their content of active agents should be standardized by distillation (Delaquis et al., 2002). As a general observation, spice extracts are less antimicrobial than the whole spice but little quantitative data are available (Shelef, 1983). Four studies are relevant. With oregano, Charai et al. (1996) found whole (ground) plant material had greater antimicrobial activity than essential oil at the same concentration. Lachowicz et al. (1998) found crude essential oil of basil more effective than components linalool and methyl chavicol either separately or together. Vardar-Unlu et al. (2003) found similar results following fractionation of extracts from thyme. In aqueous extracts from oregano or thyme there was little antimicrobial activity. Thus there appear to be interactive effects among constituents not extractable in the water-soluble phase and these components do not appear to be the phenolics normally considered to show the major antimicrobial activities. In contrast with the three studies above, Delaquis et al. (2002) found that individual fractions of cilantro and dill essential oils had greater antimicrobial activity than did the whole oil. In addition, they found cilantro fractions deficient in phenolics but enriched in long chain (C_6–C_{10}) alcohol and aldehydes that were particularly active against Gram-positive bacteria including *L. monocytogenes*. To broaden the antimicrobial spectrum, a fraction from cilantro oil with no activity against Gram-negative bacteria was combined with a eucalyptus fraction having broader activity. Additive or synergistic action was reported against all Gram-positive bacteria plus *Yersinia enterocolitica* and the mixture was antagonistic to *P. fragi*, *E. coli* O157:H7 and *Salmonella* Typhimurium.

A large number of studies have examined the in vitro antimicrobial activity of spices, herbs and naturally occurring compounds from other sources. The reader is referred to recent reports to understand the scope and variety of studies completed (Shelef, 1983; Lis-Balchin et al., 1998b; Smith-Palmer et al., 1998; Hammer et al., 1999; Davidson and Naidu, 2000; Naidu, 2000).

Plant essential oils have been widely tested against both Gram-positive and -negative bacteria. For example, Farag et al. (1989) examined the antimicrobial activity of the oils of sage, thyme and rosemary leaves, caraway fruits, clove flower buds, and cumin fruits against three Gram-negative bacteria (*P. fluorescens*, *E. coli*, and *Serratia marcescens*) and four Gram-positive bacteria (*S. aureus*, *Micrococcus* spp., *Sarcina* spp., and *B. subtilis*). They found that the essential oils from sage, cumin, rosemary and their principal components had no or very little effect against Gram-negative bacteria, but oil of caraway was moderately effective against this group. Oils from clove and thyme were highly active at a concentration of 0.75–1.5 mg ml^{-1} against *S. aureus* and *Micrococcus* spp., while only small inhibition zones were reported for Gram-negative bacteria. In general, Gram-negative bacteria were more resistant to essential oils than Gram-positive bacteria, with the oils being effective even at low concentration (0.25–12 mg ml^{-1}) against the Gram-positive organisms. In similar work it was also found that mint essential oil was more effective against Gram-positive bacteria than against Gram-negative bacteria (Sivropoulou et al., 1995; Iscan et al., 2002). Delaquis et al. (2002) also reported that Gram-positive bacteria were more sensitive to the essential oils of dill, cilantro, coriander and eucalyptus than Gram-negative bacteria.

Recognition of new foodborne pathogens has changed our understanding of the epidemiology of foodborne diseases in the last two decades. Bacterial pathogens which have gained prominence over the last 20 years include *Salmonella* Enteritidis, *Campylobacter* spp., *E. coli* O157:H7 and related verocytoxigenic (VTEC) spp. The psychrotrophs *L. monocytogenes* and *Y. enterocolitica* are known to be transmitted by refrigerated foods with extended shelf-life. Nonetheless, *S.* Typhimurium, *Clostridium perfringens* and *S. aureus* remain threats to the safety of the food supply (Meng and Doyle, 1998). *Campylobacter* spp. are responsible for the greatest number of cases of gastrointestinal illness in humans from all sources including food. Of 15 recognized *Campylobacter* species, 12 have

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been associated with human disease. However, *C. jejuni* is most frequently associated with foodborne illness. The unusual conditions required for the growth of *Campylobacter* place limitations on the range of foods in which they can survive or grow. *Campylobacter* spp. are microaerophilic, do not grow at $<30\,^{\circ}C$ and are sensitive to both drying and $\geqslant 2\%$ NaCl. Infections caused by *Campylobacter* in humans are considered to be the result of ingestion of contaminated foods of animal origin, mainly poultry products and raw milk, or untreated water (Moore et al., 2002; Park, 2002). Successful steps to reduce the occurrence of *Campylobacter* on poultry could have a major effect on reduction of foodborne illness. In a recent study, a proprietary mixture of herbs (Protecta II) at 2% (w/v) was used in poultry chill water and reduced the numbers of both *Campylobacter* and *E. coli* by $2\log_{10}$ cfu ml^{-1} in carcass rinses (Dickens et al., 2000). Friedman et al. (2002) evaluated 96 different naturally occurring plant oils and oil compounds against *C. jejuni* in iron-supplemented brucella agar. The oils of marigold taegetes, ginger root, jasmine, patchouli, and gardenia were most effective with bactericidal activity (BA) assessed as BA50's (concentration of oil at which a 50% reduction of total cfu was observed) ranging from 0.003% to 0.007%. Like plant essential oils and oil-derived compounds, garlic-derived organosulphur compounds have also shown antimicrobial activity. When evaluated against *C. jejuni* in ground beef, diallyl sulphide and diallyl disulphide at 20 μM showed a significant reduction with final viable numbers of 1.63 log cfu g^{-1} and 1.26 log cfu g^{-1}, respectively, compared to 7.54 log cfu g^{-1} in untreated controls during 6 d storage at 15 °C (Yin and Cheng, 2003).

Recently there has been significant interest in the development of secondary preservation steps that could reduce *L. monocytogenes* viability and growth in refrigerated ready-to-eat foods (Rocourt et al., 2003). Four recent studies examined the effects of different natural antimicrobials on this organism in broth media. Of the agents tested isoeugenol was most effective, giving a $4.6\log_{10}$ reduction of *L. monocytogenes* numbers at 100 ppm in conjunction with use of freeze thaw cycles at −20 °C (Cressy et al., 2003). Cilantro oil was more effective than hydroxycinnamic acids, with MICs of cilantro against *L. monocytogenes* of 0.02–0.07% (v/v) in BHI broth at 24 °C (Gill et al., 2002) as compared with MICs of 0.2–0.27% (w/v) for 4 hydroxycinnamic acids (Wen et al., 2003). Annatto (a GRAS carotenoid pigment used in butter and cheese), was least effective (MIC 1.25% v/v) against this organism (Galindo-Cuspinera et al., 2003). Differences in strain susceptibility were evident and cilantro oil was ineffective against *L. monocytogenes* when used on the surface of inoculated ham at a concentration of 6% (v/v) of the enrobing gel (Gill et al., 2002).

It is apparent that the generally greater resistance of Gram-negative bacteria to essential oils (Moleyar and Narasimham, 1992; Wan et al., 1998; Davidson and Naidu, 2000; Lambert et al., 2001; Walsh et al., 2003) is likely to be due in part to the greater complexity of the double membrane-containing cell envelope of these organisms in contrast with the single membrane-glycoprotein/ techoic acid, or membrane-glycoprotein/ β-glucan-based structures of Gram-positive bacteria and yeast, respectively. Sterols present in membranes of yeasts and fungi but absent from prokaryotic cells do not confer resistance against these antimicrobials (Vardar-Unlu et al., 2003). Resistance also seems to be related to the rate and extent of antimicrobial dissolution or ability to partition in the lipid phase of the membrane as previously discussed (Lambert et al., 2001), although this is not the complete explanation. In attempting to explain differences in the sensitivity of Gram-positive and -negative cells, differences in cell surface hydrophobicity have been suggested as contributing factors (Helander et al., 1998; Chao et al., 2000). It is notable that glycerol monolaurate (a lipophilic surface active agent) enhanced the inhibitory effects of eugenol against *E. coli* O157:H7 (Blaszyk and Holley, 1998). However, it is more likely that the effects of differences in hydrophobicity between these two bacterial groups (Rozgonyi et al., 1985; Zita and Hermansson, 1997; Annuk et al., 1999), with Gram-negative cells having more hydrophobic surfaces (Gotoh et al., 1989) can be offset by the presence of porin proteins in the outer membrane of Gram-negative cells. These can create channels large enough to allow restricted passage of small molecular mass compounds ($<200\,M_w$), like the substituted phenolics in essential oils, allowing their access to the periplasmic space, the glycoprotein layer and the cytoplasmic membrane. In addition, the good solubility of lipopolysaccharide in phenols and its routine isolation from bacterial cell walls using phenolic-based solvents (Helander et al., 1998) suggest that the differences in susceptibility of the two groups may result from the interaction of a number of factors including differences at the cytoplasmic membrane/glycoprotein interface as well as greater physicochemical complexity of the Gram-negative cell wall. Enhancement of essential oil antimicrobial action against both types of bacteria by ethylene diamine tetra acetic acid (EDTA) suggests a role for metal cations in resistance (Naidu, 2000; Walsh et al., 2003).

While it is possible that structural differences in cell envelopes may contribute to resistance or sensitivity to essential oils, metabolic differences may also be important. Among the generally sensitive Gram-positive bacteria, the lactic acid bacteria (LAB) are the most resistant (Shelef, 1983; Blaszyk and Holley, 1998). Their ability to generate ATP by substrate level phosphorylation (oxidative phosphorylation was inhibited in *E. coli*

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by tea tree terpenoids, Cox et al., 2000) may contribute to this resistance. Differences in internal levels of ATP in *L. monocytogenes* and *Lactobacillus sakei* after challenge by eugenol or cinnamaldehyde support this explanation (Gill and Holley, 2003). It is also possible that the greater resistance of the LAB is related to their better ability to deal with conditions of osmotic stress and respond more effectively to K^+ efflux caused by many of these antimicrobials. This will be discussed in the next section.

Among the Gram-negative bacteria examined, pseudomonads consistently show high or often the highest resistance to these antimicrobials (e.g. pseudomonads and linalool/chavicol, Smith-Palmer et al. (1998); *P. aeruginosa* and terpenoids/carvacrol/thymol, Griffin et al. (1999); pseudomonads and oregano, Skandamis et al. (2002); *P. aeruginosa* and *Capsicum* or bell pepper, Careaga et al. (2003); *P. fluorescens* and annatto, Galindo-Cuspinera et al. (2003)). Nonetheless, since pseudomonads are so frequently responsible for spoilage of food stored at low temperatures they have often been used as targets, and at high concentrations some essential oil components have been reported to be effective (Careaga et al., 2003).

In contrast with phenolic-based essential oils, the main component of horse radish/mustard essential oil, AIT, shows strong activity against *Salmonella* and *E. coli* O157:H7 (Delaquis and Mazza, 1995; Ward et al., 1998), but has little activity against LAB. We have used this difference to inhibit *E. coli* O157:H7 in inoculated hamburger stored under nitrogen at 4 °C without affecting development of the normally dominant lactic acid microflora (Muthukumarasamy et al., 2003).

2.3. Mechanism(s) of antimicrobial action

As mentioned above plant oils and extracts, primarily from clove, oregano, rosemary, thyme, tea tree (*Melaceuca alternafolia* from Australia) and sage have shown significant inhibitory activity, while less potent activity was shown by other plant materials. Expression of antimicrobial activity is often very clear, but the mechanism of antimicrobial action is incompletely understood. One of the more dramatic effects of inhibitory action appears in two separate reports where the outer of the two cell membranes of *E. coli* and *S.* Typhimurium disintegrated following exposure to carvacrol and thymol (Helander et al., 1998). Similar observations were made by Lucchini et al. (1990) with these agents using a different strain of *E. coli* and *P. aeruginosa*. Yeast and Gram-positive bacteria showed no such changes in cell wall morphology. This was probably due to the solubility of lipopolysaccharides (LPS) in the outer membrane in phenolic-based solvents.

There is overwhelming consensus that aromatic and phenolic compounds exert their antimicrobial effects at the cytoplasmic membrane by altering its structure and function (Sikkema et al., 1995). Efflux of K^+ is usually an early sign of damage (Walsh et al., 2003) and is often followed by efflux of cytoplasmic constituents (Cowan, 1999; Griffin et al., 1999; Ultee et al., 2002; Davidson and Naidu, 2000; Cox et al., 2000; Lambert et al., 2001) including ATP (Brul and Coote, 1999). The loss of the differential permeability character of the cytoplasmic membrane is frequently identified as the cause of cell death. Some workers have explored this further, reasoning that loss of membrane function is only part of the explanation for antimicrobial activity (Walsh et al., 2003). Other events which could lead to membrane dysfunction and subsequent disruption include dissipation of the two components of the proton motive force in cells (the pH gradient and the electrical potential) either by changes in ion transport or depolarization through structural changes in the membrane; interference with the energy (ATP) generation system in the cell; or enzyme inhibition preventing substrate utilization for energy production (Helander et al., 1998; Lambert et al., 2001; Ultee et al., 2002). In addition, Cox et al. (2000) showed that tea tree oil which contains terpinen-4-ol (a cyclic monoterpene believed primarily responsible for the antimicrobial activity) inhibited oxidative respiration in *E. coli*, *S. aureus* and a yeast at the MIC; and also induced membrane swelling and increased membrane permeability.

Certainly, the ability of phenolics to interfere with cellular metabolism through a number of mechanims (substrate complexing, membrane disruption, enzyme inactivation and metal chelation) is well known (Cowan, 1999). It is also evident that their ability to preferentially partition from water to membrane structures and penetrate the membrane are important factors which have a bearing on the sensitivity or resistance of exposed cells (Helander et al., 1998; Griffin et al., 1999; Cox et al., 2000; Lambert et al., 2001).

Fluorescent probes have been used to study membrane changes. Propidium iodide (Cox et al., 2000), the nucleic acid dye ethidium bromide (Lambert et al., 2001) as well as the self-quenching probe rhodamine *B* (Ultee et al., 2002) were used to monitor membrane integrity and uptake of essential oil components. An acetate-succinyl ester of carboxy fluorescein was used by Lambert et al. (2001) to follow changes in cytoplasmic pH following carvacrol challenge.

Work with fluorescent probes showed that essential oils increased membrane permeability and that oil components actually dissolved in the membranes causing swelling and reduced membrane function. Although important, the extent of dilution in the membrane of the antimicrobial was unrelated to their overall antimicrobial activity in the case of carvacrol and its precursor *p*-cymene (Ultee et al., 2002). In this study, Ultee et al. (2002) concluded that to be effective against vegetative

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cells of *B. cereus* antimicrobials should have both a hydroxyl group on the phenolic ring as well as a system of delocalized electrons (i.e. presence of α–β double bonds) to elicit strong antimicrobial activity. Ultee et al. (2002) developed a model for carvacrol inhibition of bacterial cells where it acted as a protonphore uncoupler, facilitating K^+ efflux and destruction of the internal pH gradient. With the loss of the gradient, ATP levels were depleted and this led to cell death.

The results of our own experiments (Gill and Holley, 2003) with eugenol and cinnamaldehyde against *L. monocytogenes* and *Lb. sakei* are not consistent with a protonphore uncoupler mechanism. Eugenol, like carvacrol, is a substituted phenolic compound and cinnamaldehyde is a substituted aromatic compound. Treatment of un-energized cells of *L. monocytogenes* with bactericidal concentrations of eugenol (5 mM) or cinnamaldehyde (40 mM) prevented cellular ATP pools from increasing following addition of glucose. The treatment of energized *L. monocytogenes* cells with eugenol had no significant effect on cellular ATP pools, but the cellular ATP of cinnamaldehyde treated cells was rapidly depleted. The ATP pools of *L. monocytogenes* cells treated with 10 μM of the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) responded identically to cells treated with cinnamaldehdye, not like eugenol treated cells. *Lb. sakei* responded to bactericidal eugenol treatment (10 mM) like *L. monocytogenes*, but was unresponsive to $\leqslant$0.4 M cinnamaldehyde or 100 μM CCCP.

If eugenol functioned as a protonophore it would affect *L. monocytogenes* like CCCP. Protonophores rapidly deplete the cellular ATP of *L. monocytogenes* as the cell makes a futile attempt to reestablish the membrane proton motive force by exporting hydrogen ions with the F_1F_0 ATPase (Shabala et al., 2002). Though cinnamaldehyde does behave like CCCP, it cannot function as a protonophore as it does not possess a hydroxyl or acid group to act as a proton carrier. Since measurements of extracellular ATP were inconclusive, our results (Gill and Holley, 2003) would be consistent with either membrane disruption by cinnamaldehyde or inhibition of either glucose uptake or utilization by both compounds.

These differences in response to eugenol and cinnamaldehyde by *L. monocytogenes* and *Lb. sakei* may be due in part to differences in the solubility or permeability of the agents in the cell membrane. It is conceivable that small differences in antimicrobial concentration internally may determine the biochemical event that dominates to inhibit cell growth or cause death. It is clear that the bactericidal response of *Lb. sakei* and *L. monocytogenes* to eugenol and cinnamaldehyde involves energy generation by the cells.

In other relevant work on the mode of action of natural antimicrobials Delaquis et al. (2002) found, in a study of fractionated hydroxycinnamic acids used against *L. monocytogenes*, that the presence of hydroxyl groups on long chain alcohols was correlated with inhibitory activity. This supports observations reported by Helander et al. (1998). Fitzgerald et al. (2003) in other work with yeast found that the antimicrobial functionality of vanillin was due to its aldehyde group. In their study of the mode of action of cinnamaldehyde against *Enterobacter aerogenes*, Wendakoon and Sakaguchi (1993) concluded that the antimicrobial inactivated decarboxylase enzymes in the cell.

The non-phenolic isothiocyanates are also potent antimicrobials and have activity against a wide range of microorganisms. Their antimicrobial activity is believed to be due to the inactivation of extracellular enzymes through cleavage of disulfide bonds (Delaquis and Mazza, 1995).

2.4. Activity in food systems

The modulating influence of food composition upon the antimicrobial effectiveness of essential oils is an important area of study. Shelef (1983) noted that while much of the early in vitro work with essential oils and their components showed they had substantial activity, when used in food systems amounts required were high (1–3%) and these levels were often higher than would normally be organoleptically acceptable (Lis-Balchin et al., 1998a). The presence of fat, carbohydrate, protein, salt and pH reaction influence the effectiveness of these agents in foods. Their antimicrobial potency is also reduced in foods with lower water activity. Some examples from in vitro work also show these effects are varied. While bovine serum albumin (BSA) neutralized the antimicrobial activity of thymol, oil of clove and tea tree oil were not substantially affected by organic matter or BSA, respectively (Davidson and Naidu, 2000). In a food system, Gill et al. (2002) found cilantro oil at 6% with glycerol monolaurate or lecithin in a gelatin gel coating on ham became ineffective against *L. monocytogenes* even though cilantro showed considerable antilisterial action in broth. There are many examples of this type of antimicrobial inactivation in the literature, some of which are outlined below, but there are also two examples where spice/herbal materials have been successfully used as either a dip on poultry carcasses (Dickens et al., 2000) or as a surface coating on salt water fish (Asian sea bass; Harpaz et al., 2003) to extend shelf-life. Instances where these antimicrobials have been used in foods and the levels at which they were effective are shown in Table 1.

Smith-Palmer et al. (1998) reported that the oils of clove, cinnamon and thyme were effective against *L. monocytogenes* and *S.* Enteritidis in tryptone soya broth (TSB). The oils had MICs of 0.04%, 0.075% and 0.03%, respectively, against *L. monocytogenes*.

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Table 1
Effectiveness of natural antimicrobials from plants when used in foods; (a) bactericidal, (b) bacteriostatic, (c) little or no effect

Antimicrobial agent	Concentration	Food[a]	Organism	Effect	Reference
Allyl isothiocyanate (AIT)	20 μg ml^{-1}	Roast beef	*E. coli* O157:H7	a	Ward et al. (1998)
			S. Typhimurium	a	
			L. monocytogenes	a	
			S. aureus	a	
AIT	0.1% (w/w)	Acidified chicken meat	*Lb. alimentarius*	a	Lemay et al. (2002)
			E. coli	a	
AIT	1 μl	Rye bread	*A. flavus*	a	Neilsen and Rios (2000)
			P. commune	a	
			P. corylophilum	a	
			P. discolor	a	
			P. polonicum	a	
			P. roqueforti	a	
			Endomyces fibulige	a	
AIT	1300 ppm	Ground beef	*E. coli* O157:H7	a	Muthukumarasamy et al. (2003)
Basil	1% (w/v)	Spagetti sauce	*Shigella* spp.	b,c	Bagamboula et al. (2003)
Basil oil	1%	Tomato juice	*Lb. curvatus*	a	Lachowicz et al. (1998)
			S. cerevisiae	a	
Capsicum (bell pepper)	<3 ml/100 g	Minced beef	*Salmonella*	a	Careaga et al. (2003)
			P. aeruginosa	a	
Carvacrol	3%	Fish cubes	*S.* Typhimurium	a	Kim et al. (1995b)
Cilantro oil	6% in film	Ham	*L. monocytogenes*	a	Gill et al. (2002)
Cinnamaldehyde	0.5%	Dried beef	Inoculated Gram-positive, natural Gram-negative microflora	a	Kim et al. (2001)
		Cream puff			
Cinnamaldehyde	1% (w/w) film	Cured meat	*Enterobacteriaceae*	a	Ouattara et al. (2000)
Cinnamon oil	1% (v/v)	Full fat cheese	*L. monocytogenes*	a	Smith-Palmer et al. (2001)
Cinnamon powder	0.3% (w/v)	Apple juice	*L. monocytogenes*	a	Yuste and Fung (2002)
Cloves	1% (w/v)	Beef	*L. monocytogenes*	c	Ting and Deibel (1992)
Clove (eugenol)	2%	Cooked chicken	*A. hydrophila*	a	Hao et al. (1998b)
			L. monocytogenes	a	
Clove oil	500 μg ml^{-1}	Cooked pork	*A. hydrophila*	b	Stecchini et al. (1993)
Clove oil	0.5% + 2% NaCl	Mackerel broth	*E. aerogenes*	b	Wendakoon and Sakaguchi (1993)
Clove oil	1% (v/v)	Low-fat cheese	*L. monocytogenes*	a	Smith-Palmer et al. (2001)
Diallyl sulfide	20 μM	Ground beef	*C. jejuni*	a	Yin and Cheng (2003)
Diallyl disulfide	20 μM	Ground beef	*C. jejuni*	a	Yin and Cheng (2003)
Eugenol	2%	Cooked beef	*A. hydrophila*	a, b	Hao et al. (1998a)
			L. monocytogenes	a, b	
Garlic	4%	Sausages	Natural microflora	b, c	El-Khateib and Abd El-Rahman (1987)
		Beef hamburger			
Horseradish oil	20 μl l^{-1}	Cooked roast beef	*L. monocytogenes*	a	Ward et al. (1998)
			S. aureus	a	
			E. coli O157:H7	a	
			S. grimsii	a	
Methyl chavicol (basil)	0.1%	Lettuce extract	*A. hydrophila*	a	Wan et al. (1998)
Mint oil	2% (v/w)	Tzatziki[b]	*S.* Enteritidis	a	Tassou et al. (1995)
			L. monocytogenes	a	
Oregano	0.05% (v/v)	Whole fish	Natural microflora	b	Harpaz et al. (2003)
Oregano	1%	Beef	*L. monocytogenes*	c	Ting and Deibel (1992)
Oregano	≤0.1%	Mayonnaise	*E. coli* O157:H7	c	Skandamis et al. (1999)
Oregano oil	0.8% (v/w)	Beef fillet	*S.* Typhimurium	a	Skandamis et al. (2002)
Protecta II (herbal mix)	2%	Chicken broilers	Natural microflora	b	Dickens et al. (2000)
Pimento leaf	2%	Cooked beef	*A. hydrophila*	a, b	Hao et al. (1998a)
			L. monocytogenes	a, b	

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Table 1 (*continued*)

Antimicrobial agent	Concentration	Food[a]	Organism	Effect	Reference
Rosemary (α-pinene)	0.5% ground rosemary or 1% rosemary oil	Fresh pork sausage	*L. monocytogenes*	b	Pandit and Shelef (1994)
Sage	⩽2.5% (w/w) ground	Baby food (noodles, beef), boiled rice	*S.* Typhimurium	b	Shelef (1983)
			S. aureus	b	
			B. cereus	b	
Thyme	0.05% (v/v)	Whole fish	Natural microflora	b	Harpaz et al. (2003)
Thyme	1% (w/v)	Spaghetti sausage	*Shigella* spp.	b, c	Bagamboula et al. (2003)
Thyme	2%	Cooked beef	*A. hydrophila*	c	Hao et al. (1998a)
			L. monocytogenes	c	
Vanillin	3 mg ml^{-1}	Strawberry puree	Natural microflora, yeasts	b b	Cerrutti and Alzamora (1996)
Wasabi	60 mg ml^{-1}	Fatty tuna meat suspension	*V. parahaemolyticus*	a	Hasegawa et al. (1999)

[a]See Shelef (1983) for early work with foods.
[b]Product containing stirred yoghurt, cucumbers, garlic, clove oil and salt.

Similarly, concentrations of 0.075%, 0.1%, and 0.04% were required to inhibit the growth of *S.* Enteritidis in TSB. On the other hand, when Smith-Palmer et al. (2001) evaluated clove, cinnamon, thyme and bay oil for their activity against *L. monocytogenes* and *S.* Enteritidis in both low (16%) and high fat (30%) cheese it was observed that the oils of clove and cinnamon were highly effective against *L. monocytogenes*. However, a 1% concentration of the oils was required to inhibit *L. monocytogenes* and reduce its number to $<1.0\log_{10}$ cfu ml^{-1} within 3 d in low fat cheese. Clove oil at 1% was the only oil able to reduce viable numbers to $<1.0\log_{10}$ cfu ml^{-1} in high-fat cheese. Generally they found that *L. monocytogenes* was more rapidly inhibited in low fat cheese than in high fat cheese. At 0.5%, all oils gave initial inhibition (ranging from $<1.0\log_{10}$ cfu ml^{-1} for clove oil to $2.3\log_{10}$ cfu ml^{-1} for bay oil) of *S.* Enteritidis, but this was followed by recovery of the bacteria during the subsequent storage period. However, at 1%, all oils were able to completely inhibit *S.* Enteritidis, reducing numbers to below the detection limit in both low fat as well as in full fat cheese. The need for higher concentrations of oils in foods than in laboratory media to achieve inhibition may be related to the more complex nature of food. All the plant essential oils tested were more effective in low than in high fat cheese. This may have resulted from the fat in the product providing a protective layer around the bacteria, or the lipid fraction may have absorbed the antimicrobial agent and thus decreased its concentration and effectiveness in the aqueous phase. Even though the oils did not eliminate large numbers of *L. monocytogenes*, Smith-Palmer et al. (2001) concluded that plant essential oils could be used as natural antimicrobial agents in dairy products, since they prevented growth and reduced viability of *L. monocytogenes* and *S.* Enteritidis in both low fat as well as full fat cheese.

Minimally processed fruits and vegetables in MAP packaging are becoming popular in the marketplace. Products are usually held at refrigerator temperature during their manufacture, storage and distribution. However, this provides opportunity for growth of psychrotolerant pathogens and spoilage bacteria. Essential oil components have been used in vegetable-based food systems to explore their value as secondary preservatives (Shelef, 1983). In a recent study Wan et al. (1998) evaluated the antimicrobial activity of basil sweet linalool (BSL) and basil methyl chavicol (BMC) oil against a wide range of bacteria, yeasts and moulds in filter sterilized lettuce supernatant, with emphasis on inhibition of *A. hydrophila* and *P. fluorescens*. *A. hydrophila* is a psychrotroph and potential pathogen, which may occur in minimally processed foods at 4–6 $\log_{10}$ cfu g^{-1} and may produce cytotoxins. *P. fluorescens* is a psychrotrophic spoilage bacterium which can reduce the shelf-life of refrigerated fruits and vegetables. Both BMC and BSL were shown to have inhibitory effects against Gram-positive and Gram-negative bacteria, yeasts and moulds. While the growth of *A. hydrophila* was delayed by 0.063% (v/v) BMC, at 0.125% (v/v) this oil completely inhibited growth of the organism. BSL was less effective as an antimicrobial than BMC and had an MIC of 1% (v/v) against *A. hydrophila*. The MIC of BMC against *P. fluorescens* was 2% (v/v), while that of BSL was >2%. Thus, *P. fluorescens* was again substantially more resistant to these agents.

Lachowicz et al. (1998) examined the inhibitory action of basil oil against the acid-tolerant food microflora. When foods are packaged in MAPs or vacuum, lactobacilli, *Leuconostoc* species or yeasts and

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moulds are often the major cause of spoilage. Commercial basil oil and oils prepared from four other varieties of basil (Anise, Bush, Cinnamon and Dark Opal) were used by the investigators. Anise basil oil (containing 44% linalool and 27% methyl chavicol) was tested against *Lactobacillus curvatus* and *S. cerevisiae* which can grow at low pH and spoil tomato-based foods. Growth of these two organisms at pH 4.2 was determined using an indirect impedance method. The addition of 0.1% anise oil increased the time for detection of growth (TDG) by about 51 h, while at the 1% level the growth of both organisms was completely inhibited over a 99 h test period. The antimicrobial activity of anise oil was then evaluated against these two organisms in tomato juice at 15 °C. At 0.1% (v/v) anise oil, *Lb. curvatus* was reduced from $4\log_{10}\text{cfu ml}^{-1}$ to $<1\text{cfu ml}^{-1}$ at the end of first week and the inhibition was maintained during 4 weeks of further incubation. At the same concentration, anise oil was able to inhibit *S. cerevisiae* for only one week; by the second and third weeks the numbers of the yeast increased to 6 and $7\log_{10}\text{cfu ml}^{-1}$, respectively. At 1% (v/v) oil, both organisms were completely inhibited and there was no growth of either organism during subsequent incubation.

Allyl isothiocyanate (AIT), a major antimicrobial component in mustard and horseradish oil, has been used in a number of foods against a variety of organisms. It has been found to be generally more effective against Gram-negative bacteria with less or no effect on LAB. In a recent study, Hasegawa et al. (1999) found AIT more effective in fatty (20.8%) than lean (0.4%) tuna meat suspension against 4 strains of *V. parahaemolyticus*. After 24 h of incubation, AIT at $152.6\,\mu\text{g ml}^{-1}$ was able to inhibit only one strain in the lean suspension, but it reduced all strains below $10\,\text{cfu ml}^{-1}$ in the fatty suspension. At $101.7\,\mu\text{g ml}^{-1}$, AIT inhibited 3 of the strains to the same level in the fatty suspension. The higher activity of AIT in fatty tuna meat flesh may be related to the high level of unsaturated fat. The main fatty acids of tuna flesh are *cis*-vaccenic, palmitic and docosahexaenoic acid, which may stabilize AIT in tuna tissue suspensions. AIT possesses strong antimicrobial activity against *E. coli* O157:H7 as well as *V. parahaemolyticus*. Nadarajah et al. (2002) killed $3.6\log_{10}\text{cfu g}^{-1}$ *E. coli* O157:H7 in ground beef with AIT (200–300 ppm) after 21 d at 4 °C. The antimicrobial effectiveness of AIT against *E. coli* O157:H7 varied with storage temperature and inoculation level. There was very little inhibitory effect on the natural microflora or LAB.

In subsequent work, Muthukumarasamy et al. (2003) examined the effectiveness of AIT at 1300 ppm in ground beef stored at 4 °C under nitrogen with *Lactobacillus reuteri* against *E. coli* O157:H7. As an ingredient, AIT by itself eliminated $3\log_{10}\text{cfu g}^{-1}$ *E. coli* O157:H7 within 15 d and reduced $6\log_{10}\text{cfu g}^{-1}$ by $4.7\log_{10}\text{cfu g}^{-1}$ during 25 d storage. AIT did not interact synergistically with *Lb. reuteri* against *E. coli* O157:H7. When AIT was used in acidified chicken meat (0.1% w/w), it failed to exert a significant effect on the growth of *Brochothrix thermosphacta*, but it was able to delay growth of some LAB and aerobic mesophilic bacteria for at least 2 d (Lemay et al., 2002). In another similar study, when AIT was evaluated for its effectiveness in precooked roast beef against pathogenic bacteria (*E. coli* O157:H7, *L. monocytogenes*, *S.* Typhimurium, and *S. aureus*) and spoilage bacteria (*Serratia grimesii* and *Lb. sakei*), it was found that pathogenic bacteria were inhibited by AIT at a concentration in the head space of $20\,\mu\text{l l}^{-1}$. *E. coli* O157:H7, *S. aureus* and *S.* Typhimurium were most sensitive. The resistance of the *Lactobacillus* strain was notable (Ward et al., 1998). While AIT has been shown to be an effective antimicrobial in several different foods it is known, along with other thiocyanates, to react with thiols and sulphydryls as well as terminal amino acids, and these reactions may contribute to its loss from products during storage (Ward et al., 1998).

Tassou et al. (1995) examined the antimicrobial activity of mint essential oil in three food systems with different composition to study effects of composition on activity. The food system containing beef required a higher concentration of mint oil for microbial inhibition and this was believed to be due to the higher concentration of fat and protein present. Mint oil is hydrophobic in nature and so is more soluble in the lipid fraction of food while microorganisms are partitioned in the hydrophilic portion. These reactions may reduce opportunity for contact between the antimicrobial and the target organism.

In addition to fat and protein, the pH of food systems is an important factor affecting the activity of oils. At low pH, the hydrophobicity of some essential oils (for example, thyme oil and the phenolic oleuropein) increases and while they may tend to partition in the lipid phase of the food, they can also dissolve more easily in the lipid phase of the bacterial membrane and have enhanced antimicrobial action.

As previously mentioned, Smith-Palmer et al. (2001) found hydrophobic plant essential oils were more effective against *L. monocytogenes* in low fat (16%) that in high fat (30%) cheeses. Hasegawa et al. (1999) reported that AIT was more effective against *V. parahaemolyticus* in high fat (20.8%) than in low fat (0.4%) tuna tissue. The potential for intrinsic fat levels in food to moderate the antimicrobial activity of essential oils is clear, and results from these two studies showed that interference can be expected at fat levels in food of $>16\%$.

The oils extracted from plants of the genus *Origanum* have been shown to have antimicrobial activity in vitro

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and in food (Charai et al., 1996; Aligiannis et al., 2001). Sivropoulou et al. (1996) reported that the oil from *Origanum vulgare* at 1/4000 dilution reduced the level of *S. aureus* from 8 $\log_{10}$ cfu ml^{-1} to zero within 60 min of exposure in nutrient broth. When diluted 1/10000, 24 h was required to bring about complete destruction of all viable cells in nutrient broth. This oil has the highest concentration of carvacrol and thymol (82.03%) among all the oils from this genus. The oil was also evaluated against *S.* Typhimurium in meat packaged under MAP. The latter technique has become popular in the food industry because it controls growth of aerobic bacteria and reduces the rate of growth of anaerobic spoilage organisms like *B. thermosphacta* (Skandamis and Nychas, 2001). In conjuction with vacuum packaging, oregano oil (0.8% v/w) gave complete elimination of *S.* Typhimurium from meat after 8 d storage at 5 °C when inoculated at 3.5 $\log_{10}$ cfu g^{-1} (Skandamis et al., 2002). Other recent examples where plant-derived antimicrobials have been used with varying degrees of success in food are listed in Table 1.

Essential oils and fractions are also formulated in shampoos, toothpaste, disinfectants, topical ointments and cosmetics. However, when used in foods, highly volatile plant essential oils are sometimes lost during processing operations. Microencapsulation technology is one way these losses of essential oils by volatilization can be prevented. This technique is being widely used in the pharmaceutical industry for controlled delivery of drugs. It is also currently used in the food industry for flavour stabilization. By encapsulating antimicrobial essential oils, not only can they be protected from heat, but they also can be released in products at a controlled rate to deliver effective inhibitory concentrations over extended periods and thereby extend shelf-life.

2.5. Antimycotic activity

Due to their ability to grow in almost all food products, yeasts and moulds can generate off-flavours, produce toxin, and cause discolouration and proteolysis through the action of various enzymes like lipases and proteases. The most important feature of moulds from a food safety perspective is their ability to produce mycotoxins, such as aflatoxins, which are toxigenic secondary metabolites. *Aspergillus ochraceus* produces ochratoxin A (OTA) which is responsible for nephropathies in pigs and humans. Fortunately, essential oils derived from plants are also known to possess antifungal activity (Salmeron et al., 1990; Filtenborg et al., 1996; Sivropoulou et al., 1996; Mangena and Muyima, 1999; Schnurer et al., 1999; Soliman and Badeaa, 2002), and generally they are more active against fungi than they are against Gram-positive bacteria (Shelef, 1983). As an example of antimycotic activity, the oil of *Ocimum gratissimum* leaves was fungicidal at 78 ppm for *Microsporum gypseum* and *Trichophyton rubrum*, but a concentration of 312 ppm was required to inhibit growth of *Candida albicans* and *Cryptococcus neoformans* (Amvam Zollo et al., 1998).

Chao et al. (2000) evaluated antifungal activity of 45 different plant oils against *C. albicans*, *Aspergillus niger* and *Rhizopus oligosporus*. Of the 45 oils, those of coriander, cinnamon bark, lemongrass, savory and rosewood were effective against all three microorganisms. Other oils showed selective activity against the three organisms. Some of them were effective against *C. albicans* only. Angelica and pine oils used in this study were not effective against *A. niger* and *R. oligosporus* which have a symbiotic relationship with the mycorhizae associated with the plants from which the oils were isolated (*Angelica archangelica* L. and *Pinus sylvestris* L.), respectively. In addition to inhibition of vegetative growth, the other oils also inhibited production of mycotoxins by fungi. Thyme, anise and cinnamon oils were able to inhibit production of aflatoxins, ochratoxins A and fumonisin in broth at 2%. Anise, fennel and caraway oils also showed fungicidal effects against *A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*. Anise oil showed fungicidal activity at 500 ppm, while caraway and fennel oil at 2000 ppm were required to inhibit all four fungi (Soliman and Badeaa, 2002). Some plant essential oils have also been shown to inhibit mycelial growth and conidial germination. The oils of thyme, oregano, dictamus and marjoram totally inhibited mycelial growth at 250–400 μg ml^{-1}, while 250 μg ml^{-1} inhibited conidial germination of *Penicillium digitatum*. The oils of lavender, rosemary and sage gave 29.5%, 24.0% and 9.0% (% of untreated control) mycelial inhibition, respectively, at 1000 μg ml^{-1}. Thyme, oregano and dictamus oils were fungitoxic and this may have been due to formation of hydrogen bonds between the hydroxyl group of oil phenolics and active sites of target enzymes (Daferera et al., 2000).

Fungi are the main agents of spoilage of bakery products. Apart from visible growth, they also produce off-flavors and mycotoxin production can be a concern. As with other foods, bakery products which contain natural preservatives are becoming more common. However, as with bacteria, fungi are more resistant to these natural antimicrobials when challenged in foods (Lopez-Malo et al., 2002). Active packagings with the packaging materials delivering antimicrobials, can play an important role in satisfying current requirements because inhibitors are more effective when delivered in this manner. When AIT was used as an antimicrobial agent in active packaging of rye bread, it was found that 1 μl AIT completely inhibited the growth of *A. flavus*, *Penicillium commune*, *Penicillium corylophilum*, *Penicillium discolor*, *Penicillium polonicum*, *Penicillium roqueforti* and *Endomyces fibulige* (Nielsen and Rios, 2000).

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3. Antimicrobial activity of liquid smoke

Smoke application has historically been successfully used for food preservation, often in conjunction with other hurdles such as cooking and drying. Traditional methods originally developed in the 1880s are still used to impart flavour, colour and aroma to foods. Natural wood smoke, generated by controlled smouldering of wood in the absence of or at reduced oxygen levels, is a suspension of vapours, solid particles and liquid droplets. Because of concern regarding the presence of potentially carcinogenic benzopyrenes and because of convenience issues, in the 1970s liquid smoke was developed and has become popular. For the manufacture of liquid smoke, smoke from smouldering wood is passed to a condensing tower where it is captured in water. Poorly soluble solutes precipitate during a holding period >10 d and commercial liquid smoke is prepared from the solution after filtration to further remove polymeric hydrocarbons known to be carcinogenic (Pszczola, 1995; Vitt et al., 2001).

Smoke flavours are considered to be Generally Regarded As Safe (GRAS), so they can be used in foods as an additional barrier to prevent microbial growth at levels which comply with good manufacturing practice. Food composition plays an important role in determining the overall antimicrobial activity of smoke solutions (Sunen et al., 2001). In addition to food composition, processing conditions and hygienic or sanitary conditions used during product manufacture as well as during smoke application play important roles in determining the nature and size of the final bacterial population in the product. For example, processing during manufacture of frankfurters before cooking can increase the population of total aerobic and sporeforming bacteria as well as the population of moulds and yeast (Heiszler et al., 1972). Other parameters such as product handling, smoking temperature and relative humidity in smokehouses can be controlled to exert additional pressure on microbial contaminants (Himelbloom and Crapo, 1998).

The antimicrobial activity of liquid smoke is attributed to the presence of compounds like phenols, carbonyls and organic acids (Vitt et al., 2001). Studies have been carried out to evaluate the antimicrobial activity of a variety of smoke preparations and some are noted in Table 2. For example, Sunen et al. (2001) evaluated the antimicrobial activity of four different commercial smoke preparations. One dried (S) and three liquid (L1, L2, L3) formulations were used against *L. monocytogenes*, *Y. enterocolitica* and *A. hydrophila* in agar (TSA). The smoke preparations were evaluated at 1% for S, 0.4% for L1, 0.6% for L2 and 4% for L3. Preparation S was most effective against *A. hydrophila* preventing recovery of the organism after 2 d whereas L1 and L2 took 14 and 12 d to bring about 99% inactivation of the organism. *Y. enterocolitica* was only inhibited by S and L1. Preparation S was also the most effective against *L. monocytogenes*, exerting bacteriostatic activity.

Liquid smoke usually contains acetic acid, which is regarded as being responsible for a large portion of its bacteriostatic properties. In a study where a series of commercial liquid smoke preparations (Red Arrow International, Manotowoc, WI) were analysed for their antimicrobial activity against pathogens including *E. coli* and *S. aureus*, as well as *P. aeruginosa* and *Weissella viridescens*, it was found that CharSol C-6 at 0.25% (v/v) in nutrient agar showed 72% inhibition of growth (reduction in numbers over the untreated controls) of *S. aureus*. It caused 33%, 52% and 99% reduction in recovery of *E. coli*, *P. aeruginosa* and *W. viridescens* (Wendorff, 1981). When a series of liquid smoke extracts were evaluated against *L. monocytogenes*, it was found that CharSol-10, Aro-Smoke P-50 and CharDex Hickory had strong antilisterial activity, with complete inhibition of cell recovery occurring at 0.5% with all preparations in 4 h. CharSol PN-9 and CharOil Hickory showed inhibition of *L. monocytogenes*, but this effect was weaker compared to the other three extracts. The higher inhibitory activity of some liquid smokes is believed to be due to the higher

Table 2
Antimicrobial effects of smoke preparations when used in foods; bactericidal (a), bacteriostatic (b)

Antimicrobial agent	Concentration	Product	Organism	Effect	Reference
CharSol H-6	0.0005–0.001 M	Cheddar cheese	*A. oryzae*	a	Wendorff et al. (1993)
CharSol M-10	0.0005–0.001 M		*A. oryzae*	a	
CharSol LFB	0.0005–0.001 M		*A. oryzae*	a	
CharSol C-10	Undiluted	Salmon	*L. monocytogenes*	a	Poysky et al. (1997)
Isoeugenol	150 ppm	Wiener exudates	*L. monocytogenes*	b	Faith et al. (1992)
CharSol suprim	0.5%	Cold smoked Salmon	*L. monocytogenes*	a	Vitt et al. (2001)
Commercial	0.4–0.6%	Rainbow Trout	*A. hydrophila* *L. monocytogenes*	a b	Sunen et al. (2003)
Zesti-Smoke	0.0005–0.001 M	Cheddar cheese	*A. oryzae*	a	Wendorff et al. (1993)

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concentration of polar phenolic compounds present. Since they have higher water solubility, these polar phenolics have greater opportunity to contact and interact with target organisms and exert higher levels of kill (Messina et al., 1988).

A more recent development in smoke technology with food applications has been the commercialization of liquid smoke preparations which are either soya or canola oil rather than aqueous-based. For their preparation, the oils are used to extract flavours from liquid smoke, facilitating transfer of oil-soluble phenolics but leaving behind the less soluble, tangy acids. While aqueous smokes can be dried and applied as a powder to accentuate smoke flavour in products like potato chips, oil-based smokes have a very mild flavour since a larger portion of the carbonyls is not extracted from the aqueous fraction into the oils. Their higher levels of phenolics serve as better antioxidants and prevent deterioration of lipids in high fat foods like bacon and some fish. The lower level of carbonyls in the oil-based smokes also means that they have reduced capacity to generate colour. Oil-based smokes have milder flavour and find application in foods with delicate flavour character like fermented sausage and cheese (Dekker, 2003). Although these new products can be expected to be antimicrobial there are no reports describing this activity.

3.1. Activity in food systems

L. monocytogenes is an important pathogen for the seafood as well as the poultry industry. It is frequently isolated from many types of seafood where it can grow and cause illness following consumption. Not surprisingly, liquid smoke was evaluated for its antilisterial activity in salmon, and it was found that smoking for 4 h resulted in a 1.5-log-reduction of *Listeria innocua* (used as a model for *L. monocytogenes*). When smoking was done for 12 h, it gave a 3-log-cycle reduction in viable *L. innocua* (Sabanadesan et al., 2000). When smoking was applied together with other physical hurdles like salting (Niedziela et al., 1998), growth of two *L. monocytogenes* strains (s_1 and s_2) was prevented. In these tests, fillets inoculated with the two strains were first either dry salted or brined. After salting, the fillets were cold smoked at 25 °C and 65% RH. There was inhibition of both strains by the treatments with no difference in result due to the methods of salt application. Smoke treatment had a bacteriostatic effect on *L. monocytogenes*. It was also found that addition of salt plus phenolics in the form of smoke had additive but not synergistic inhibitory effects.

When smoke was applied to rainbow trout together with salt and the fish was then vacuum packaged, smoke had a synergistic inhibitory effect on both *L. monocytogenes* and *A. hydrophila* (Sunen et al., 2003). Of four different commercial smoke preparations used (L1, L2, L3 and S), L3 and S were highly effective against *A. hydrophila*, causing its reduction from day 0 to below the detection limit. On the other hand, extracts L1 and L2 were the only ones able to reduce *L. monocytogenes* to below the detection limit. It is clear that not all smoke preparations were equally effective, and inhibitory effects in food were less than in agar (Sunen et al., 2001). The phenolic concentrations in smoke preparations together with organic acid levels and carbonyls play an important role in controlling the growth of microorganisms. In the preceding work, for example, extracts L1 and L2 had higher concentrations of phenolics and these were the most effective against both test organisms. The extract S was effective against *A. hydrophila* but was only bacteriostatic against *L. monocytogenes*.

The use of smoke reduced the minimum heat required to kill *L. monocytogenes* in salmon steaks (Poysky et al., 1997). When salmon steaks were only heat processed, *L. monocytogenes* was detected in almost all samples unless the internal temperature reached $\geqslant 82.8$ °C. Addition of smoke throughout the heat treatment resulted in elimination of *L. monocytogenes* at $\geqslant 67.2$ °C, but they were detected in samples processed at < 67.2 °C. When smoking was done only during the last half of the heat cycle, *L. monocytogenes* was able to survive at $\leqslant 80$ °C, but the organism was eliminated from samples, which were processed $\geqslant 80.6$ °C for 3 h. CharSol C-10 was the most effective antilisterial smoke preparation among those tested and when used at 50% dilution, *L. monocytogenes* was eliminated at 65.6 °C. Undiluted, the same smoke preparation reduced the temperature needed to eliminate *L. monocytogenes* to 58.9 °C. The reduced effectiveness of smoke when it was applied only during the last half of processing was due to surface drying and formation of a coagulated protein layer (pellicle). The pellicle that formed enveloped the bacteria on the surface, providing protection against smoke compounds as well as heat.

In addition to smoking, the presence of LAB can also control the growth of *L. monocytogenes*. Lactic acid bacteria, including carnobacteria, are the dominant microflora in cold-smoked salmon. Some of these organisms such as *Carnobacterium piscicola* are able to produce bacteriocins that inhibit *L. monocytogenes* (Nilsson et al., 1999).

Smoking is not usually inhibitory to most LAB. In cold smoked sausage, the use of liquid smoke had no influence on the viability of LAB, but there was a delay in initiation of LAB growth. In the presence of liquid smoke LAB cells entered the exponential phase after 5 h of fermentation, which was 1 h longer than in non-smoked samples at 38 °C. In order to inhibit pathogenic bacteria, it is essential for LAB to enter the exponential growth phase as soon as possible. In the traditional

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sausage making process, smoking may delay growth of LAB and retard production of desired amounts of acid in sausage (Donnelly et al., 1982) although the benefits from smoke application in terms of pathogen inhibition outweigh its inhibitory influence on acid production by LAB.

Moulds are one of the major groups of spoilage agents in cheese. They not only affect colour and texture, but can also produce undesirable toxic substances. *Penicillium*, *Aspergillus* and *Mucor* species are the major spoilage moulds in cheese and their growth on cheese can be inhibited by smoke (Wendorff and Wee, 1997). Phenolic compounds present in smoke are also reported to show complete or appreciable inhibition of aflatoxin production. In earlier work, Wendorff et al. (1993) determined the effect of various commercial smoke solutions on the growth of moulds (*Penicillium camemberti*, *A. oryzae* and *P. roqueforti*) on cheese. They evaluated the effect of Zesti-smoke (natural Hickory smoke flavoring), Charsol H-6, Charsol M-10 and Charsol LFB commercial products. Samples were dipped in liquid smoke solution for 30 s and then allowed to drain for 1 min on a sterile rack. All four smoke solutions prolonged the lag phase of the three moulds tested and reduced the growth rate of *P. camemberti*, although *P. roqueforti* grew faster on liquid smoke-treated cheese, once growth was initiated. With this exception, smoke components were able to retard the growth of spoilage moulds on the surface of cheddar cheese. Wendorff et al. (1993) also evaluated the major phenolic compounds in liquid smoke for their inhibitory potentials. All three organisms were inhibited by isoeugenol, a major component of smoke. *P. camemberti* was also inhibited by *m*-cresol and *p*-cresol while *A. oryzae* was inhibited by 5 of the 8 phenols tested. It was concluded that phenolic compounds in wood smoke have antifungal properties that are useful in food preservation.

4. Conclusions

Resistance or sensitivity to naturally occurring cyclic hydrocarbons appears to be related in part to cell wall structure. The complex envelope of Gram-negative bacteria (with a dual membrane) appears to afford protection, and among this group the pseudomonads show the greatest resistance. Gram-positive bacteria are more sensitive but the LAB are the most resistant among this group. Yeast and fungi are generally more sensitive than bacteria. The antimicrobial effectiveness of cyclic hydrocarbons depends upon a variety of factors, and there is a need for more information on modes of action. Some information about the chemical features that promote activity of these antimicrobials is available. The ability of cyclic hydrocarbons to partition or dissolve in the lipid phase of the cytoplasmic membrane is important for activity, but greater solubility does not mean greater antimicrobial action. Compounds having a hydroxyl group plus a system of delocalized electrons in the phenolic ring structure have high activity. The ability to be involved in hydrogen bond-catalysed reactions also appears important for antimicrobial activity. The aldehyde group of vanillin was believed responsible in part for its antimicrobial activity, and this may also be true for cinnamaldehyde which does not have a free hydroxyl group. Among the alcohols, longer chain (C_6–C_{10}) molecules are more effective.

There is overwhelming evidence that many of these antimicrobials act at the cytoplasmic membrane, altering its function and in some instances structure, causing swelling and increasing its permeability. A consistent observation is an increase in K^+ and often cytoplasmic content efflux from cells in response to antimicrobial challenge. These effects may develop as a result of membrane depolarization by altered ion transport or through changes in membrane structure, inhibition of energy (ATP) generation by interference with glucose uptake or inhibition of enzymes involved in oxidative or substrate level phosphorylation. Increases in cytoplasmic membrane permeability appear to be a consequence of the loss of the cellular pH gradient, decreased ATP levels and, loss of the proton motive force, which lead to cell death.

It is also evident that all essential oil antimicrobials do not necessarily act in the same manner. With any one agent, changes in its concentration may change the nature of the inhibitory action. These effects may be related to the influence of concentration upon solubility in the membrane. Some examples of different inhibitory effects follow. Tea-tree terpenoids inhibited oxidative respiration in both *E. coli* and *S. aureus* at the MIC. Carvacrol acted as a protonphore uncoupler in *B. cereus* causing K^+ efflux, destroying the pH gradient, and the proton motive force, yielding inhibition of ATP generation and cell death. Similar effects were caused by thymol and carvacol against *S. aureus* and *P. aeruginosa*. Eugenol and cinnamaldehyde had significant inhibitory effects on energy generation in *L. monocytogenes* and *Lb. sakei*. Eugenol, but not cinnamaldehye, at lethal concentrations caused slow leakage of ATP from treated *L. monocytogenes* cells, but neither compound caused ATP leakage from *Lb. sakei* at lethal levels. These differences could be related to the cytoplasmic membrane solubility of the antimicrobials or differences in susceptibility of host enzyme systems responsible for maintenance of energy levels. Results with *Lb. sakei*, where continued substrate level phosphorylation was able to sustain ATP levels following challenge, suggest a possible explanation for the greater resistance of LAB among the Gram-positive group. Since LAB have the

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Table 3
Potential use of natural antimicrobial and smoke extracts in foods

Natural antimicrobial or smoke extract	Major antimicrobial component(s)	Sensitive microorganisms	Potential food application
Basil	Linalool (44%) Methyl chavicol (27%)	*L. curvatus* *B. cereus* *S.* Typhimurium *S. cerevisiae*	Tomato juice and other tomato-based foods, oral and dental care products, confectionary, baked goods, condiments, meats
Cinnamon	Cinnamic aldehyde	*S.* Enteritidis *L. monocytogenes* *L. innocua* *C. botulinum*	Marinades and sauces (poultry, pork, beef, seafood), cheeses, bakery products, seasoning
Clove	Eugenol (85.3%)	*L. monocytogenes* *L. innocua* *S.* Enteritidis *S.* Typhimurium *Micrococcus* spp. *V. vulnificus* *C. botulinum* *B. subtilis*	Marinades and sauces (poultry, pork, beef, seafoods), salads and sauces (celery, lettuce, parsley, radish, mushroom, cabbage, fennel, spinach, bean sprouts, cucumber), cheeses, bakery products and seasonings
Garlic	Allicin	*C. botulinum* *E. coli* O157:H7	Marinades and sauces, beef, pizza, cheeses,
Horseradish, Mustard	Allyl isothiocyanate (95%)	*E. coli* O157:H7 *L. monocytogenes* *V. vulnificus*	Beef, seafood, bakery products, pork, fruit juices, salad dressing
Liquid smoke	Phenols Acetic acid Carbonyl derivatives	*L. innocua* *A. hydrophila* *P. camemberti* *P. roqueforti* *L. monocytogenes*	Cheese, seafoods, beans
Mint	Menthol (28–42%), Menthone (18–28%)	*L. monocytogenes* *S. aureus* *S. epidermidis* *Pseudomonas* spp.	Ice cream, confectionary, chewing gum
Rosemary	Borneol (26.5%) α- terpinene (15.6%) α- pinene (12.7%)	*L. monocytogenes* *E. coli* *S. aureus* *Sarcina* spp.	Marinades and sauces, cheese, beef, bakery products

same wall structure as other, more sensitive Gram-positive bacteria, metabolic differences such as a greater capacity to maintain the pH gradient under osmotic stress may contribute to their greater resistance to these antimicrobials. Inhibition of glucose uptake and inhibition of metabolic enzymes (decarboxylases) have also been reported and may play a role in the overall effectiveness of essential oils against micro-organisms.

In contrast to cyclic hydrocarbons, the effectiveness of thiocyanates and thiosulfinates from mustard/horse-radish and onion/garlic, respectively, appears to result from their interaction with sulphydryl groups in enzymes essential for metabolic activity of cells. The sensitivity of *E. coli* O157:H7 to AIT is the subject of active study in a number of laboratories.

There is no doubt that more effective antimicrobials will be useful in the safe extension of refrigerated food shelf-life as well as in meeting future changes and present challenges of the marketplace. Not only can natural antimicrobials meet many of these requirements, but they can also increase the type and number of food products available for sale (Branen, 1983). Table 3 shows where plant essential oils and liquid smoke may be used to inhibit the growth of potential pathogens and spoilage organisms on or in food products.

While both plant essential oils and wood smoke can serve as natural antimicrobial agents for a variety of foods, it is unlikely that a single natural antimicrobial from each group can be used at one concentration in all food products and be effective against all kinds of organisms. Different concentrations of even the most

potent compounds are required in different food products to show desirable antimicrobial activity. The optimum concentration will depend on the type and number of problematic bacteria, as well as the type of food and food storage temperature. If these natural antimicrobials are used above certain concentrations, they may create flavour or other organoleptic problems in foods. However, these effects can be moderated to some extent through the judicious assessment of flavour compatibility of the antimicrobial with specific food uses and the inclusion of ingredients such as vinegar, salt or sugar to soften flavour notes. Essential oils and liquid smoke ingredients can be good sources of potent natural antimicrobial agents for foods, but further research is required to optimize their stability in food products and better understand their key mechanism of antimicrobial action.

5. Future work

There is a need to better understand how essential oil components and other natural antimicrobials interact with cells to cause bacteriostatic or bactericidal effects. It should be determined whether changes in membrane permeability are primary effects or are a consequence of: direct effects on the energy (ATP) generation system; inhibition of substrate uptake or utilization; or inhibition of enzymatic reactions critical to maintenance of cellular viability and normal metabolism.

Work needs to be undertaken to understand reasons for the generally greater resistance of pseudomonads to essential oil components and, among the Gram-positives, why LAB are more resistant. Experimental systems are available where these organisms show sensitivity and tests could be used to develop new approaches for increasing the sensitivity of these and other more troublesome organisms in foods by taking advantage of synergies among the antimicrobials.

Use of groups of natural antimicrobials with small differences in molecular structure has proven to be a valuable approach in understanding the molecular basis for component antimicrobial activity. Further work along these lines should allow better understanding of the basis for microbial species resistance or sensitivity.

Newly developed smoke products in lipid matrices contain a lower proportion of organic acids than the aqueous-based products and may prove to be less antimicrobial. Comparative inhibitory studies in food products using these materials are yet to be published.

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