

The Use of Essential Oils and Bacteriocins as Natural Antimicrobial and Antioxidant Compounds

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ABSTRACT

Food-borne illness resulting from consumption of food contaminated with pathogenic bacteria has been of vital concern to public health. To reduce the health hazards and economic losses due to food-borne microorganisms, the use of natural products as antimicrobial compounds seem to be an interesting way to control the presence of pathogen in food. Spices are rich in phenolic compounds such as flavonoids and phenolic compounds, which exhibit a large range of biological effects, including antimicrobial and antioxidant properties. Lactic acid bacteria (LAB) have been also used for centuries as a preservation method of food using fermentation process. Antimicrobial activities of LAB have been demonstrated in various species and their antagonist actions are demonstrated against numerous intestinal and food-borne pathogens. The preservative ability of LAB in foods is attributed to the production of anti-microbial metabolites. Bacteriocins are antimicrobial metabolites category, recognized as small peptides and designated as bacteriocins. Bacteriocins have attracted attention as potential substitutes for antibiotics to cure and/or prevent bacterial infections and are widely employed in food preservation. The inclusion of essential oils and bacteriocins in food products should lead to beneficial effects such as improved safety, quality and flavour; and potential in the biopreservation of food. This review highlights the mechanism of action of these antimicrobial and antioxidant compounds, and their application in food systems.

Keywords: antibacterial, peptides, polyphenols, terpenoids, lactic acid bacteria

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INTRODUCTION

In spite of modern technologies and safety concepts, such as HACCP, the reported numbers of food-borne illnesses and intoxications are still increasing (O'Sullivan *et al.* 2002). According to the According to the United Nations, more than 30% of the mortality rate world-wide is caused by alimentary diseases. According to Health and Welfare Canada, the annual costs to treat food borne illness are estimated at \$1 billion in Canada and from \$5 to \$86 billion in the United States. The estimated cost from five bacterial foodborne pathogens total 6.9 billion/year in 2000 (Anon 1994; USDA 2000). *Campylobacter*, *Listeria*, *Shigella*, *Escherichia coli* and *Salmonella* are the most important bacteria responsible for food-borne illness in Canada. However, *Salmonella*, *Campylobacter jejuni*, *E. coli* 0157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium botulinum* can contaminate meat, poultry, eggs, seafood and dairy products.

The desire of most countries to make food safer for consumption requires better food preservation and production

techniques. In this regard, the use of natural antimicrobial compounds is an interesting alternative to be considered.

Essential oils (EOs) have long served as flavouring agents in food and beverages, and due to their versatile content of antimicrobial and antioxidant compounds; they possess potential as natural agents for food preservation (Conner 1993; Oussalah *et al.* 2007a). Lactic acid bacteria (LAB) can produce important antimicrobial metabolites called bacteriocins. This paper will review some applications of these natural compounds in food systems in order to kill pathogens and also to increase their shelf life.

ESSENTIAL OILS

EOs are secondary metabolites of the plant and are used in many applications of the spice and food industry. Each variety has a different physiological and biochemical form with special enzymatic equipment, genetically codified, which directs biosynthesis by a preferential formation of the components (Russo *et al.* 1998). They are commonly concentrated in one particular region such as leaves, bark or fruit,

and when they occur in various organs in the same plant, they frequently have different composition profiles. EOs have long served as flavouring agents in food and beverages, and due to their versatile content of antimicrobial compounds, they possess potential as natural agents for food preservation (Connor 1993).

Steam distillation is the most commonly used method for producing EOs on a commercial basis (Burt 2004). Extraction by liquid carbon dioxide under low temperature and high pressure seem to produce a more natural organoleptic profile and may also influence antimicrobial properties (Moyler 1998) and the use of hexane for the extraction have been shown to exhibit greater antimicrobial activity than corresponding steam distillation (Packiyasothy and Kyle 2002). It was also established that the antioxidative properties of most herb extracts were greatly dependent on the extraction solvent used (Yanishlieva *et al.* 2006) and supercritical carbon dioxide extraction can also protect the antioxidant property of the extract (Tipsrisukond *et al.* 1998). The major active compounds present in EOs are terpenoids and phenolic compounds (Oussalah *et al.* 2007a).

Antimicrobial properties

EOs from spices and herbs have been shown to possess antimicrobial functions and could serve as a source of antimicrobial agents against food pathogens (Janssen *et al.* 1985; Deans and Ritchie 1987; Kim *et al.* 1995). More particularly, EOs and their components are known to be active against a wide variety of microorganisms. Depending of the Eos and the microorganism evaluated a minimal inhibitor concentration of 1 mM for active compounds to 3% for less active compound was observed when tested against *E. coli* or *S. Typhimurium* (Kim *et al.* 1995; Helander *et al.* 1998; Skaltsa *et al.* 2003). The use of EOs as natural antibacterial compounds seem to be an interesting way to control the presence of pathogenic bacteria and to extend the shelf life of food. According to Cragg (1997) over than 78% of the drugs from natural origins are for antibacterial uses. Jantova *et al.* (2000) observed an inhibition of 42% of *S. aureus* growth when in presence of *Philadelphus microphyllus* (Philadelphaceae). This extract also inhibited 32% of *Enterococcus faecalis*. According to his study, *Gymnocladus dioica*, *Amelanchier ovalis*, *Exochorda racemosa*, *Holodiscus discolor*, *Philadelphus microphyllus*, *Philadelphus coronaries* and *Pelargonium tabulare* had the strongest antimicrobial effect. Billing and Sherman (1998) compared the antimicrobial properties of 43 spices traditionally used in the meat-based cuisines in 36 different countries. According to these authors, spices help cleanse foods of pathogens and thereby contribute to the health, longevity and reproductive success of people. Between the 30 spices evaluated, some of them (garlic, onion, allspice, oregano) inhibited all bacteria evaluated (more than 35 bacterial species), 80% of spices inhibited more than 50% of bacteria tested, 50% of spices inhibited more than 75% of bacteria tested (Table 1). In general, Gram-positive bacteria are more sensitive than Gram-negative and *Pseudomonas* species are least sensitive to bioactive agents (Ceylan and Fung 2004). Huhtanen (1980) reported that 31 ppm alcoholic extracts of mace and

achiote (*Cannato*, *Bixa orellana*) were the most inhibitory spices among 33 spices tested against *Clostridium botulinum* in culture media. Nutmeg bay leaf (*Myristica fragrans* Houtt), white and black peppers (*Piper nigrum* L.) (125 ppm) were moderately inhibitory while paprika (*Capsicum annuum* var. *angulosum* Mill.), rosemary (*Rosmarinus officinalis*), cloves (*Syzygium aromaticum* L.), oregano (*Origanum vulgare*), turmeric (*Curcuma domestica* Loir.) and thyme (*Thymus serpyllum*) (500 ppm) were slightly inhibitory. Shelf-life of chicken, beef and mutton cuts sprayed with water extracts of ginger (*Zingiber officinale* Rosc.), garlic (*Allium sativum*) and onion (*Allium amaryllis*) were extended at ambient temperature (Ziauddin *et al.* 1996). Garlic was the most effective showing a longer shelf-life than ginger and onion treatments (Ziauddin *et al.* 1996). Garlic juice was effective to eliminate the growth of *Staphylococcus*, *Streptococcus*, *Vibrio cholerae* and the growth of some zoopathogenic fungi and many strains of yeast (Block 1985). A concentration of garlic juice (1%) can inhibit the growth of *E. coli*, *S. Typhosa* and *S. aureus*. Addition of onion is less effective. A concentration of 4% is needed to eliminate completely *S. aureus* and to reduce by 48% the growth of *E. coli* (Al-Delaimy and Ali 1970). Various organic sulphur compounds related to the flavour of garlic and onion are believed to be antimicrobial (Sharma *et al.* 1979; Garlic and Health Group 2007). Stecchini *et al.* (1993) have shown that clove extract can inhibit the growth of *Aeromonas hydrophila* in cooked pork. Cinnamon (*Cinnamomum cassia*) and clove have a greater inhibition on the growth of *L. monocytogenes* compared to garlic, onion and mustard (Bahk *et al.* 1990). Eugenol and pimento extract (one-tenth ml of ethyl alcohol extract/25-g beef sample) exhibited a great inhibition of *A. hydrophila* and *L. monocytogenes* in cooked beef (Hao *et al.* 1998). The use of methyl chavico, a compound present in basil is bactericidal against *A. hydrophila* and *Pseudomonas fluorescens* at a respective concentration of 0.125 and 2%, respectively (Wan *et al.* 1998). A decoction of 100% sage (*Santolina* sp.) or thyme reduces significantly the population of *Yersinia lipolytica* on chicken (Ismail *et al.* 2001). The furocoumarins present in parsley (0.12-8%) was found to have antimicrobial properties against *L. monocytogenes*, *E. coli* 0157:H7 and spoilage microorganisms (Manderfeld *et al.* 1997). Also, EOs containing monoterpenes, eugenol, cinnamaldehyde, thymol, citronellol, limonene and geraniol and phenolic compounds present in carrots seem to have strong antilisterial activity (Lis-Balchin and Deans 1997).

EOs have also antifungal properties. When antibiotics are not employed, chicken can be contaminated by the growth of *Candida* and *Rhodotorula*. Clove, coriander (*Coriandrum sativum* L. Vernacular), cinnamon, cardamom (*Elettaria cardamomum* L.), thyme, tea tree (*Melaleuca alternifolia*), marjoram (*Pelargonium* spp.), ho leaf (*Acanthopanax sieboldianus*), rosemary, peppermint (*Mentha × piperita*), palmarosa (*Cymbopogon martinii*), lemon grass (*Cymbopogon citratus*) and sage EOs in the range of 50–500 µg/ml possessed stronger antimicrobial activity against yeast than bacterial cultures (Hili *et al.* 1997). Vanillin (2000 ppm), a major constituent of vanilla beans, has a significant inhibitory effect against spoilage yeast like *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii*, *Debaryomyces hansenii* in apple puree (Cerutti and Alzamora 1996). Garlic culture inhibits the growth of *Candida*, *Cryptococcus*, *Thodotorula torulopsis* and *Trichosporon* (Moore and Atkins 1977). The addition of ground oregano, thyme and their extract in growth media inhibit the production of aflatoxins (B₁ and G₁) of *Aspergillus parasiticus* (Salmeron *et al.* 1990). Thymol and carvacrol (0.0025 and 0.05%) at pH 5.5 can inhibit completely the growth of *Aspergillus flavus*, *Aspergillus niger*, *Geotricum candidum*, *Mucor* spp., *Penicillium* spp. in potato dextrose agar (Akgul and Kivanc 1988). Cinnamon at 0.02, 0.2, 2.0 and 20% inhibited the growth of *Aspergillus parasiticus* by 16, 23, 31 and 100% and aflatoxin production by 25, 68, 97, and 100%, respectively (Bullerman 1974). Mustard oil can inhibit the growth

Table 1 Proportional inhibitory property of spices against bacterial strains tested¹.

Percent bacterial inhibition	Spice
75-100%	Garlic, onion, allspice, oregano, thyme, cinnamon, tarragon, cumin, cloves, lemon grass bay leaf, capsicums, and rosemary.
50-75%	Marjoram, mustard, caraway, mint sage, fennel, coriander, dill and nutmeg.
Less than 50%	Basil, parsley, cardamom, pepper (black and white), ginger, anise seed, celery seed, lemon, and lime.

¹ Adapted from Billing and Sherman 1998.

of *Saccharomyces ellipsoideus*, *Saccharomyces cerevisiae* and *Mycoderma vini* in pickles and sauerkraut (Shelef 1984).

Several other studies were done on the antimicrobial activity of EOs (Conner and Beuchat 1984; Carson and Riley 1993; Panizzi *et al.* 1993; Helander *et al.* 1998; Cox *et al.* 2000; Nielsen and Rios 2000; Delaqui *et al.* 2002; Mejholm *et al.* 2002; Araujo *et al.* 2003; Burt and Reinders 2003). According to these studies, the antimicrobial activity of EOs is assigned to a number of small terpenoids and phenolic compounds, which also in pure form have been shown to exhibit higher antibacterial or antifungal activity (Karapınak and Aktung 1987; Conner 1993; Didry *et al.* 1993; Juven *et al.* 1994; Oosterhaven *et al.* 1996; Suppakul *et al.* 2003). According to Cosentino *et al.* (1999) an essential oil rich in phenolic compounds presents higher antimicrobial properties as compared to EOs rich in terpenoids. However, some minor components can play a role by producing a synergistic effect with other components. This has been observed with sage (Marino *et al.* 2001), certain species of thyme (Marino *et al.* 1999), oregano (Paster *et al.* 1995; Turgis *et al.* 2007) and clove (Turgis *et al.* 2007). EOs of spices damage the structural and metabolic enzymes, and inhibit the repair of heat-injured yeasts (Conner and Beuchat 1984). The antibacterial properties of the compounds present in EOs are normally associated with their lipophilic character, leading to accumulation in membranes and to subsequent attack of the integrity of the membrane, energy depletion (Conner 1993; Sivropoulou *et al.* 1996), a significant damage of the external envelope (Rhayour *et al.* 2003) or plasmic membrane (Lambert *et al.* 2001). The hydrophilic/lipophilic balance, presence of a phenolic-OH group that can easily react and form hydrogen bonds with enzymes and other factors could also determine the extent of the inhibition of EOs (Farag *et al.* 1989). The presence and location of a hydroxyl group on the molecule, the lipid solubility and the degree of steric hindrance also determines the antimicrobial activity of phenolic antioxidants (Raccach 1984). Aliphatic aldehydes with one or more double bonds conjugated to their carbonyl group had much higher antifungal activity than those that did not have double bonds (Kurita *et al.* 1981). The presence of alkyl group on benzene ring of phenol or guaiacol enhanced the antifungal activity of the phenolic compounds (Ceylan and Fung 2004). Allicin is the principal antibacterial compound in garlic and acts as an inhibitor towards -SH group enzymes (Beuchat and Golden 1989). Allicin also affects fatty acids, lipid biosynthesis and RNA synthesis of microorganisms, inhibits acetyl-CoA synthetase in fatty acid biosynthesis, acetate kinase and phosphotransacetylase enzyme systems (Focke *et al.* 1990). Adetumbi *et al.* (1986) reported that garlic extract interfered with enzymes in lipid biosynthesis. Ghanoum (1988) has also observed that the structure and the integrity of the outer surface of *Candida albicans* was affected, that there was a loss of intracellular components and morphological changes, a decrease of the lipid content, a higher proportion of phosphatidylserines and lower proportions of phosphatidylcholines. Oussalah *et al.* (2006a) evaluated the mechanism of the antimicrobial action of Spanish oregano, Chinese cinnamon and savory (*Satureja montana*) EOs. Cell membranes and walls of bacteria was studied by the measurement of the intracellular pH (pH_{in}) and ATP concentration, the release of cell constituents and the electronic microscopy observations of the cells when these EOs at their minimal inhibitory concentration (MIC) were in contact with *E. coli* O157:H7 and *L. monocytogenes*. *E. coli* O157:H7 and *L. monocytogenes* as Gram-negative and Gram-positive bacterial models. Treatment with these EOs at their MIC (from 0.025 to 0.05%) was able to affect the membrane integrity of bacteria and to induce depletion of the intracellular ATP concentration. An increase of the extracellular ATP concentration was observed only when Spanish oregano and savory oils were in contact with *E. coli* O157:H7 and *L. monocytogenes*. Also, a significantly higher ($P \leq 0.05$) cell constituents release was observed in

the supernatant when *E. coli* O157:H7 and *L. monocytogenes* cells were treated with Chinese cinnamon and Spanish oregano oils. Chinese cinnamon oil was more effective to reduce significantly the pH_{in} of *E. coli* O157:H7, whereas Chinese cinnamon and Spanish oregano decreased more significantly the pH_{in} of *L. monocytogenes*. Electronic microscopy observations revealed that except for the cells treated with cinnamon, the cell membranes of both treated bacteria was significantly damaged. These results suggested that degradation of the cytoplasmic membrane involves the toxic action of EOs. The concentration of membrane muropeptide of higher molecular weight was 6 times higher in the murein of cells treated with EOs. Also, the concentration of muropeptide of lower molecular weight in the presence of the EOs at their MIC was 2 times weaker than in the presence of 0.5 of the MIC concentration (Caillet *et al.* 2005, 2006a). Transmission electron microscope observations revealed that EOs significantly affect the cell wall structure. The authors concluded that the murein is not disrupted by antimicrobial treatments, but its composition and relative percentage of muropeptides were severely modified so that they became critical for the physical integrity of the cell wall. Ultee *et al.* (2000a, 2000b) also observed that carvacrol, one of the major components of oregano and thyme, can interact with the cell membrane, where it dissolves the phospholipid bilayer and is assumed to align between the fatty acid chains. 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.* 2002a, 2000b). Some EOs can inhibit the production of enzymes. For example, eugenol present in clove inhibits the production of amylase and proteases by *Bacillus cereus* (Farag *et al.* 1989). The hydroxyl group of eugenol is thought to bind to proteins, preventing enzyme action in *Enterobacter aerogenes* and cell wall deterioration and high degree of cell lysis were also noted (Thoroski *et al.* 1989; Wendakoon and Sakaguchi 1995). Other compounds do not affect the cell wall. For example, cinnamaldehyde inhibit the growth of *E. coli*, *Salmonella* and *E. coli* O157:H7 but do not disintegrate the outer membrane or deplete the intracellular ATP pool (Helander *et al.* 1998; Oussalah *et al.* 2006a). The carbonyl group is thought to bind to proteins, preventing the action of amino acid decarboxylases in *E. aerogenes* (Wendakoon and Sakaguchi 1995).

Chemical analysis of the most efficient oils like thyme, oregano and cinnamon had shown that the principal active constituents are principally carvacrol, thymol, citral, eugenol, 1,8-cineole, limonene, α -pinene, linalool and their precursors (Lataoui and Tantaoui-Elaraki 1994; Sikkema *et al.* 1995; Juliano *et al.* 2000; Demetzos and Perdetzoglou 2001). There are often large differences in the reported antimicrobial activity of oils from the same plant. The reasons of this variability can be due to the different geographical sources, the harvesting season, the genotype, the climate, the drying procedure and the distilled part of the plant. All of these variabilities influence the chemical composition and the relative concentration of each constituent in the EOs (Salzer 1972; Rhyu 1979; Arrebola *et al.* 1994; McGimpsey and Douglas 1994; Viollon and Chaumont 1994; Juliano *et al.* 2000). A number of EOs constituents exhibit significant antimicrobial properties when tested separately (Kim *et al.* 1995; Ultee *et al.* 1998; Ultee *et al.* 2000a, 2000b; Lambert *et al.* 2001). However, there is evidence that EOs are more strongly antimicrobial than is accounted for by the additive effect of their major antimicrobial components; minor components appear, therefore, to play a significant role (Lataoui and Tantaoui-Elaraki 1994; Paster *et al.* 1995).

Even though the essentials oils are generally regarded as safe (GRAS) (Kabara 1991; Lambert *et al.* 2001), their use is often limited to organoleptical criteria. For this reason, it is necessary to determine the MIC of pathogenic bacteria. The EOs that exhibit a low MIC have the potential to be added to food as an antimicrobial compound without affecting its sensorial quality. Oussalah *et al.* (2007a) evaluated

Table 2A Plant species, common name, origin, plant part source and main compounds of selected essential oils against pathogenic bacteria.

Plant species	Common name	Origin	Distilled part	Main compounds (Area %)
<i>Cinnamomum cassia</i>	Chinese cinnamon	China	Leaf-branch	Cinnamaldehyde (65), methoxy-cinnamaldehyde (21)
<i>Cinnamomum verum</i>	Ceylon cinnamon	Skri Lanka	Bark	Cinnamaldehyde (87)
<i>Cinnamomum verum</i>	Ceylon cinnamon	Madagascar	Leaf	Eugenol (63), β -caryophyllene (5)
<i>Coriandrum sativum</i>	Coriander	Russia	Fruit	Linalool (70), α -pinene (6)
<i>Corydothymus capitatus</i>	Spanish oregano	Spain	Flowering plant	Carvacrol (76)
<i>Cymbopogon citratus</i>	Lemongrass	Guatemala	Herb grass	Geranial (45), neral (32), limonene (9)
<i>Cymbopogon flexuosus</i>	Indian lemongrass	India	Herb grass	Geranial (46), neral (31)
<i>Cymbopogon martinii var. motia</i>	Indian palmarosa	India	Herb grass	Geraniol (80), geranyl acetate (9)
<i>Cymbopogon nardus</i>	Ceylon citronella	Skri Lanka	Herb grass	Geraniol (19), limonene (10), camphene (9)
<i>Cymbopogon winterianus</i>	Java citronella	Vietnam	Herb grass	Citronnellal (34), geraniol (21), Citronnellol (11)
<i>Eugenia caryophyllus</i>	Clove	Madagascar	Flower bud	Eugenol (78), eugenyl acetate (14)
<i>Inula graveolens</i>	Sweet inula	France	Flowering plant	Bornyl acetate (51), borneol (23), camphene (7)
<i>Lavandula hybrida reydovan</i>	reydovan lavandin	France	Flowering plant	Linalool (51), linalyl acetate (19), camphor (8)
<i>Lavandula latifolia spica cineolifera</i>	Spike cineole lavender	France	Flowering plant	Linalool (34), 1,8-cineole (22), camphor (15)
<i>Melaleuca linariifolia</i>	Narrow melaleuca	Australia	Leaf	Terpine ol4 (30), γ -terpinene (19), 1.8 cineole (14)
<i>Origanum compactum</i>	Oregano	Morocco	Flowering plant	Carvacrol (22), γ -terpinene (23), thymol (19)
<i>Origanum heracleoticum</i>	Greek oregano	France	Flowering plant	Carvacrol (54), paracymene (14), γ -terpinene (14)
<i>Origanum majorana</i>	Sweet marjoram	Egypt	Flowering plant	Terpinene-4-ol (26), γ -terpinene (12), thuyanol (10)
<i>Pimenta dioica</i>	Allspice	Antilles	Leaf	Eugenol (48), myrcene (27), geraniol (10)
<i>Satureja hortensis</i>	Summer savory	France	Flowering plant	Carvacrol (41), γ -terpinene (33), <i>p</i> -cymene (6)
<i>Satureja montana</i>	Winter savory	Slovenia	Flowering plant	Thymol (43), <i>p</i> -cymene (12), γ -terpinene (9)
<i>Thymus mastichina</i>	Borneol thymol	Spain	Flowering plant	1.8 cineole (47), linalool (24), limonene (7)
<i>Thymus satureoides</i>	Mother of thyme	Morocco	Flowering plant	Borneol (26), camphene (9), carvacrol (7)
<i>Thymus serpyllum</i>	Common carvacrol thyme	Albania	Flowering plant	Cavacrol (23), <i>p</i> -cymene (20), γ -terpinene (18)
<i>Thymus vulgaris carvacroliferum</i>	Common carvacrol thyme	France	Flowering plant	Cavacrol (32), <i>p</i> -cymene (24), thymol (12)
<i>Thymus vulgaris linaloliferum</i>	Common linalol thyme	France	Flowering plant	Linalool (60), linalyl acetate (10)
<i>Thymus vulgaris thuyanoliferum</i>	Common Thyjanol thyme	France	Flowering plant	Thujanol 4 (44), mycene 8-ol (13)
<i>Thymus vulgaris thymoliferum</i>	Common thymol thyme	France	Flowering plant	Thymol (38), <i>p</i> -cymene (19), γ -terpinene (17)

Adapted from Oussalah et al. (2007a).

Table 2B Minimal Inhibitory Concentration (MIC) and Maximal Tolerate Concentration (MTC) of selected essential oils against pathogenic bacteria.

Plant species	A	B	C	D
	MIC/MTC	MIC/MTC	MIC/MTC	MIC/MTC
<i>Cinnamomum cassia</i>	0.05/0.013	0.025/0.013	0.025/0.013	0.05/0.025
<i>Cinnamomum verum</i>	0.025/0.006	0.05/0.025	0.025/0.013	0.05/0.013
<i>Cinnamomum verum</i>	0.1/0.013	0.1/0.013	0.05/0.013	0.2/0.006
<i>Coriandrum sativum</i>	0.2/0.006	0.2/0.003	0.2/0.1	>0.8/0.2
<i>Corydothymus capitatus</i>	0.025/0.003	0.025/0.006	0.013/0.006	0.025/0.013
<i>Cymbopogon citratus</i>	>0.8/0.1	0.8/0.1	0.1/0.05	0.4/0.006
<i>Cymbopogon flexuosus</i>	>0.8/0.4	0.4/0.1	0.1/0.025	0.4/0.1
<i>Cymbopogon martinii var. motia</i>	0.2/0.1	0.2/0.025	0.1/0.025	0.2/0.013
<i>Cymbopogon nardus</i>	>0.8/0.1	0.8/0.013	0.4/0.1	0.8/0.013
<i>Cymbopogon winterianus</i>	>0.8/0.05	0.4/0.1	0.05/0.025	0.4/0.2
<i>Eugenia caryophyllus</i>	0.1/0.013	0.1/0.025	0.05/0.025	0.2/0.006
<i>Inula graveolens</i>	>0.8/0.013	>0.8/0.4	0.2/0.1	0.8/0.1
<i>Lavandula hybrida reydovan</i>	>0.8/0.1	0.4/0.1	0.8/0.4	>0.8/0.2
<i>Lavandula latifolia spica cineolifera</i>	>0.8/0.025	0.8/0.2	0.2/0.1	>0.8/0.4
<i>Melaleuca linariifolia</i>	>0.8/0.025	0.8/0.4	0.4/0.2	>0.8/0.1
<i>Origanum compactum</i>	0.025/0.006	0.05/0.006	0.013/0.006	0.1/0.013
<i>Origanum heracleoticum</i>	0.025/0.006	0.05/0.013	0.013/0.006	0.05/0.006
<i>Origanum majorana</i>	>0.8/0.013	0.4/0.025	0.2/0.05	>0.8/0.8
<i>Pimenta dioica</i>	0.1/0.025	0.1/0.025	0.1/0.025	0.2/0.006
<i>Satureja hortensis</i>	0.05/0.006	0.05/0.003	0.013/0.006	0.1/0.006
<i>Satureja montana</i>	0.05/0.013	0.05/0.013	0.013/0.006	0.05/0.013
<i>Thymus mastichina</i>	>0.8/0.003	>0.8/0.4	0.8/0.4	>0.8/0.006
<i>Thymus satureoides</i>	0.2/0.05	0.2/0.025	0.05/0.025	0.4/0.1
<i>Thymus serpyllum</i>	0.1/0.025	0.1/0.05	0.05/0.025	0.2/0.006
<i>Thymus vulgaris carvacroliferum</i>	0.05/0.003	0.05/0.006	0.025/0.013	0.1/0.013
<i>Thymus vulgaris linaloliferum</i>	>0.8/0.025	0.2/0.1	0.1/0.05	>0.8/0.4
<i>Thymus vulgaris thuyanoliferum</i>	0.8/0.05	0.4/0.1	0.4/0.2	>0.8/0.4
<i>Thymus vulgaris thymoliferum</i>	0.05/0.003	0.1/0.013	0.025/0.006	0.2/0.013

^aAccording to the data of the gas chromatography analysis of essential oils provided by the manufacturer.A: *E. coli* O157:H7B: *S. typhimurium*C: *S. aureus*D: *L. monocytogenes*

twenty eight EOs for their antibacterial properties, against four pathogenic bacteria: *E. coli* O157:H7, *L. monocytogenes* 2812 1/2a, *S. Typhimurium* SL 1344 and *S. aureus*.

Between them, *Corydothymus capitatus*, *Cinnamomum cassia*, *Origanum heracleoticum*, *Satureja hortensis*, *Satureja montana*, and *Cinnamomum verum* (bark) showed the low-

est MIC ($\leq 0.05\%$) for all bacteria tested. *Thymus vulgaris thymoliferum*, *Thymus serpyllum*, *Thymus satureioides*, *Cymbopogon martinii*, *Pimenta dioica*, *Cinnamomum verum* (leaf) and *Eugenia caryophyllus* showed a lower antimicrobial activity showing a MIC ranging between 0.05% and 0.4% (v/v) against the four bacteria tested. Also thirteen others EOs were less active showing a MIC value $\geq 0.8\%$ (v/v) (Table 2).

Even if the organoleptic impact is important, storage and cooking can enhance the degradation of EOs permitting the use of a higher concentration of EOs. It was observed for example that the addition of 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 MAP and vacuum at 5°C (Skandamis and Nychas 2001). The flavour of oregano also gradually decreased during storage at 2°C (Mejlholm and Dalgaard 2002). Fish cubes inoculated with *S. Typhimurium* (10^7 CFU/mL) were treated by immersion with carvacrol, citral or geraniol at 0.5, 1.5 and 3% (w/v) and a dose of 3% showed a complete inhibition of the bacteria in the presence of carvacrol and geraniol (Kim *et al.* 1995b). The addition of 0.9% (v/w) in a coating formulation sprayed on cooked shrimps had no detrimental effect on the appearance and flavour; however at higher concentration (1.8%) the coating decreased significantly the acceptability of shrimps (Ouatara *et al.* 2001). Carvacrol also affects the taste of fish (Kim *et al.* 1995b). It was found also that some compounds develop a more pronounced odour during storage. This phenomenon is observed after addition of thyme and oregano on whole sea bass at 0.05% (Mejlholm and Dalgaard 2002).

The food composition, the pH of the food, the temperature of storage and of the process, the external condition of storage (e.g. atmosphere packaging) affect significantly the efficiency of the EOs. Some compounds (e.g. mint oil) are effective against *S. enteritidis* in low fat yoghurt and cucumber salad (Tassou *et al.* 1995). It seems that the EOs are more effective in vegetables since these have a low fat content (Singh *et al.* 2002). The antimicrobial activity of sage increased with an increase in water content but decreased with an increase in fat and protein content (Shelef *et al.* 1984). Inhibition of *S. Typhimurium*, *S. aureus*, and *Pseudomonas* sp. was higher in broth (minimal inhibitor concentration (MIC) = 0.1-1%) and diminished in rice (MIC = 0.4->2.5%) and little or no inhibition was observed in meat at <2.5%. According to Skandamis and Nychas (2000), the activity of EOs depends on the pH, the storage temperature and EO concentration. A higher antimicrobial activity was observed at high temperature (15°C as compared to 10 and 5°C) and low pH (pH 4 as compared to pH 4.5 and 5).

Also, some compounds are not effective alone, however, when combined together, a synergistic effect could be found. For example, the use of spice blends such as chilli powder (red pepper, onion, paprika, garlic, cumin and oregano) and oriental five (pepper, cinnamon, anise, fennel, and cloves) in food produced powerful antimicrobial effects (Ceylan and Fung 2004). Lemon juice can synergistically enhance the antimicrobial effects of other spices (Mahrou *et al.* 2003a). In acidic environments the synergistic effect of lemon was higher due to the high amount of undissociated form of the acid (Ceylan and Fung 2004). The addition of essential oil under acidic conditions can also dissolve and/or attack to the lipid phase of the bacterial membrane (Skandamis and Nychas 2000). *p*-Cymene is not an effective antimicrobial compound, however, when combined with carvacrol, synergism has been observed against *B. cereus* (Ultee *et al.* 2000a, 2000b). It seems that *p*-cymene, incorporated in the lipid bilayer of bacteria, facilitates transport of carvacrol across the cytoplasmic membrane (Ultee *et al.* 2002a, 2002b). A mixture of cilantro, coriander, dill and eucalyptus EOs at different ratios resulted in an additive synergistic or antagonist effects (Delaquis *et al.* 2002). According to Moleyar and Narasimham (1992) a mixture of

cinnamaldehyde and eugenol can inhibit *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp., and *Enterobacter* sp. for more than 30 days completely at a concentration as low as 500 µg/ml. Rosemary extract have little bactericidal effect on the growth. A concentration of 5% was needed to obtain a bactericidal effect of *S. aureus* in mechanically deboned poultry meat (Farbood *et al.* 1976). However, the addition of sage and citric acid to rosemary can inhibit Gram-positive bacteria, *L. monocytogenes*, *L. innocua*, *S. aureus* and *Lactobacillus brevis* at 400 ppm in culture media. The use of EOs can also reduce the level of chemical additives. The addition of 0.08% spice extracts in mechanically deboned chicken frankfurters to substitute for nitrites and nitrates increased the lag phase of microbial growth for an extended period of time when stored at 7.2°C (MacNeil and Mast 1973). The use of carvacrol, cinnamaldehyde, thymol, and oregano oil at 0.1-2% can inhibit the growth of toxin production by *Clostridium perfringens* during chilling time of ground poultry (Juneja and Friedman 2007). Finally, it seems that most of the EOs are slightly more active against Gram-positive than Gram-negative bacteria (Lambert *et al.* 2001; Harpaz *et al.* 2003). It seems that the outer membrane surrounding the cell wall of Gram-negatives restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (Ratledge and Wilkinson 1988; Vaara 1992). However, a study testing 50 commercially available EOs against 25 genera found no evidence for a difference in sensitivity between Gram-positive and Gram-negative organisms (Deans and Ritchie 1987).

Since the active compounds are not stable over time and during processes, some studies have evaluated the possibility to protect the compound during storage and also to assure a controlled release during storage time. One study found that encapsulated rosemary oil was much more effective than standard rosemary EOs against *L. monocytogenes* in pork liver sausage; however the effect of EOs concentration was not evaluated in this study (Pandit and Shelef 1994). More recently, milk protein-based edible films containing oregano and/or piment EOs were applied on beef. Oregano-based films (1% w/v) were the most effective films to protect against the growth of *E. coli* 0157:H7 or *Pseudomonas* sp. (Oussalah *et al.* 2004). Spanish oregano, Chinese cinnamon and winter savory EOs were used to protect the quality of bologna and ham. The EOs were immobilized in alginate-based films and applied on prepared meat. The results showed that the polymer was able to protect and assure a control release of the active compounds present in the EOs and protect the meat against pathogen contamination during storage (Oussalah *et al.* 2007b).

Antioxidant properties

Lipid oxidation is one of the major concerns in food technology (Yanishlieva *et al.* 2006). With the growing interest in natural antioxidants found in plants EOs from spices and herbs are the most important targets. The antioxidant properties of herb extracts vary depending of the food system. This is due in part to the composition of the extract and the food. It seems that the more polar antioxidants are more active in pure lipids and non-polar antioxidants are more active in a polar substrate (Frankel *et al.* 1996). Rosemary is one of the most effective spice extracts widely used in food processing. Rosemary was considered as both lipid antioxidant and meat chelator. According to Bracco *et al.* (1981), the antioxidant properties of rosemary is related essentially to the presence of carnosol and carnosic acid. Several other diterpenes such as rosmaridiphenol and rosemaniquinone have also been reported to contribute to the antioxidant properties of rosemary extract. Some of these compounds have greater antioxidant properties than synthetic compounds. For example carnosic acid is more active than BHT and BHA (Auroma *et al.* 1992); rosmanol and carnosol are more effective than BHA, BHT and α -tocopherol (Nakatani and Inatani 1983; Yanishlieva *et al.* 2006). According to the authors, rosemary extract inhibits

the formation of polar substances and polymers and the decomposition of polyunsaturated triacylglycerols. Rosemary antioxidants were found suitable for deep-frying of edible oils (Gordon and Kourimska 1995a), especially in presence of ascorbil palmitate (Gordon and Kourimska 1995b). Sage extract is also in the same family as rosemary (*Labiatae*). It is not surprising to find the presence of carnosol, carnosic, rosmadial and rosmanol as well as an important antioxidant property (Schwarz and Ternes 1992; Cuvelier *et al.* 1994). Oregano has both antioxidant and antimicrobial properties. It is primarily composed of carvacrol and thymol (Lagouri *et al.* 1993). It was found that thymol was a better antioxidant for triacylglycerols of sunflower than those of lard (Yanishlieva *et al.* 1999). Thyme and oregano are rich in thymol and carvacrol and are also known to inhibit lipid peroxidation (Yanishlieva *et al.* 1999). Mild oregano, strong oregano, sage and mint showed also good antioxidant properties (Caillet *et al.* 2007). Spanish oregano, pimento and oregano-pimento (1:1) EOs were immobilized in milk proteins-based films for meat preservation (Oussalah *et al.* 2004). The antioxidant properties of the films were evaluated and showed that oregano-based films were able to stabilize lipid oxidation in beef muscle, whereas pimento-based films presented the highest antioxidant activity. Salmieri and Lacroix (2006) have incorporated Spanish oregano, Chinese cinnamon or winter savory as natural antioxidant and antimicrobial agents in alginate/polycaprolactone-based films. The antioxidant properties have shown that the oregano-based films had the highest antiradical properties. Oregano-based films showed also a better level of active compounds in films during storage of whole beef, ham and bologna as compared to cinnamon or savory based-films (Oussalah *et al.* 2006b, 2007b). These results mean a better control release of the active compounds during storage. Nutmeg, mace and turmeric also have good antioxidant properties. Some examples have been reported on lard (Chipault *et al.* 1952, 1955) and on oil for the protection of α -tocopherol (Beddows *et al.* 2000). A strong synergistic effect was found between the rosemary extract (0.02%) and α -tocopherol (0.05%) in sardine oil for protection against lipid oxidation at 30°C and in frozen-crushed meat models (Wada and Frang 1992). The combination of Butylated HydroxyToluene (BHT) and rosemary or a combination of sage and citric acid has a synergistic effect for the prevention of lipid oxidation (Jaswir *et al.* 2004). Oleoresin rosemary and sage were also found to be effective phytochemical antioxidants, protecting palm olein against oxidative deterioration during frying (Jaswir *et al.* 2005). Other compounds like lecithin, ascorbyl palmitate, and amino acid salts of phosphatic acids, amino acids and lower peptides could be used to act in synergy with the herb or spice extracts. The activities of the extracts are higher in food containing protein, as sulphur and amino groups of the polypeptide chains interact with hydroperoxides, thus decreasing the free radical level (Pokorny 1999).

Essential oils in combined treatments

The activities of spice extracts could be increased by synergistic activity of other harmless compounds. Simultaneous application of a bacteriocin (nisin) and an essential oil (carvacrol or thymol) caused a larger decline in viable counts for strains of *B. cereus* than was observed alone. The use of spice extract in combination with sodium chloride, sugar and organic acids might provide a synergistic effect during the thermal process in controlling microbial growth (Shelef *et al.* 1980). When mild heat treatment (45°C, 5 min.) was applied a higher reduction of viability could be achieved (Periago *et al.* 2001). Mild heat treatment (45°C, 30 min.) in the presence of carvone could permit a 1.31 log reduction of viable cell numbers of *L. monocytogenes*. According to the authors, the high degree of unsaturation causes the cell membranes to be more fluid at 45°C than the membranes of cells grown at 8°C. This higher 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). Low oxygen levels can enhance the antibacterial efficiency of EOs. The use of vacuum packing increase the efficiency of oregano when applied on beef fillets (Paster *et al.* 1990). The lethal effect of clove and coriander EOs is more pronounced on *Aeromonas hydrophila* on pork loin when packed under vacuum (Stecchini *et al.* 1993). The application of oregano before packing minced beef under modified atmosphere conditions can delay the microbial growth as compared to the packing under air where no shelf life extension was found (Skandamis and Nychas 2001) and modified atmosphere is effective in controlling the growth of *Clostridium jejuni* (Paster *et al.* 1990). Low oxygen tension might yield less oxidative changes in the oils and/or anaerobic conditions might increase the sensitivity of bacterial cells to the antimicrobial activity of the oils (Ceylan and Fung 2004). The antimicrobial activity of thyme oil or thymol against *S. aureus* and *S. Typhimurium* was greater under anaerobic conditions (Naijre *et al.* 1996). The addition of EOs before a cold pasteurization can significantly increase the radiosensitivity of bacteria in meat. Mahrouir *et al.* (2003) observed that a marinade treatment before irradiation is highly effective, and has an additive effect with gamma irradiation (0, 3 and 5 kGy) to reduce the microorganism load, and to control the proliferation of the total endogenous microorganisms during storage of poultry. No *Salmonella* was observed during 12 days of storage in marinated and irradiated chicken at 3 kGy. A dose of 5 kGy was needed to eliminate *Salmonella* in non-marinated poultry. More recently, Chiasson *et al.* (2004, 2005) evaluated the effect of thyme or its principal constituents (carvacrol or thymol) addition on ground beef, on the radiation D_{10} values for *E. coli* and *S. Typhi*. The results of these studies have demonstrated that the addition of carvacrol significantly reduced the radiation D_{10} of both bacteria. An additive effect of carvacrol addition and packaging under modified atmosphere was also observed on bacterial radiosensitization. Compared to the samples packed under air, the relative sensitivity was increased from 2 to 2.7 for *E. coli* and to 10 for *S. Typhi*. Caillet *et al.* (2006b, 2006c) obtained similar results when irradiation (0.07-2.5 kGy) was done on carrots coated with edible coating containing *trans*-cinnamaldehyde in order to eliminate *L. monocytogenes* ready to use carrots. Moreover, the results indicated that elimination of *L. monocytogenes* by irradiation was done at a lower dose when irradiation was done under modified atmosphere. These results indicated that the combination of natural antimicrobial compounds, irradiation and modified atmosphere play an important role in the radiosensitization of *L. monocytogenes*. A marinated mixture containing thyme and rosemary in lemon juice was also able to protect against the phospholipid oxidation present in chicken legs and preserve their sensorial quality during irradiation treatment (Mahrouir *et al.* 2003b). Lacroix *et al.* (2000) reported that natural antioxidants from rosemary and thyme caused a substantial reduction in the generation of volatile hydrocarbons from arachidonic and linoleic acids generated during irradiation at 3 and 9 kGy.

BACTERIOCINS

Fermentation is a well known process in order to preserve food quality. LAB have been used for centuries in the fermentation of variety of dairy products (O'Sullivan *et al.* 2002). LAB include the genera *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Carnobacterium* and *Propionibacterium* (Nettles and Barefoot 1993; Savadogo *et al.* 2007). Antimicrobial activities of LAB have been demonstrated in various species and their antagonist actions are demonstrated against numerous intestinal and food-borne pathogens such as *E. coli*, *L. monocytogenes*, *S. aureus*, *Clostridium difficile* and others (Heik-

kila and Saris 2003; Mahoney and Henriksson 2003; Ghairi *et al.* 2004; Plummer *et al.* 2004; Millette *et al.* 2007a). The preservative ability of LAB in foods is attributed to the production of anti-microbial metabolites. These metabolites include organic acids, diacetyl, carbonyl dioxide, hydrogen peroxide, reuterin, derivatives of lactic acid such as hydroxyl lactic acid and also small peptides designated bacteriocins (Millette *et al.* 2007b). Bacteriocins have attracted attention as potential substitutes for antibiotics to cure and/or prevent bacterial infections and are widely employed in food preservation (Riley and Wertz 2002). Bacteriocins have been shown to have potential in the preservation of meat, dairy products, canned food, fish, alcoholic beverage, salads, egg products, high-moisture bakery products, and fermented vegetables (Chen and Hoover 2003). When screened for food applications, the producing strain should have GRAS; the bacteriocin should have a broad spectrum of inhibition; the bacteriocin should be heat-stable and have no associated health risks; the inclusion of the bacteriocin in the food products should lead to beneficial effects such as improved safety, quality and flavour; it should have high specificity and potential in the biopreservation of food (Cotter *et al.* 2005).

Bacteriocins are divided into three or four groups (Nes *et al.* 1996; Cleveland *et al.* 2001). Class I bacteriocins are small peptides that have from 19 to 38 and sometimes more than 50 amino acids and are characterized by their unusual amino acids such as lanthionine, dehydrobutyrine and dehydroalanine. Generally the elongated amphiphilic cationic antibiotics (e.g. nisin) are active through the formation of pores, leading to the dissipation of membrane potential and the efflux of small metabolites from sensitive cells. Globular antibiotics (e.g. mersacidin) act through enzyme inhibition. However, the binding of nisin to lipid II facilitates a dual mechanism of action involving the prevention of peptidoglycan synthesis and pore formation (Cotter *et al.* 2005). Class Ia consist of cationic and hydrophobic peptides that form pores in target membranes and have flexible structure compared to the more rigid class Ib. Compared to Class Ia bacteriocin, Class Ib bacteriocins are globular peptides and have no charge or a net negative charge (Altena *et al.* 2000). Class II bacteriocins contains small (< 10 kDa) and heat-stable, non modified peptides. The majority of class II bacteriocins are active in the nanomolar range by inducing membrane permeabilization and the subsequent leakage of molecules from target bacteria (Cotter *et al.* 2005). Class IIa bacteriocins includes pediocin- active peptides with a conserved N-terminal sequence Tyr-Gly-Asn-Gly-Val and two cysteines forming an S-S bridge in the N-terminal half peptide. Pediocin-like bacteriocins have a narrow spectrum of activity but display a high specificity against *L. monocytogenes* (Herchard and Sahl 2002). Class III bacteriocins are normally large and heat labile. Their mechanism of action is distinct from that of bacteriocins as they function through the lysis of sensitive cells by catalyzing cell-wall hydrolysis (Cotter *et al.* 2005). The Class IV consist of bacteriocins forming complexes with other macromolecules (Klaenhammer 1993).

Antimicrobial properties

The incorporation of bacteriocins as a biopreservative ingredient into model food systems has been studied extensively and has been shown to be effective against the growth of pathogens and spoilage microorganisms in foods. Numerous bacteriocins with potential biopreservatives including various nisins, lacticins, lactostrepcins, lactococcins and lactocins (Carolissen-Mackay *et al.* 1997; Moll *et al.* 1999; Twomey *et al.* 2002), sakacin B (Samelis and Roller 1994), plantaricin C (Gonzalez *et al.* 1994), leucocin A-UAL187 (Leisner and Greer 1996), enterocin A (Ennahar and Deschamps 2000), carnocin U149 (Stoffels and Sahl 1993), piscicolin 126 (Jack *et al.* 1996), divercin V41 (Duffes *et al.* 1999) are produced by LAB. Several strains of *Pediococcus* genera such as *P. acidilactici*, *P. pentosa-*

ceus and *P. parvulus* were found to produce pediocins. Pediocins thus far described have been reviewed by Ray and Miller (2000). Millette *et al.* (2007c, 2007d) were also the first to isolate bacteriocin-producing strains of *P. acidilactici* MM33 and *Lactococcus lactis* subsp. *lactis* MM19 from human intestine. Their study showed that the supernatant of the culture have important antimicrobials properties. The antimicrobial metabolites were heat-stable, and were active at a large pH range (2-10). The supernatant from *Lactococcus lactis* culture inhibited *Enterococcus faecium*, various species of *Lactobacillus* and *S. aureus* while those in the supernatant from the *P. acidilactici* culture inhibited *Enterococcus* spp., some lactobacilli and various serotypes of *L. monocytogenes*. The minimal inhibitor concentration obtained for *L. monocytogenes* was 200 AU/ml showing that these strains might be useful for control of enteric and food pathogens (Millette *et al.* 2007d). Purification of the bacteriocin was performed by cationic exchange chromatography followed by a reverse phase step. Biochemical, mass spectrophotometry, plasmid and amino acid sequence analysis showed that the bacteriocin secreted by *P. acidilactici* is identical to pediocin PA-1 (Millette *et al.* 2007d).

These antimicrobial metabolites can inhibit or destroy target microorganisms such as molds, yeasts, vegetative bacteria and bacterial spores. However, bacteriocins are produced in the ribosome and kill related bacteria (Klaenhammer 1993). Also, studies applied on food systems should be done to confirm their efficiency in presence of different nutrients and under different media, processes and storage conditions. Liu and Hansen (1990) have observed that nisin is significantly more soluble at pH 2 than pH 8. Davies *et al.* (1999) have observed that lower fat content is correlated with higher nisin activity. It seems that the effective concentration of nisin depends also of the food product. For example, the effective concentration of nisin in kimchi (lactobacilli), ricotta cheese (*L. monocytogenes*), bologna (*Lactobacillus sake* and *Lactobacillus curvatus*), cottage cheese (*L. monocytogenes*), and skim milk (*B. cereus* spores) are respectively (100, 100, 1000, 2000, 4000 IU/ml) (Ferreira and Lund 1996; Davies *et al.* 1997; Davies *et al.* 1999; Wandling *et al.* 1999; Choi and Park 2000).

Among the studies done, pediocins (Class IIa bacteriocins) were evaluated for their effectiveness to control *L. monocytogenes* contamination in food, for example during manufacture of chicken summer sausage, and also for the preservation of wine and baked products (Baccus-Taylor *et al.* 1993; Schoeman *et al.* 1999). Pediocins were also evaluated on cheese sauce and showing that when 100 AU/ml is added, a 5 log reduction of *L. monocytogenes* was observed (Pucci *et al.* 1998). Leucocin showed effectiveness to delay the spoilage of meat by *L. sake* for up to 8 weeks (Leisner *et al.* 1996). Enterocin showed a good ability to control *L. monocytogenes* growth in ham, pork, chicken breast, pate and sausage under several conditions (Aymerich *et al.* 2000a, 2000b). Lacticin 3147 was also able to control *L. monocytogenes* in cottage cheese. A 99.9% reduction in the numbers of *L. monocytogenes* was observed within 5 days at 4°C (McAuliffe *et al.* 1999). Sakacin A was also used to control *L. monocytogenes* in ground beef (Schillinger *et al.* 1991). Nisin is an antimicrobial peptide or Class Ia bacteriocin, produced by several strains of *Lactococcus lactis*. This bacteriocin is approved for use in over 40 countries and has been in use as a food preservative for more than 50 years (Cleveland *et al.* 2001). Nisin is also recognized as GRAS by the United States Food and Drug Administration as stated in the Code of Federal Regulations (CFR section 184.1538). This bacteriocin can be added directly to cheese to prevent the growth of *Clostridium* and *Listeria* (Hirsch *et al.* 1951; Chung *et al.* 1989; Siragusa *et al.* 1999; Cleveland *et al.* 2001) and liquid egg ingredients (Siragusa *et al.* 1999) or combating contamination in wines by *Leuconostoc* and *Pediococcus* (Radler 1990a, 1990b; Daeschel *et al.* 1991). Nisin has been used throughout the world to preserve salad dressings (Delves-Broughton *et al.* 1996), canned foods

(Thomas *et al.* 2000) or meat (Cutter and Siragusa 1998). Nisin addition (2000 IU/g) in long-life cottage inoculated with 10^4 cfu/g of *L. monocytogenes* can reduce by 1000-fold the content of this bacteria after 7 days storage at 20°C (Ferreira and Lund 1996) and a complete growth inhibition of this bacteria was observed during 55 days in ricotta cheese stored under refrigeration when 2.5 mg/l of nisin was added in presence of 10^3 cfu/ml. Nisin is now known to form poration complexes in target cell membranes through a multi-step process that includes binding of the C-terminal via electrostatic interaction, then, the N-terminal part of nisin is inserted into the lipid phase of the bilayer. A depletion of the transmembrane potential ($\Delta\psi$) and/or the pH gradient, resulting of the leakage of cellular materials (Okerke and Montville 1992). It then results in the rapid efflux of small cytoplasmic compounds, e.g. amino acids, potassium, inorganic phosphate, and ATP, and finally to the cell death (Bauer and Dicks 2005). However, the effectiveness of nisin on meat surface is dependent of the meat system and the type of micro-organisms (Davies *et al.* 1999). Antimicrobial efficacy of nisin is dependent upon numerous factors including salt and fat content, basic pH, presence of curing agents and food particle size (Jung *et al.* 1992). Binding of nisin to food components make it unavailable to inhibit microorganisms or reduce its solubility and its dispersion throughout the foodstuff which decrease its antimicrobial capacity. Nisin is also relatively insoluble due to its hydrophobic nature and loses its efficacy at pH >5 (Scannell *et al.* 1997; Pol and Smid 1999). Storage temperature, MAP, state of the meat (raw or cooked) and the presence of other preservatives can also affect nisin efficacy (Murray and Richards 1998). Organisms normally sensitive to nisin *in vitro* are not necessarily sensitive to nisin in a meat system. For example, the concentration needed to inhibit *C. botulinum* spores is significantly higher in cooked meat than in nutritive media (Scott and Taylor 1981). According to Rose *et al.* (1999), nisin could be inactivated by the presence of small amount of glutathione, a low molecular mass thiol compound present in fresh raw ground beef. Cutter and Siragusa (1996) observed a lack of effectiveness to control the growth of *Brochothrix thermosphacta* when nisin was applied directly on beef carcass surface for a long period of storage. Nisin can also bind sulfudryl groups or meat particles and interacts strongly with phospholipids which limit its activity in meat with a high fat content (Henning *et al.* 1986; Chung and Dickson 1989). However, nisin is stable at low temperature and could be used for meat preservation. Also, nisin in combination with lower concentrations of nitrate can prevent the growth of *Clostridium* in meat products (Rayman *et al.* 1981, 1983). In order to protect the nisin against deleterious agents in fresh beef and to improve the efficiency of this bacteriocin, nisin (500 or 1000 IU/mL) was immobilized into palmitoylated alginate-based films or in activated beads in order to control a pathogen in meat product. Results demonstrated a 2 log reduction of *S. aureus* after one week of storage of slice meat covered by the film containing 1000 IU/mL of nisin (Millette *et al.* 2007b). The palmitoylation of alginate can be used to produce hydrophobic and water-vapor resistant beads and films. These matrices act also as a control delivery vehicle or to protect the bioactivity of the immobilized compound (Ie Tien *et al.* 2004). Nisin was also immobilized in plastic film for food application. Their results have shown that nisin-activated film may inhibit *Micrococcus luteus* ATCC 10240 in tryptic soya broth and control bacterial growth in raw and pasteurized milk during storage. The release of nisin was pH and temperature dependant but was able to maintain food quality, safety and extending the shelf life of milk when stored at 4°C during 7 days (Mauriello *et al.* 2005).

Bacteriocins in combined treatments

The narrow inhibitory spectrum, the poor penetration and solubility in meat, and the possible degradation by native proteolytic enzymes in meat are the major limits

uses in food (Mahapatra *et al.* 2005). Food treated with bacteriocins could not be safer just by the utilization of bacteriocins. The use of combined treatments with bacteriocins could have synergistic effects and the combination may be effective in controlling the microorganisms in food and assure the sensorial quality of foods. For example, high hydrostatic pressure inactivation of *E. coli* and *L. innocua* inoculated in liquid whole egg was improved significantly when done in presence of nisin at 1.25 and 5 mg/l. A reduction of almost 5 log¹⁰ units for *E. coli* and more than 6 log¹⁰ units for *L. innocua* was obtained at 450 MPa and 5 mg/l of nisin (Ponce *et al.* 1998). The combined effects of moderate heat (60°C, 5 min or 65°C, 2 min) in presence of nisin (25 mg/kg) increase the sensitivity of *L. monocytogenes* in cans of cold-pack lobster (Budo-Amoako *et al.* 1999). The use of bacteriocins and EOs combination is also an effective treatment. According to Singh *et al.* (2001), the use of nisin (0-200 IU/ml) and garlic extract (0-6 mg/ml) could help to overcome the problem of nisin resistance in Gram-positive bacteria. It seems that the presence of garlic extract can improve the activity of nisin in broth media and in food system (hummus). This phenomenon was also observed in presence of other food additives and spices extracts (James and Johnson 2001). Other natural additives like organic acids (polylactic and lactic) at 2% are more effective when added in presence of bacteriocins (nisin) at 200 IU/mL. This combined treatment was effective to lower the microbial population of psychrotrophic, *Enterobacteriaceae*, *Pseudomonas* and *Lactobacillus* and to inhibit *L. monocytogenes* for 42 d in vacuum-packaging beef (Ariyapitipun *et al.* 1999, 2000). Nisin (500 IU/g) was also mixed with 1.5% (sodium lactate or sodium citrate) and this combined treatment was also effective to control the growth of *Clostridium perfringens* DSM 756, *Salmonella Kentucky* AT 1, non-pathogenic *L. innocua* DPC 1770 and increase protection against common pathogenic contaminants in fresh pork sausage (Scannell *et al.* 2000). The used of enzymes (lactoperoxidase) and other proteins (lysosyme, lactoferrin) could be useful to increase the bacterial sensitivity in food system i.e. milk to inhibit *L. monocytogenes*, to inhibit *E. coli*, *Salmonella* and *Campylobacter* in beef and dairy products (Boussouel *et al.* 2000; Davidson and Harrison 2002). The use of bacteriocins in combination with irradiation was also evaluated to determine their efficiency to eliminate *L. monocytogenes* on frankfurters. An addition of 6000 AU of pediocin and irradiation of 2.3 kGy was effective for inhibition of the pathogen for 12 weeks at 4 or 10°C (Chen *et al.* 2004).

CONCLUSION

A number of EOs and some of their constituents have important antimicrobial properties against food-borne pathogens. A high concentration of phenolic compounds is a key factor for the efficiency of the biological activity of the EOs. The organoleptic quality could be protected by selection of the extracts or the compounds, the determination of the maximal concentration that could be added in the product, the treatment applied and the storage conditions of the food. Synergistic effects could be obtained by combination of compounds and/or treatments. As alternative to the traditional chemical preservatives, bacteriocins have also considerable promise. However, bacteriocins do not have a broad host range. The use of EOs in combination with bacteriocins and with a combined treatments could be effective in synergistic effects and the combination may be effective in controlling the microorganisms in food, assure the safety of food and protect the sensorial quality of foods.

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