Plant Spices as Sources of Natural Antimicrobials for Food

Guadalupe Virginia Nevárez-Moorillón

ABSTRACT

Plant spices are described as those parts of the aromatic plants which have special aroma and flavor compounds that can be extracted in aromatic essential oils. The chemical nature of antimicrobials in spices suggests that they act in the natural defense mechanism of plants. Spices have been used in food preparation in warm climate countries, where they are commonly found and used in spicy traditional cuisine. Worldwide concern on the use of toxic preservatives in food has led to the combination of mild antimicrobial barriers, according to the hurdle technology concept. Spices and their essential oils are one of the most important natural antimicrobials considered in this application. Studies on the antimicrobial effect of spices as natural antimicrobials have been conducted in two areas: growth inhibition in broth or agar cultures, and the addition of spices or essential oils to food systems. When the spice or the essential oil is added to a food system, the study of the antimicrobial effect is complex, since there are interactions of the natural antimicrobials with the food components. Most of the time, the concentration of the natural antimicrobial that is inhibitory or bactericidal under controlled culture conditions, is not efficient when added to a complex food system. Therefore, even though there are multiple references on the antimicrobial effect of spices, there are still many questions to be answered, from the mechanism of action and the best conditions for their use in food systems, to the study of those not-yet analyzed spices around the world.

Keywords: antimicrobial tests, food systems, hurdle technology

INTRODUCTION

An ancient quest of humanity is to seek the best method for food preservation, so that it can be transported, distributed and consumed safely. Man has developed many food preservation methods, from the antique use of sugar for fruit preservation, to the use of salt or drying of meat. Along with sugar and salt, spices have been used as food additives. Development of food preservation methods includes the use of physical or chemically based methods, and recently biological based methods are important in food industry (Gould 1996).

Currently, the food industry uses a very large amount of chemical antimicrobials, and their use will continue to grow. The most common antimicrobials used include salt, sugar, organic acids and benzoates. Many of those antimicrobials, especially organic acids, have limited spectra of usage. Their antimicrobial action is determined by physicochemical characteristics of food, especially pH. Chemical antimicrobials can be synthesized or can be obtained from natural sources, but excess use of artificially synthesized antimicrobials has generated rejection from the consumer, who is constantly being informed of collateral effects of an abuse in chemical additives in food. Among the problems reported are food allergies and risk analysis data that correlate some food additives such as nitrate, to cancer. The ideal characteristics of a good antimicrobial are: a wide antimicrobial spectrum, not toxic to humans, effective at low concentrations, minimally affected by the food pH and no notorious effects on odor, color or flavor at the concentration required for its antimicrobial action. The antimicrobial will be more valuable if it is presented as a powder, soluble in water, not corrosive, stable during storage, but above all, not expensive (Doyle and Beuchat 2007).

Worldwide tendencies on minimal food processing and the reduction on the use of synthetic additives, has revitalized the search for new antimicrobials, especially those obtained from natural sources. Efforts have now concentrated on the cultural traditions of ethnic groups, especially in gastronomy and traditional medicine. In food preparation, the importance of spices as food additives has been particularly recognized. Besides the sensorial improvement of food flavor due to the addition of spices, their antimicrobial effects can help to increase the food shelf life (Nielsen and Rios 2000; Ates and Erdogan 2003). Spicy food plates are common in warm climates, where they also function as food antimicrobials (Sherman and Flaxman 2001).

More than 1,300 plants with recognized antimicrobial
properties have been documented (Velluti et al. 2003), and approximately 80 food products of plant origin have a high concentration of antimicrobials, including basil, cinnamon, clove, garlic, leek, mustard, pepper, thyme, oregano and rosemary (Cowan 1999; Draughon 2004). In recent years, the search for new antimicrobials has increased, especially considering the development of alternative food treatment technologies and the growing market for minimally processed food products (Ponce et al. 2004; Leite de Souza et al. 2005).

The interest in spices has also increased, especially in the characterization of its components and its effects on biological systems for applications in a wide variety of areas, including pharmaceutical applications (Adam et al. 1998; Ates and Erdogruz 2003; Lin et al. 2005), alternative medicine and natural therapies (Kalemba and Kunicka 2003; Nostro et al. 2004), in control of phytopathogens (Bouchra et al. 2003; Daferera et al. 2003; Jajakumar et al. 2007) by the use of plant extracts, or as additives in the food industry (Cutter 2000; Ceylan et al. 2004; Dorman and Deans 2000; Leite de Souza et al. 2005). In particular in food, the restrictions imposed by regulatory agencies on the abuse of chemicals added preservatives (Guynot Leite de Souza 2007) found a different composition in the essential oil of different aromatic plants, depending on their collection dates. It has also been reported variation on the antifungal activity of the essential oil during different seasons, being more potent the essential oil obtained from May to November (Mishra and Dubey 1994). European oregano (Origanum vulgare spsh. Hirtum) composition has also qualitative and quantitative differences depending on the growth conditions and the time of collection. Fluctuations are so important, that concentrations of the main components, carvacrol, thymol and cymene, vary up to 10 times among samples (Jerkovic et al. 2001).

In another report, the concentration of thymol in plants of Thymus vulgaris from Brazil varied from 31 to 52% depending on the harvesting time. The percentage of recovered oil also varied from 0.2 to 0.3% (Ahti-Santos et al. 2004). The biological activity and composition of savory (Satureja pannassica, Satureja thymbra) essential oil was also affected by harvesting time, as reported by Chorianopoulos et al. (2000). In these experiments, moisture content did not have a significant effect on the content and composition of essential oil in Mexican oregano (Lippia berlandieri Schauer). Plant age, on the other hand, did have an effect on the amount of essential oil recovered; being the youngest plants the ones that contained a higher concentration (Dunford and Silva 2005).

Even though the antimicrobial activity of essential oils is attributed mainly to the components present in larger quantities, it is important to consider the antagonistic or synergistic effect of minor components that affect the overall effect. Each component in the essential oil has its own contribution on the biological effect of the whole mixture; it is well known that the essential oil mixture is more effective than the components added separately (Daferera et al. 2003). Although usually one or two components are responsible for most of the antimicrobial activity, the effect of individual compounds is usually done to identify the main compounds responsible for the biological activity (Bouchra et al. 2003).

The analysis of the components present in spices’ essential oils can also be used as a tool to characterize different plant species. In many cases, plants of different genera or even different plant families are recognized as the same or similar spice. The use of chemical analysis for taxonomic purposes has been proven for identification of oregano from marjoram (Baranska et al. 2005), as well as for oregano from different geographical locations (Kokkin et al. 2004). The variability of oregano species were evaluated by D’Antouno et al. (2000) considering their growth areas, and the essential oil was classified into three groups according to its composition: the first group had a high content of compounds derived from the metabololic pathways of carvacrol and thymol; the second group was characterized by a different composition of sesquiterpenes and a large proportion of linalool, and the third group had abundant quantities of sesquiterpenes. In the search for optimization of essential oil extractions, new methodologies such as supercritical extraction have been studied. Sub-critical and super-critical fluid extraction using CO₂ as solvent has been tested on European aromatic plants, since it is not toxic or explosive and is easily removed from the essential oil (Gaspar et al. 2000; Areila-Lozano et al. 2004). The use of supercritical CO₂ gives higher yields of essential oils, and a total of 24-27 compounds were found in the essential oil of oregano; the distribution of the compounds extracted was a function of the extraction time (Alves Rodrigues et al. 2004; Santoyo et al. 2005, 2006).

When the components that are responsible for the biological activity of a spice are well known, the development of methodologies to increase their concentration in the volatile

**SPICES AND THEIR ESSENTIAL OILS**

The word spices derive from the Latin word *species*, meaning “earth fruits”. Technically, it refers to the hard parts of aromatic plants, such as seeds, crust, buds or fruits, although the aromatic leaves of some plants (which should be referred as herbs) are also included (Doyle and Beuchat 2007). Spices have always been related to magic, as they are considered as a witch’s tool for their enchantments, because they have been related to aphrodisiac, healing and magical properties. In antiquity, spices were considered as treasures, and were even used as money for exchanging merchandise. Each spice has a unique flavor and aroma, which is the result of the presence of different chemical compounds, derived from the plants’ secondary metabolism. Pigments, simple phenols, polyphenols, phytolaxines, terpenes, terpenoids, are among the different chemical compounds related to the spices particular properties (Cowan 1999).

The product of hydro-distillation of spices is known as essential oil, and contains many of the compounds related to aroma and flavor. Essential oils are usually soluble in alcohols and other organic solvents, and are widely used in the perfume and beverage industries (Hammer et al. 1999; Kalemba and Kunicka 2003). Many of the compounds present in the essential oils are terpenes and terpenoids, derived from isoprene units, including some 12,000 chemical compounds that represent almost 10% of the total substances produced by plants (Cowan 1999; Daferera et al. 2000).

The physiological role in plants of the compounds present in essential oils can be related to protection mechanisms of the plant against microbial plant pathogens, as well as against insects. Some other compounds are responsible for aroma; quinones and tannins are plant pigments, and many other compounds are related to flavor. If spices or their compounds can inhibit or destroy predator organisms of plants, they can also protect humans from infections, and can also protect foods from microbial deterioration (Sherman and Flaxman 2001).

**CHEMICAL COMPOSITION OF ESSENTIAL OILS**

Chemical composition of plants’ essential oils depend on a large number of parameters, including environmental and climatic conditions, season of collection, dehydation process and storage conditions (Daferera et al. 2000; Baydar et al. 2004). As expected, antimicrobial activity of essential oils also varies according to the phenologic stage of the plant at the time of collection and the climatic conditions present during the plant growth. Müeller-Riebau et al. (1997) found a different composition in the essential oil of different aromatic plants, depending on their collection dates. It has also been reported variation on the antifungal activity of the essential oil of lemon grass during different seasons, being more potent the essential oil obtained from May to November (Mishra and Dubey 1994). European oregano (Origanum vulgare spsh. Hirtum) composition has also qualitative and quantitative differences depending on the growth conditions and the time of collection. Fluctuations are so important, that concentrations of the main components, carvacrol, thymol and cymene, vary up to 10 times among samples (Jerkovic et al. 2001).
fraction can be applied. Castillo-Herrera et al. (2007) de-veloped a method for extraction of essential oil from Mexican oregano, enriching the phenolic content, regardless of the geographical origin of the plant used.

**ANTIMICROBIAL EFFECTS OF ESSENTIAL OILS**

Although the antimicrobial activity of herbs and spices has been identified a long time ago, the scientific literature on their biological activity has intensified in recent years (Tables 1, 2). This antimicrobial effect has been studied for either bacteria or fungi and yeast, responsible for food deteri-oration as well as for food-borne pathogens.

**Table 1** includes some examples of antimicrobial analysis of several spice essential oils against a wide variety of bacterial strains related to foods. The effect against the most common food-borne pathogens, such as *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter* spp. and *Shigella* spp. are found in many scientific reports. There is also a wide variety of essential oils tested, but there are common species such as clove, thyme, oregano, anise, basil, coriander, cinnamon, lemongrass and marjoram. The antimicrobial effect is higher for Gram negative bacteria, but *Bacillus cereus* is one of the most sensitive bacterial species (Ulte et al., 1999, 2002). Kim et al. (1995) reported the antibacterial activity of 11 essential oils, along with thymol and carvacrol against five food-borne bacteria, and demonstrated that carvacrol, citral and geraniol presented strong bactericidal activity.

The approach of antimicrobial analysis is either the evaluation of many essential oils against one or two different microbial species, or the evaluation of a wide variety of bacterial and fungal species against few essential oils or their main components (Tables 1, 2). Spices such as oregano, thyme, rosemary and clove, are the ones that show the better effects. There are also differences on the antimicrobial effect by the strains tested. *Staphylococcus*, *Escherichia*, *Salmonella*, *Listeria* and *Bacillus* are among the most reported bacterial genera, and they show a large variability on their susceptibility to the same essential oil; this effect could be due to differences in response of the isolates to environmental stresses or pathogenic factors. There are not many reports that address this question.

The antimicrobial effect of spices has also been reported for yeasts, and one of the pioneer works was reported by Conner and Beuchat (1984), that demonstrated a strong effect of several essential oils against 13 yeast of impor-tance to foods. The antifungal effect of several essential oils of plants from Greece was also evaluated by inhibition of radial growth and germination of *Penicillium digitatum*. According to the results, the essen-tials oils of oregano, thyme, dictamus, and marjoram completely inhibited fungal development at relatively low concentra-tions (250-400 mg/L). The authors suggest that the antifungal effect of the essential oils is the result of a syner-gistic interaction amongst their components (Dafarera et al. 2003).

The effect of different essential oils was also studied by Basilio and Basilico (1999) showing that oregano (*Origanum vulgare*), mint (*Menta arvensis*) and basil (*Ocimum basilicum*), had an antifungal effect on *Aspergillus ochraceus* growth, as well as on the inhibition of ochratoxin production; small concentrations of the essential oil, however, presented an increased mycelial growth, but inhibited mycotoxin production. A similar effect was observed by Portillo et al. (2005) that evaluated Mexican oregano against fungi and yeasts related to food spoilage. Four dif-ferent effects were observed when 25 fungal strains were analyzed: no fungal growth inhibition; a concentration-dependent inhibition of fungal growth; an increase in fungal growth by low concentrations of oregano but inhibition at higher concentrations; an increase in fungal growth by high concentrations of oregano, but inhibition by low concentra-tions.

The antifungal effect of several essential oils has been studied on the growth of specific fungal strains of concern for a particular food group. Guynot et al. (2003) evaluated the effect of volatile compounds of 16 different essential oils versus fungi related to bread product contamination, such as *Eurotium herbariorum* and *E. rubrum*. In order to better simulate the effect of the essential oils, the volatile fraction was tested in a medium containing wheat flour, adjusted to different water activities. Suhr and Nielsen (2001) also tested the essential oil against bread-contaminat-ing fungi, but this time, the essential oil was added to the agar plates, and the antifungal effect was evaluated as inhibition of radial growth (Table 2).

When the antimicrobial activity of essential oils is studied, the effect is considered as the result of the interaction of all the compounds present; in order to understand the effect of the predominant compounds, some authors have also studied the effect of individual compounds (Table 1). Main components such as carvacrol, thymol, p-cymene and α-terpene were evaluated for antimicrobial activity against *E. coli* O157:H7, carvacrol-thymol combinations showed additive effects (Burt et al. 2005). A high concentration of carvacrol in European oregano essential oil was significant in the inhibition of food-borne pathogens such as *E. coli*, *S. typhimurium*, *S. aureus* and *B. subtilis*, among others (Siv-ropoulou et al. 1996).

When carvacrol was mixed with food stabilizers, such as agar or carrageenan, the antimicrobial activity of the pheno-lic compound was improved; therefore, the components of the essential oils can interact with food components, increasing or possible decreasing their biological activity (Burt et al. 2005). Lambert et al. (2001) reported that is possible to obtain the same inhibition of *P. aeruginosa* and *S. aureus* with either thymol-carvacrol combinations or with oregano essential oil. The authors suggest that the anti-microbial effect of those compounds is due to damages to the cell membrane, with an effect on the pH and inorganic ions’ cell equilibrium.

**MECHANISM OF ACTION**

Since the essential oils of spices are a complex mixture of compounds with different structures, it is difficult to assess the mechanism of action of the mixture. Therefore, research has focused on the mechanism of action of some of their main components, and as a consequence, the mechanism of action of the essential oil. Still, there are few reports on the effects of the spices’ essential oils on microbial cells. It has been suggested that the antimicrobial activity is the result of a damaged enzymatic cell system, especially those associated with energy metabolism and the synthesis of structural compounds (Gill and Holley 2004, 2006). It has also been reported that the effect of phenolic compounds on microbial growth and the production of toxins could be due to the ability of these compounds to modify the microbial cell permeability, allowing the loss of macromolecules from within. Furthermore, they can interact with membrane proteins, causing structural and functional deformity (Rhayour et al. 2003).

In Gram positive bacteria, the action of the phenolic compounds involves the leakage of potassium ions from the cell, with a decrease in the internal pH, a collapse on the membrane potential and inhibition of ATP generation; all those events lead to cell lysis and death (Ulte et al. 1999; Lambert et al. 2001). Under the microscope, those effects are shown as cytoplasm coagulation and breakage of the cell wall (Jussalaj et al. 2006a). Spices essential oil also inhibits DNA and RNA synthesis, as well as protein and polysaccharide synthesis in bacteria and fungi. In the later, the changes are similar to the effects of medical antibiotics (Kalemba and Kunicka 2003).

Several authors have reported that the degree of microb-ial inhibition of spices’ essential oils can be attributed to the presence of compounds with an aromatic ring in its structure (Velluti et al. 2003; Sokmen et al. 2004). The high antimicrobial activity of the phenolic compounds can be ex-
Table 1 Antimicrobial analysis of spices against foodborne bacteria.

<table>
<thead>
<tr>
<th>Spices</th>
<th>Microorganisms</th>
<th>Method used</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Black pepper (Piper nigrum), clove (Syzygium aromaticum), geranium (Pelargonium graveolens), nutmeg (Myristica fragans), oregano (Origanum vulgare) thyme (Thymus vulgaris)</td>
<td>Twenty five bacterial strains, including foodborne, animal and plant pathogens, food spoilng</td>
<td>Well-diffusion test (4 mm diameter, 15 µL of essential oil added)</td>
<td>Dorman and Deans 2000</td>
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<td>European oregano (Origanum scabrum and Origanum microphyllum) Twelve different essential oils, including anise, basil, cardamom, coriander, fennel, parsley, oregano, and rosemary</td>
<td>S. aureus, S. epidermidis, E. coli, E. cloacae, K. pneumoniae, P. aeruginosa L. monocytogenes, S. aureus, E. coli O:157:H7, Y. enterocolitica, P. aeruginosa, L. plantarum</td>
<td>MIC by microplate assay.</td>
<td>Aligiannis et al. 2001</td>
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<td>Dill (Anethum graveolens), coriander seeds and leaves (Coriandrum sativum), Eucalyptus (Eucalyptus dives) Essential complete and fractionated oil</td>
<td>E. coli O157:H7, S. typhimurium, L. monocytogenes, S. aureus, P. fragilis, S. grimesii, E. agglomerans, Y. enterocolitica, B. subtilis Sp. A</td>
<td>MIC in microplates. Individual fractions and mixtures were analyzed</td>
<td>Delaquis et al. 2002</td>
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<tr>
<td>96 essential oils and 23 oil compounds</td>
<td>C. jejuni, E. coli O157:H7, L. monocytogenes, S. enterica</td>
<td>Bactericidal activity (BA50) was defined as the percentage of the sample in the assay mixture that resulted in a 50% decrease in CFU relative to a buffer control.</td>
<td>Friedman et al. 2002</td>
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<td>Bay (Pimenta racemosa), clove bud (Eugenia caryophyllata), Oregano (Origanum vulgare) and red and light thyme (both Thymus vulgaris) Thymus species from Portugal Thymus masticha, T. camphoratus, T. luteocephalus 17 spices, including cloves, thyme, oregano, allspice, basil, rosemary, and marjoram</td>
<td>E. coli</td>
<td>Disc diffusion and colorimetric determination of MIC using Alanar Blue</td>
<td>Burt and Reinders 2003</td>
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<td>Cinnamon bark, Thyme (Thymus vulgaris), Perilla, Lavender</td>
<td>S. aureus E. coli</td>
<td>Minimal concentration of vapor phase to inhibit growth of the microorganisms; Plate dilution test</td>
<td>Inouye et al. 2003</td>
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<td>Anise (Pimpinella anisum), Coriander (Coriandrum sativum), Chinese cinnamon (Cinnamomum cassia), juniper (Juniperus oxycedrus)</td>
<td>B. brevis, B. cereus, B. megaterium, B. subtilis, E. faecalis, K. pneumoniae. L. monocytogenes, M. luteus, M. smeagmas, P. aeruginosa, S. aureus, Y. enterocolitica</td>
<td>Disk agar diffusion test (3 µL in 6 mm paper disks)</td>
<td>Falheiro et al. 2003</td>
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<td>Ginger (Zingiber officinale), fingerroot (Boswellia pungens) and turmeric (Curcuma longa) essential oil extracted with ultrasound assisted-solvent-extraction</td>
<td>L. monocytogenes, S. Typhimurium DT 104</td>
<td>Spices added to either Mueller-Hinton broth or agar (1%); visual observation of growth. Determination of MIC by agar dilution method. Effect of other factors such as pH, s, NaCl by agar dilution method</td>
<td>Bagamboula et al. 2003</td>
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<tr>
<td>Fourteen essential oils (simple or mixed)</td>
<td>S. aureus and C. albicans</td>
<td>MIC by OD and metabolic dye test (INT). Disk diffusion and hole-plate diffusion assays</td>
<td>Donaldson et al. 2005</td>
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<td>Cumin (Cuminum cyminum) stored for 30 years</td>
<td>S. epidermidis, S. aureus, B. subtilis, B. pumilus, E. coli, P. aeruginosae, Salmonella ahony, S. cerevisiae, C. albicans, A. niger</td>
<td>Agar diffusion cup</td>
<td>Jirovetz et al. 2005</td>
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<td>Basil (Ocimum basilicum), oregano (Origanum vulgare), thyme (Thymus vulgaris) Satureja spinosa</td>
<td>Thirty bacterial strains</td>
<td>Hole-plate agar diffusion test</td>
<td>Bozin et al. 2006</td>
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<td></td>
<td>S. aureus, E. coli O157:H7, L. monocytogenes, S. enteric serovar Enteritidis, B. cereus</td>
<td>Conductance method and optical density measures of growth</td>
<td>Chorianopolous et al. 2006b</td>
</tr>
<tr>
<td>18 different essential oils</td>
<td>Vibrio parahaemolyticus</td>
<td>NIC by microplate serial dilution</td>
<td>Yano et al. 2006</td>
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<tr>
<td>Basil (Ocimum basilicum), Lemon balm (Melissa officinalis), Marjoram (Origanum majorana) Oregano (Origanum vulgare), Rosemary (Rosmarinus officinalis), Sage (Salvia trilobata), Thyme (Thymus vulgaris)</td>
<td>B. cereus, E. coli, L. monocytogenes, P. aeruginosae.</td>
<td>Spot-on-agar test, microplate dilution assay for individual essential oils. For combinations of the essential oils tested, a check board method was used, to test for interactions.</td>
<td>Gutierrez et al. 2008</td>
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Explained by the alkyl substitution in the aromatic ring. The importance of the hydroxyl group was confirmed by comparing the efficiency of carvacrol with cymene, which lacks the hydroxy group in its structure. Carvacrol antimicrobial activity was higher than cymene, suggesting the destabilization of the cytoplasmic membrane, reducing the pH gradient across the cytoplasmic membrane (Ultee et al. 2002; Veldhuizen et al. 2006). Recently, Xu et al. (2008) demonstrated that the antimicrobial effect of thymol and carvacrol against E. coli is caused by an increase in cell membrane permea-
Aldehydes, such as citral, are also known to have a strong antimicrobial activity that can be attributed to the double bond in its structure, which gives to the molecule a high electronegative potential. The electronegative compounds can interfere with biological processes that involve electron transfer and can also react with proteins and nucleic acids (Dorman and Deans 2000). Another component commonly found in essential oils is terpenes, which are highly lipophilic, and they are reported to interfere with membrane enzymes, such as those involved in the respiratory chain. Some other components present in essential oils interfere with protein translocation across membranes, and with membrane-bound enzymes (Cowan et al. 1999).

Bennis et al. (2004) reported the cell wall and cell membrane damage of Saccharomycyes cerevisiae cells by eugenol and thymol, as demonstrated by electron microscopy and release of organic molecules. The authors placed yeast cells in contact with fungicidal concentrations of eugenol (3 mM) and thymol (1.8 mM) and determined viable cells, as well as the release of organic material from the destroyed cells (260 nm OD). By electron microscopy, the cells showed severe damage in their cell surface, with a morphological damage by the presence of thymol. The effect of oregano essential oil and irradiation on E. coli O157:H7 and L. monocytogenes were observed by changes in the composition of murein and the internal and external ATP concentration. Those organisms were chosen because of their

### Table 2 Antimicrobial analysis of spices against foodborne fungi.

<table>
<thead>
<tr>
<th>Spices</th>
<th>Microorganisms</th>
<th>Method used</th>
<th>Reference</th>
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<tr>
<td>Black cumin (Nigella sativa), coriander (Coriandrum sativum), cumin (Cuminum cyminum), dill (Anethum graveolens), laurel bay (Laurus nobilis), oregano (Origanum onites), parsley (Petroselinum sativum), spearmint (Mentha spicata), sweet basil (Ocimum basilicum), white mustard (Brassica oleracea)</td>
<td>Aspergillus flavus, Aspergillus niger, Geotrichum candidum, Macor spp., Penicillium roqueforti and 3 Penicillium spp.</td>
<td>Spore suspension spread over agar plates containing different concentration of spices. Results were expressed as days until visual fungal growth and spore germination was observed.</td>
<td>Akgül and Kivanc 1988</td>
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<td>Oregano (Origanum vulgare), mint (Menta arvensis), Basil (Ocimum basilicum), sage (Salvia officinalis) and coriander (Coriandrum sativum)</td>
<td>Aspergillus ochraceus and ochrotaxin production</td>
<td>Growth in YSE broth added with essential oil. After incubation, ochrotaxin production and dry mycelium mass was determined.</td>
<td>Daferera et al. 2000</td>
</tr>
<tr>
<td>Thyme (Thymus vulgaris), Oregano (Origanum vulgare), Dictamus (Origanum dictamus), Marjoram (Origanum majorana), Lavander (Lavandula angustifolia), Rosemary (Rosmarinus officinalis) Salvia (Salvia fruticosa)</td>
<td>Penicillium digitatum</td>
<td>Inhibition of radial growth, conidial germination.</td>
<td>Nielsen and Rios 2000</td>
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<tr>
<td>Mustard essential oil, cinnamon oleoresin, oregano oleoresin, clove oleoresin, vanilla oleoresin, garlic essential oil</td>
<td>Aspergillus flavus, Endomyces fibuliger, Penicillium commune, Penicillium corylophilum, Penicillium discolor, Penicillium patulans, Penicillium polonicum, Penicillium roqueforti, Penicillium solitum, Pichia anomala</td>
<td>Agar plates were inoculated with vegetative fungal material. Essential oils were placed in filter paper on the lid of the plate and growth was recorded.</td>
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<tr>
<td>Twelve different essential oils, including anise, basil, cardamom, coriander, fennel, parsley, oregano, and rosemary</td>
<td>A. niger, Geotrichum, and Rhodotorula.</td>
<td>Filter paper disk diffusion test (24 mg essential oil in each disk), followed by Microbial Lethal Concentration (MLC) by twofold dilutions</td>
<td>Elgayyar et al. 2001</td>
</tr>
<tr>
<td>Melissa (Melissa officinalis), lavender (Lavandula angustifolia), Spearmint (Mentha piperita)</td>
<td>Five food spoilage yeasts, S. cerevisiae was used as reference</td>
<td>Disc diffusion test and broth dilution assay, determining deltaOD600 by the addition of essential oil to broth cultures</td>
<td>Araújo et al. 2003</td>
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<tr>
<td>Cinnamon bark, Thyme (Thymus vulgaris), Perilla, Lavender</td>
<td>T. mentagrophytes A. fumigatus C. albicans</td>
<td>Minimal concentration of vapor phase to inhibit growth of the microorganisms.</td>
<td>Inouye et al. 2003</td>
</tr>
<tr>
<td>Bay, cinnamon leaf, clove, lemongrass, mustard, orange, sage, thyme and rosemary essential oils</td>
<td>Penicillium roqueforti, Penicillium corylophilum, Eurotium repens, Aspergillus flavus</td>
<td>Agar plates added with flour and at different water activities, inoculated with fungal spores. Essential oils were placed in filter paper on the lid of the plate.</td>
<td>Suhr and Nielsen 2003</td>
</tr>
<tr>
<td>Cumin (Cuminum cyminum) stored for 30 years</td>
<td>S. cerevisiae, C. albicans, A. niger</td>
<td>Agar diffusion cup</td>
<td>Jirovetz et al. 2005</td>
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<tr>
<td>Basil (Ocimum basilicum), oregano (Origanum vulgare), thyme (Thymus vulgaris)</td>
<td>Six fungal strains</td>
<td>Antifungal concentrations of essential oil in microplates, inoculated with known concentrations of spores. (microdilution assay).</td>
<td>Bozin et al. 2006</td>
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<td>Anise (Pimpinella anisum), Boldus (Piumus boldus), Peppermint (Mentha piperita), Oregano (Origanum vulgare), Peperina (Minthostachys verticillata)</td>
<td>Aspergillus section Flavi</td>
<td>Spore germination and germ-tube elongation of fungal strains in agar plates with different concentrations of essential oils. Fungal growth in plates with essential oil and different water activities</td>
<td>Bluma et al. 2008</td>
</tr>
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</table>
Gram reaction, since it is well established that essential oils are more effective against Gram negative bacteria. In both cases, changes in the composition of murein cell wall components were observed; the murein was not disrupted by the treatment of oregano alone, but the composition and relative percentage of several muropeptides were modified, with the consequent effect on cell wall integrity. The effect of irradiation was more evident on the disequilibrium of ATP concentrations inside and outside the cell wall, and the effect of both factors was synergistic (Caillet et al. 2005; Caillet and Lacroix 2006).

Oussalah et al. (2006a) showed that Spanish oregano (Corydathymus capitatus), Chinese cinnamon (Cinnamomum cassia), and savory (Satureja montana) essential oils create an increase in cell wall permeability in E. coli O157:H7 and L. monocytogenes, with the consequent release of cell constituents, a decrease in ATP concentration and a decrease in the internal pH. On the other hand, Rasooli et al. (2006) demonstrated the deleterious effect of two essential oils of the Thymus genus, against Aspergillus niger. The authors report a strong antifungal effect, and when cells were observed by electron microscopy, MIC of the essential oil caused severe damage to the cell wall, cell membrane, and cellular organelles. The changes could also be observed visually, with changes in mycelial morphology and spores.

ANTIMICROBIAL COMPOUNDS NOT FOUND IN ESSENTIAL OILS

The essential oil of spices concentrates low molecular weight volatile compounds that are usually responsible for odor and flavor of the aromatic plants. Most of the antimicrobial activity of spices has been related to the compounds found in their essential oil; however, the strong odor and flavor can be an impediment for the use of essential oils as food additives. The study of spices has also focused on the identification of other compounds, different than those found in the essential oils that can also have antimicrobial activity.

Hydrosols, also known as floral water, are the co-products of hydrodistillation of aromatic plants. Hydrosols are complex mixtures of traces of the essential oil components and water-soluble compounds. Sagdic (2003) studied hydrosols from oregano (Origanum vulgare L., Origanum onites L., Origanum majorana L.) and thyme (Thymus vulgaris L., Thymus serpyllum L.) grown in Turkey and found that they were inhibitory at 10-25 mL/100 mL concentrations, and bactericidal at 50 mL/100 mL when tested against E. coli O157:H7, S. aureus and Yersinia enterocolitica. On the other hand, it has been demonstrated that volatile compounds of European oregano with antimicrobial capacity, include a series of compounds attached to glucosidic residues that are not found in the essential oil (Mastelic et al. 2000). Cilantro essential oil was shown to be an effective antilisterial; furthermore, the essential oil was separated by fractional distillation and fractions of cilantro essential oil that were deficient in phenolic compounds have a strong antilisterial activity (Delaquis et al. 2002).

The variety of chemical compounds found in plants that have antimicrobial activities is extensive. Although many of the most recognized compounds are present in essential oils, there are many other chemicals from non-aromatic plants that also have antimicrobial properties. Cowan (1999) wrote an excellent review on the nature of chemical antimicrobials found in plants.

THE STUDY OF NATURAL ANTIMICROBIALS

The chemical nature of spices essential oils, especially its high volatility and low water solubility, represent a significant problem when assessing biological activities, including antimicrobial properties. Much has been discussed on the appropriate method for determination of antimicrobial activity of essential oils, on the reproducibility of results, as well as on the correlation between in situ and in vitro studies. This is especially important, since most of the microbiological methods includes the use of water-based culture media, were essential oils are only partially soluble.

Methods used to test biological activity of essential oil includes qualitative techniques such as disk and well diffusion assays (Elgayyar et al. 2001; Ates and Erdogrun 2004; Mimica-Durik et al. 2004), as well as quantitative methods based on dilution of the test culture or those used to determine the Minimal Inhibitory Concentration (MIC) and the Minimal Bactericidal (or Fungicidal) Concentration (MBC, MFC) (Delaquis et al. 2002; Thongson et al. 2004; Bozin et al. 2006). Although diffusion assay methods are fast and are usually compared to dilution methods, they are considered not reliable, since do not consider factors such as the differential diffusion of oil constituents through agar (Hood et al. 2005). On the other hand, dilution methods include agar dilution, broth dilution and microplate-based microdilution, but there are some reports on the carry-over of volatile compounds between the wells in a microplate, when highly volatile compounds are tested (Donaldson et al. 2005). Dilution-based methods also have the disadvantage of the difficulty of obtaining homogeneous mixture of oil in the test medium. To overcome this problem, the use of surfactants such as Tween 80 or chemical solvents such as ethanol or dimethyl sulfoxide have been used, but for many microorganisms, the solvent rather than the oil tested, are the ones that exhibits antimicrobial activity (Hood et al. 2003; Donaldson et al. 2005). Also, the use of dye indicators of microbial activity, such as Alamar blue or fluorescent dyes have been used to help on the determination of MIC, when suspension of antimicrobial compounds are turbid and determination of bacterial growth is difficult (Burt and Reinders 2003; Donaldson et al. 2005; Xu et al. 2008).

Lambert and Pearson (2000) first proposed the use of Optical Density (OD) data and application of a mathematical model to obtain MIC and NIC (Non-Inhibitory Concentration) values. The authors used the OD data to generate areas-under-the-curve values and fractional areas that were used in a model derived from the Gompertz equation to determine MIC and NIC. They first reported the use of their model on the dose response of S. aureus, E. coli and P. aeruginosa against several inhibitors. However, OD readings can have several sources of error, including dead or not active cells that still contribute to turbidity. Marino et al. (1999) suggested the use of a bioimpedometric method that can detect metabolic activity of microorganisms, based on variations on an electrical signal supplied directly into the culture medium. The time to detect bacterial activity was used as response variable to evaluate antimicrobial properties of thyme against Gram positive and Gram negative bacteria. The method has also been used to determine MIC by Chorianopoulos et al. (2006a), using the time to detection, maximum conductance and slope of the conductance curve to obtain data and adjust it to the model proposed by Lambert and Pearson (2000). In the report, the authors used the method for determination of CMI and NIC of Satureja spinosa against several food-borne pathogens.

Determination of antifungal activities of essential oils is an even more complex task, considering the difficulties on assessment of viable fungal cells. Methods such as spreading a spore suspension over a Petri plate and addition of essential oils to wells or paper disks are used as techniques for preliminary detection of antifungal activity (Elgayyar et al. 2001; Jirovetz et al. 2001). Also, biofilm production in the presence of the antimicrobial has proven to be an effective method for filamentous fungi assays (Basilico and Basilico 1999). Among the most reliable methods used in antifungal analysis of essential oils, is the inhibition of radial growth, because data can resemble a bacterial growth curve (Portillo et al. 2005). The problem of achieving a homogeneous concentration of the essential oil in the Petri plates is a disadvantage, but it is also shared with most of the methods used to test antifungal activities. Spore germi-
nation and germ-tube elongation of fungal strains placed in contact with the essential oil has recently been reported for analysis of several essential oils against Aspergillus strains (Bluma et al. 2008). Although it can be time-consuming, the methods provide important information on the effect of essential oil compounds on fungal growth.

Suhr and Nielsen (2003) demonstrated that the antifungal activity of 10 different essential oils was dependent on the micel forming behavior. They used a culture medium with a composition similar to rye bread, and also tested the inhibition when the essential oil was spread on a slice of rye bread. In the in vitro analysis, thyme was more effective, while mustard was more effective when added to the bread slice. It has also been reported that small concentrations (50 µL) of different essential oils (cinnamon, clove, basil, thyme), applied to a bread-based agar, totally inhibited the growth of the most important bakery spoilage molds (Eurystigma, Aspergillus and Penicillium), while the same oils were not effective on mold inhibition when those were tested on cake analogues (Guynot et al. 2003). Therefore, medium composition, exposure time, as well as environmental factors (temperature, pH and water activity) are all important factors to consider in the selection of the method of evaluation for antimicrobial activity.

As stated before, the most active components of essential oils are volatiles; therefore, several authors have suggested the development of methods to test the volatile fractions of essential oils for antimicrobial activity. Inouye et al. (2003) proposed a method to test the minimal concentration of the vapor phase that inhibited the growth of bacterial strains on Petri plates. The authors reported the inhibition of S. aureus and E. coli as well fungi and yeasts (Candida albicans) by the volatile fraction of thyme, cinnamon bark, perilla and lavender, and reported the effect as MID (Minimal Inhibitory Dose per unit space). Results correlate to those obtained by MIC, but they demonstrate that very low concentrations of the antimicrobial compounds in the vapor phase are antimicrobial. Antifungal activity has also been tested by placing a filter paper impregnated with a known concentration of essential oil, on the lid of a Petri plate, and then the plate is sealed to achieve an equilibrated atmosphere saturated with volatile compounds. Inhibition of fungal growth by radial growth, or time to detection of micelial formation is used as response variables (Nielsen and Rios 2000; Guynot et al. 2003; Suhr and Nielsen 2003).

Lack of agreement between agar-based methods and evaluation of antimicrobial activity with food model systems, shows the importance of the interaction of factors such as fat and protein content in foods, and their role in water activity and chemical reactions in food (Leistner and Gorris 1995). The interaction of those factors, suggests the use of more food analogue products in standard laboratory methods (Suhr and Nielsen 2003).

**USE OF SPICES IN FOOD**

Since man has used plant extracts, including essential oils for medicinal and pharmaceutical purposes, one of the main interests in spices or their essential oils has also been food preservation. Nowadays, natural antimicrobials are a common alternative to extend the shelf life of different products, by either inhibiting their growth or by killing the microorganisms present (Dadalioglu and Evrendilek 2004; Draughon 2004). According to reports by the World Health Organization (WHO), it is estimated that up to 80% of human populations use plant extracts or their active compounds for the abovementioned reasons (Arcila-Lozano et al. 2004).

On the other hand, the ability of the microorganisms to grow and easily adjust to environmental changes, allows them to develop resistance mechanisms against antimicrobials in a short period of time; therefore, the challenge of developing new food preservation methods, is a continuous task (Dormann and Deans 2004). The challenge is to find natural antimicrobial products that can be easily added to food, without disturbing the nutritional or sensorial quality of the product, and that does not represent a significant increase in formulation, processing or storage costs (Gould 1996; Doyle and Beuchat 2007). The balance between effective dose and consumer acceptability of food products is an actual puzzle for the food industry (Skandamis and Nychas 2000). The incorporation of natural additives to foods must be evaluated in the real capacity of the products to cause a beneficial effect on the microbiological quality and the sensorial properties of the foodstuff, with the expected effect of increasing shelf life. Up to recent years, most of the reports were on antimicrobial effects of natural products that were evaluated under laboratory conditions, without considering the complex food environment where the antimicrobials would be added.

The quantitative evaluation of natural antimicrobials through the development of mathematical models done using data from food model systems can help on the prediction of the efficiency of the antimicrobial additives under real conditions (Baranyi and Tamplin 2003; Kong et al. 2007). Predictive microbiology, as the quantitative study of microbial ecology in foods, help on the description of the microbial response to environmental factors, including those present in the food system as a microenvironment: pH, water activity, temperature, etc. Predictive microbiology is based on the assumption that the microorganisms studied in the laboratory, under the same environmental conditions that are present in the food system, will have the same behavior as the one expected when the microorganism is present in food. Therefore, the mathematical models developed using data from laboratory studies, can then be used to predict the behavior of the microorganism when present in foods. Models can be confirmed by doing experiments in food systems (Devlieghere et al. 1999). Since the food matrix is very complex, predictive models usually consider only the main environmental factors that are the microbial growth barriers; but usually some other factors that are not considered crucial, have synergistic or antagonist effects, and have an important role in the final outcome of microbial growth. For that reason, mathematical models for the prediction of microbial growth in food systems have also been developed (Devlieghere et al. 1999; Skandamis and Nychas 2001; Skandamis et al. 2002).

The new developments in food preservation have as common objective, minimal food processing, in order to present to the consumer, a food product that most closely resembles the natural one. New preservation techniques such as light pulses, ultrasound, pulsed electric fields and high pressure processing among others, are methods not based on high temperature to reduce the number of microorganisms, but disturb the product that carries the antimicrobial effect. The interaction of those factors, suggests the use of more food analogue products in standard laboratory methods (Suhr and Nielsen 2003).
ditions against L. monocytogenes and B. cereus. At low nisin concentrations, the combination with oregano essential oil increased the bactericidal effect of the antibiotic, decreasing 3-log UFC/mL of the pathogens more when both antimicrobials were present than when the nisin was added alone. The effect of oregano has also been tested against Clostridium botulinum growth and toxin production. The antimicrobial activity of the oregano essential oil was high when added to vacuum packaged food, but when added to a pork model (pork meat), the concentration of oregano needed to achieve the antimicrobial effect was higher than the needed in laboratory tests, which made its use impractical. The authors also reported a synergistic effect of oregano essential oil with sodium nitrate (Ismael and Pierson 1990).

The use of spices or essential oils alone, does not guarantee the control of pathogens in food. The combination of spices and organic acids was necessary to control the growth of C. perfringens in cooked meat during cooling and storage (Sabah et al. 2004). The same research group also demonstrated that carvacrol, cinnamaldehyde, thymol and oregano essential oil, were tested on the control of C. perfringens growth during cooked ground turkey chilling process. Cinnamaldehyde was the most effective antimicrobial at low concentrations (Junjea and Friedman 2007). On the other hand, the combination of organic acids and cinnamon has also been reported to control growth of E. coli O157:H7 in apple juice (Ceylan et al. 2004).

In another study done in meat carcasses, the meat was sprayed with a commercial solution of herbal extracts, and found a reduction in the number of pathogenic bacteria recovered from the surface (Cutter 2000). The effect of the essential oil of oregano against E. coli O157:H7 was also tested using as a food model eggplant salad, showing a significant reduction in the number of bacterial cells (Skandalis and Nychas 2000). The same research group determined that the combination of controlled atmospheres or vacuum pack and oregano essential oil, controlled the growth of Salmonella Typhimurium in meat slices (Skandalis et al. 2002).

Another developing area in food preservation is the use of biodegradable and edible films, used for food packaging. Films based on biodegradable compounds such as starch or chitosan, have limited or non-antimicrobial properties; therefore, the addition of essential oils can increase the antimicrobial capacity of the films, and improve the quality of the films for food preservation. Zivanovic et al. (2005) reported the addition of essential oils of anise, basil, coriander and oregano into chitosan films, and demonstrated their efficiency on the control of E. coli O157:H7 and L. monocytogenes being oregano the oil that better controlled microbial growth. Edible films based on apple puree were added with cinnamon, lemongrass and oregano, and their antimicrobial properties were tested. The films added with antimicrobials were effective in the control of E. coli O157:H7, increased oxygen permeability and decreased water vapor permeability (Rojas-Gräu et al. 2006). In another report, alginate-based films were added with 60% of oregano, thyme, cinnamon (Cinnamomum cassia), and savory (Satureja montana), and treated with CaCl2 as well. The films were tested for effective control of E. coli O157:H7 and S. Typhimurium added to beef fillets, showing an antimicrobial activity during the five days of treatment. The authors placed special attention to the rate of active essential oil compounds released from the films (Oussalah et al. 2006b). Lopez et al. (2008) evaluated the microbial effect of the vapors released by polypropylene (PP) and polyethylene/ethylene vinyl alcohol copolymer (PE/EVOH) flexible films added with different essential oils, and found that a large proportion of cinnamon and oregano oils were needed to inhibit the growth of fungi (4% w/w), Gram positive bacteria (8-10%) and up to 12% was needed for Gram negative bacteria, but even at that high concentration, P. aeruginosa was not inhibited.

Most of the spice essential oils are considered as GRAS (Generally Recognized as Safe), but their use as food additives is limited to their efficiency, since they are mild antimicrobials and large quantities are required to achieve micbicidal effects. It is important to obtain the MIC (Minimal inhibitory concentrations) and MBC (Minimal bactericidal concentration) values, since those concentrations can lead to the determination of optimal use of spices. Furthermore, the complex environment produced by the interaction of the physicochemical properties of a food product, can modify the antimicrobial efficiency of added spices. Therefore, the optimized use of spices can balance the efficiency as food preservative, and the sensorial acceptability of those food products added with spices (Fisher and Phillips 2006).

CONCLUSION

The addition of spices to food has been a common practice in many societies, and the commerce associated with spices has been related to historical events, such as the discovery of America. With today technological advances in food preservation, but especially with the interest on the conservation of natural nutrients in foods, the use of spices as mild antimicrobials has received special attention. Despite the abundant and important research done on the use of spices as natural antimicrobials under laboratory conditions and in food systems, there are still many questions to be addressed. There is a need for standardized methods for determination of antimicrobial effect of spices, so that results can be easily compared. Also, there are many questions to be answered on the production of essential oils by plants, its regulation and the effect of environmental factors on the production of the biological active compounds. The search for new plant antimicrobials or the identification of new active compounds in the already studied essential oils is also a task to be attended. Finally, when considering the addition of spices to foods, the interaction with the other food components is important to determine the best conditions for their efficient use. The study of spices has still many areas for research, to finally apply the results obtained in the preparation of innocuous food.

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