Antimicrobial Effectiveness of Spices: an Approach for Use in Food Conservation Systems

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ABSTRACT

There has been constant an increasing the search alternative and efficient compounds for food conservation, aiming a partial or total replacement of antimicrobial chemical additives. Spices offer a promising alternative for food safety. Inhibitory activity of spices and derivatives on the growth of bacteria, yeasts, fungi and microbial toxins synthesis has been well reported, so they could be used in food conservation as main or as adjuvant antimicrobial compounds in order to assure the production of microbiologically stable foods.

Key words: Spices, essential oil, extracts, chemical compounds, antibacterial activity, antifungal activity

INTRODUCTION

Food conservation has been characterized for nutritious and microbiologically stable foods and it has been archived by controlling the growth of spoiling and pathogenic food-related microorganisms. Microbial control in foods could be assured by suppressing one or more essential factors for microbial survival (Horace, 1982). It could be possible by adding suitable substances (weak organic acids, hydrogen peroxide, chelators, organic biomolecules) and applying physical (temperature, packaging) and/or chemical procedures (pH, oxide-reduction potential, osmotic pressure) (Ray, 1996; Brull and Coote, 1999). These procedures could kill or make unviable some microorganisms.

There has been increasing concern of the consumers about foods free or with lower level of chemical preservatives because these could be toxic for humans (Bedin et al., 1999). Concomitantly, consumers have also demanded for foods with long shelf-life and absence of risk of causing foodborne diseases. This perspective has put pressure on the food industry for progressive removal of chemical preservatives and adoption of natural alternatives to obtain its goals concerning microbial safety. This resulted in increasing search for new technologies for use in food conservation systems, which include: modified atmosphere packaging, combined effect of underlethal procedures, alternative antimicrobial compounds (ecstatic or cidal effect), combination of conventional (used in low levels) and alternatives antimicrobials (Brull and Coote, 1999).

Uncontrolled use of chemical antimicrobial preservatives has been inducing factor for appearance of microbial strains more and more resistant to classic antimicrobial agents. Difficult
to control the microbial survival, showed by isolation of multi-resistant strains, has been reported all over the world. Fifty years of increasing use of chemicals antimicrobials have created a situation leading to an ecological imbalance and enrichment of multiples multi-resistant pathogenic microorganisms (Levy, 1997).

The successful story of microbial chemocontrol lies in the continuous search for new antimicrobial substances to control the challenge posed by resistant strains (Notermans and Hoogenboom-Verdegaal, 1992). Antibiotic resistance in foodborne pathogens is a reality, though substantial qualitative and quantitative differences have been observed (Teuber, 1999). Strains of resistant foodborne pathogens to a variety of antimicrobials have become a major health concern (Kiessling et al., 2002) and it could decrease the successful application of control measures on spoilage and pathogen microorganisms, many times leading for use of less safe, ineffective or expensive alternatives (Levy, 1997).

Changes in/on the antimicrobial target, inactivation by enzymes, changes in cellular permeability, antimicrobial active efflux, overproduction of target enzymes and bypass of the antimicrobial have been common mechanisms of antimicrobial resistance (McKeegan et al., 2002). Brull and Coote (1999) have reported microbial resistance for some antimicrobials used in food conservation as weak-organic acids, hydrogen peroxide, chelators and some small organic biomolecules.

Recently, there has been increasing interest in discovering new natural antimicrobials (Sagdiç et al., 2003a), this is also has been true in food microbiology. Plant products with antimicrobial properties notably have obtained emphasis for a possible application in food production in order to prevent bacterial and fungal growth (Lanciotti et al., 2004). Plant products are characterized for a wide range of volatile compounds, some of which are important flavor quality factors (Utama et al., 2002). Moreover, plant volatiles have been generally recognized as safe (GRAS) (Newberne et al., 2000). Systematic screening for biological interactions between microorganisms and plant products has been valuable source of new and effective antimicrobial substances, which could have different action ways on/in the microbial cell when compared to other conventional antimicrobials. Plants synthesize by a secondary metabolism many compounds with complex molecular structures and some of them have been related with antimicrobial properties found in plant and their derivatives. Among these secondary metabolites are found alkaloids, flavonoids, isoflavonoids, tanins, cumarins, glycosides, terpenes and phenolic compounds (Simões et al., 1999).

Being plant natural foodstuffs, spices appeal to consumers who tend to question the safety of synthetic additives (Farag et al., 1989; Sagdiç 2003b). Antimicrobial properties of spices have been documented in recent years and interest continues to the present (-El Shami et al., 1985; Akgul and Kivanç, 1988; Cosentino et al., 1999; Domans and Deans, 2000; Ristori et al., 2002; Radhakrishnan-Sridhar and Velusamy-Rajaopal, 2003). Still little information is available emphasizing the preservative and antimicrobial role of spices in the prevention of foods of the microbial action (Arora and Kaur, 1999).

Spices are recognized to stabilize the foods front the microbial deterioration. This could be observed when spices show initially high microbial charge and as time progresses, the microbial growth become progressively slower or it is eventually totally suppressed (Kizil and Sogut, 2003). Antimicrobial activity of spices depend on several factors, which includes: i) kind of spice, ii) composition and concentration of spice, iii) microbial specie and its occurrence level, iv) substrate composition and v) processing conditions and storage (Shelef, 1983; Farag et al., 1989).

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods (Germano and Germano, 1998). Spices include leaves (bay, mint, rosemary, coriander, laurel, oregano), flowers (clove), bulbs (garlic, onion), fruits (cumin, red chilli, black pepper), stems (coriander, cinnamon), rhizomes (ginger) and other plant parts (Shelef, 1983). Although, spices have been well known for their medicinal, preservative and antioxidant properties, they have been currently used with primary purpose of enhancing the flavor of foods rather than extending shelf-life (Aktug and Karapinar 1986, Ristori et al., 2002).

Spices active compounds have been included in class of naturally occurring food preservatives and have their inclusion in foods allowed by food production regulator offices (Brull and Coote, 1999). Several scientific reports describe the
inhibitory effect of spices on a variety of microorganisms, although considerable variation for resistance of different microorganisms to a given spice and of the same microorganisms to different spices has been observed (Akgul and Kivanc, 1988).

Gould (1995) has emphasized the possible use of spices and derivatives like alternatives for inclusion in a new perspective of food conservation called “natural antimicrobial system”, which could use the synergistic effect of antimicrobial compounds from animal, plant and/or microbial origin, more physical procedures in order to create an inhospitable environment for microbial survival in foods.

**ANTIBACTERIAL ACTIVITY**

Antibacterial activity of spices has been reported by several researchers. Aktug and Karapinar (1986) observed inhibitory action of thyme (*Thymus vulgaris*), mint (*Mentha piperita*) and laurel (*Laurus nobilis*) ground leaves and their extracts on *S. aureus*, *S. typhimurium* and *V. parahaemolyticus*. Thyme was most prominent antibacterial product being active up to concentration 0.5% (w/v) for ground and 5000ppm (v/v) for extracts. Pandit and Shelef (1994) tested the antilisterial effect of 18 spices and observed significant inhibitory effect of rosemary (*Rosmarinus officinalis*) (≥ 5% w/v) and clove (*Eugenia cariophyllata*) (≥ 1% w/v) on *L. monocytogenes*. Rosemary (0.5% w/w) and its essential oil (1% w/v) were when useful for pork sausage during storage at 5°C for 50 days. Grohs and Kunz (2000) observed that spices mixtures were able to inhibit the growth of various meat-spoiling microorganisms (*Bacillus subtilis, Enterococcus* spp., *Staphylococcus* spp., *E. coli* K12 and *Pseudomonas fluorescens*) providing stabilizing effect on colour and smell of fresh portioned pork meat.

Study carried out by Al-Jedah et al. (2000) analyzed the action of combined spices, including cumin (*Cuminum cyminum*), coriander (*Coriandrum sativum*), mustard (*Brassica juncea*), black pepper (*Piper nigrum*) and lemon (*Citrus aurantifolia*) on *V. parahaemolyticus*, *S. aureus*, *S. typhi* and *E. coli* count in fish sauce, which showed that the spices mixtures were able to exert static effect on all assayed bacteria when in interaction with an initial inoculum of 1.0 x 10⁴ CFU/mL, except on *S. typhi*.

Sagdic et al. (2003b) assayed the inhibitory effect of methanolic extracts of seven Turkish spices on *E. coli* 0157:H7 where myrtle (2.0% v/v), thyme (0.5, 1.0, 1.5 and 2.0% v/v), cumin (1.0, 1.5 and 2.0% v/v) and oregano (1.0, 1.5 and 2.0 v/v) and showed prominent results as bactericidal in both paper disc and agitated liquid culture assay. However, it was also found that laurel (0.5, 1.0, 1.5 and 2.0 % v/v) stimulated the growth of *E. coli* O157:H7. Leuchner and Zamparini (2002) studied the growth and survival of *E.coli* O157 and *Salmonella enterica* serovar *enteridis* in mayonnaise in presence of garlic (*Allium cepa*), ginger, mustard and ground clove. Garlic (1% w/v) and clove (1% w/v) which showed bacteriostatic and bactericidal effect, respectively, towards *S. enterica* and *E. coli* O157, and *E. coli* was more sensitive. These results are significant regarding the emergence of *E. coli* 0157 and *S. enterica* serovar *enteridis* as foodborne pathogens that have significant impact on the food industry. Moreover, these bacteria present various undesirable attributes of virulence that in combination make them some of the most serious threats for food safety (Proctor and Davis, 2000). Effect of clove extract on the production of vero-toxin by enterohemorrhagic *E. coli* (EHEC) 0157:H7 was investigated by Sakagami et al. (2000) where vero-toxin production was inhibited by garlic extract (0.5% w/v).

Sakandamis et al. (2002) assayed the effect of oregano essential oil on the behavior of *S. typhimurium* in sterile and naturally contaminated beef fillets stored under aerobic and modified atmospheres (different levels of CO₂, O₂, N₂ and vacuum packing) and found that the addition of this essential oil (0.8% v/w) provided initial reduction of 1-2 log cycles CFU/g for *S. typhimurium* in all gaseous atmospheres. Outtara et al. (1997) analyzed the antibacterial activity of selected fatty acids and spices essential oils on meat spoilage bacteria and no fatty acid presented antibacterial activity. *Brochothrix thermosphacta* was inhibited by cinnamon, clove, garlic and rosemary essential oil (1/100 v/v); *Serratia liquefaciens* by cinnamon, clove, garlic, pimento and rosemary essential oil (1/100 v/v); *Carnobacterium piscicola* by cinnamon, clove, pimento and rosemary essential oil (1/100 v/v); and *Lactobacillus sake* by cinnamon, clove, black pepper, pimento and rosemary essential oil (1/100 v/v).
Elgayyar et al. (2001) examined the effectiveness of cardamom, anise, basil, coriander, rosemary, parsley, dill and angelica essential oil for controlling the growth and survival of pathogenic and saprophytic microorganisms. The results showed inhibitory property for oregano, basil and coriander essential oil, which presented minimum lethal concentration (v/v) ranging between 8 and 50 ppm for *Pseudomonas aeruginosa, Staphylococcus aureus* and *Yersinia enterocolitica*. Arora and Kaur (1999) analyzed the antimicrobial activity of garlic, ginger, clove, black pepper and ground green chilli and their aqueous extracts on human pathogenic bacteria including *Bacillus sphaericus, Staphylococcus aureus, S. epidermidis, Enterobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa, Salmonella tiph* and *Shigella flexneri* and found that all tested bacteria were sensitive to ground garlic and its extract. Moreover, garlic extract showed considerable cidal effect on *S. epidermidis, S. tiph* and *E. aerogenes*. Kivanç et al. (1991) studied the effect of spices on starter cultures (*Lactobacillus plantarum* and *Leuconostoc mesenteroides*) considering that lactic acid bacteria are relatively resistant to toxic effect of spices and derivatives (Jansen et al., 1987) and that some spices have exerted stimulatory effect on these microorganisms resulting in enhanced acid production (Tiwari and Pandey, 1981). Cumin (0.5, 1.0 and 1.0% w/v) and its essential oil (150, 300 and 600 ppm) stimulated the growth of *L. plantarum* and *L. mesenteroides* and acid production. Oregano was able to stimulate the growth of *L. plantarum* and acid production, however this behavior was not observed in *L. mesenteroides*. 

Outtara et al. (1997) reported antimicrobial activity of many spices and classified their activities as strong, medium, or weak. Several studies (Shelef et al., 1980; Aureli et al., 1992; Conner, 1993) showed that cinnamon, clove, pimento, thyme, oregano and rosemary had strong and consistent inhibitory effect against several pathogen and spoiling bacteria. Exact mechanism of antibacterial action of spices and derivatives is not yet clear (Lanciotti et al., 2004). Although some hypothesis have been given, which involve: i) hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, followed by partition in the lipid bilayer (Juven et al., 1994); ii) perturbation of membrane permeability consequent to its expansion and increased fluidity causing the inhibition of membrane embedded enzymes (Cox et al., 2000); iii) membrane disruption (Caccioni et al., 2000); iv) destruction of electrons transport systems (Tassou et al., 2000); v) cell wall perturbation (Odhav et al., 2002). Generally, gram-negative bacteria have been reported to be more resistant than Gram-positive to essential oils antimicrobial effect because of their cell wall lipopolyssaccharide (Russel, 1991). Cell wall lipopolyssacaride may prevent that essential oils active compounds reach the cytoplasmic membrane of Gram-negative bacteria (Chanegriha et al., 1994).

**ANTIFUNGAL ACTIVITY**

Antifungal activity of spices and derivatives has been studied regarding viable cells count, mycelial growth and mycotoxins synthesis. Juglal et al. (2002) studied the effectiveness of nine essential oils to control the growth of mycotoxin-producing moulds and noted that clove, cinnamon and oregano were able to prevent the growth of *Aspergillus parasiticus* and *Fusarium moniliforme*, while clove (ground and essential oil) markedly reduced the aflatoxin synthesis in infected grains. These findings could be useful for rural communities to prevent the synthesis of fungal toxins in contaminated grains by simple measures. Karapinar (1985) analyzed the inhibitory effect of various concentrations of mint, sage, bay, anise and ground red pepper (0.5, 1.0, 2.0, 4.0, 8.0, 16.0% w/v) on the growth of *Aspergillus parasitic* NRRL 2999 and its aflatoxin production and reported that only thyme presented significant delay on the fungal growth up to 10 days at 2.0% and up to 30 days at 4.0, 8.0 and 16.0%. Basílico and Basílico (1999) studied the inhibitory effect of oregano, mint, basil, sage and coriander on the mycelial growth of *Aspergillus ochraceus* NRRL 3174 and its ochratoxin synthesis and the results showed that oregano (750 ppm) completely inhibited the fungal growth and ochratoxin A synthesis up to 14 days at 25°C. Basil (750 ppm) was effective to inhibit the mycelial growth up to 7 days. Thyagaraja and Hosono (1996) assayed the ability of chilli, coriander, pepper, cumin and asafoetida to inhibit food spoilage moulds (*Rhizopus* spp.).
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**CHEMICAL COMPOUNDS**

In addition to the studies on antimicrobial activity of spices and their extracts and essential oils, the antimicrobial effectiveness of their chemical compounds have also been investigated in order to improve the understanding about the cell targets of the molecules found in spices (Karatzas et al., 2000; Vasquez et al., 2001). Helander et al. (1998) assayed the effect of carvacrol, (+) carvone, thymol and trans-cinnamaldehyde on *E. coli* O157:H7 and *S. thyphimurium* and reported that carvacrol and thymol decreased the intracellular ATP content of *E. coli* cells while simultaneously the extracellular ATP increased. This indicated disruptive action of these compounds toward cytoplasmic membrane.

Delaquis and Mazza (1998) described antimicrobial properties of isothiocyanate derived from onion and garlic. For isothiocyanates, it was hypothesized that they inactivated extracellular enzymes through the oxidative cleavage of disulfide bonds (Brull and Coote, 1999). Delaquis and Mazza (1998) purposed that the formation of reactive thiocyanate radical could mediate the antimicrobial property.
Ramos-Nino et al. (1996) found that benzoic acids, benzaldehydes and cinnamic acid were able to inhibit the growth of *Listeria monocytogenes*. The lipophylic molecular portion of these compounds were recognized as being responsible for this antimicrobial property. Ulette et al. (1999) reported that carvacrol induced depletion of intracellular ATP pool in *Bacillus cereus* as consequence of ATP reduced synthesis or hydrolysis, but there was no change in the ATP permeability for the plasmatic membrane. Cinnamaldehyde, a non-phenolic compound from many spices essential oils showed antimicrobial properties by inhibiting amino acid decarboxylase activity (Wendakoon and Sakaguchi, 1995). Allyhydroxyxycinnamates, which presented similarity to cinnamaldehyde, was reported to inhibit *Pseudomonas fluorescens* by cellular energy depletion (Baranowski and Nagel, 1982).

Hao et al. (1998a, 1998b) reported inhibitory effect of eugenol, active principle of clove essential oil, on *L. monocytogenes* in cooked beef and poultry at 5 and 15°C. Karapinar and Aktug (1987) noted inhibitory effect of eugenol, thymol, menthol and anethole (volatile compounds found in several spices) at 50 and 500µg/mL concentrations on *Salmonella typhimurium*, *S. aureus* and *V. parahaemolyticus*. Nakatani (2003) observed that monoterpenes found in nutmeg and clove, lignans in papua mace and polymethoxylated phenols in alpinia presented promising antimicrobial activity. Campo et al. (2003) studied the most active phenolic compound from rosemary against *L. monocytogenes* regarding the influence of the phenolic compound concentration, pH and NaCl and noted that carnosic acid showed more effective than other assayed phenolic compounds (carnosol, 12-methoxy carnosic, ferulic acid, caffeic acid, rosmarinic acid, luteolin and luteolin-7-glucoside). The mode by which microorganisms are inhibited by essential oils and their chemical compounds seems to involve different mechanisms. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability, unavailability of vital intracellular constituents (Juven et al., 1994; Kim et al., 1995) and/or impairment of bacterial enzymes systems (Farag et al., 1989; Wendakoon and Skaguchi, 1995).

Reports have indicated that essential oils containing carvacrol, eugenol and thymol (phenolic compounds) had highest antibacterial performances (Lattaoi and Tantaoui-Elaraki, 1994; Kim et al., 1995). Many authors have emphasized that the antimicrobial effect of essential oil constituents has been dependent on their hydrophobicity and partition in the microbial plasmatic membrane. Effect of specific ions due to their addition in/on plasmatic membrane had great effect on the protons motive force, intracellular ATP content and overall activity of microbial cells, including turgor pressure control, solutes transport and metabolism regulation (Lanciotti et al., 2004).

**MICROBIOLOGICAL QUALITY OF SPICES**

Presence of pathogenic and spoiling microorganisms in spices could act as vehicles for microorganisms to enter in foods. Frequently, spices are grown and harvested in warm and humid areas where the growth of wide variety of microorganisms is readily supported (Mousuymi and Sarkat, 2003). As many other agricultural commodities, spices are exposed to a wide range of environmental microbial contamination during harvest, processing, and in retail markets by dust, waste water, and animal and even human excreta (Freire and Offford, 2003). The International Commission on Microbiological Specifications for Foods (1974) has set up maximum limit of $10^6$, $10^4$ and $10^3$ CFU of total aerobic mesophilic bacteria (TAMB), fungi, coliforms and *E. coli*, respectively, per gram spice (Zamboni et al., 1991). Brazilian Microbiological Standard for Foods (ANVISA, 2001) has set maximum limit of $5 \times 10^2$ e $10^2$ CFU/g for faecal coliforms and positive coagulase *Staphhylococcus*, respectively, and absence in 25g of spices for *Salmonella*. In German legislation, standard limit value for TAMB, *Bacillus cereus* and *S. aureus* is $10^2$, $10^3$ and $10^6$ CFU per gram of spices, respectively (Mousuymi and Sarkat, 2003). The microbiological quality, the load of heterotrophus or *Enterobacteriaceae* in particular, often acts as indicator of the hygienic situation of the region where the spices are produced and processed (Schwab et al., 1982).
Mousuymi and Sarkat (2003) reported the presence of various microorganisms including total heterotrophus, *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella* and toxigenic moulds in spices. Thus, there is strong need to evaluate and control the microbial quality of spices including bacterial and mycological analyses and presence of microbial toxic metabolites (Fernández et al., 1984; Zamboni et al., 1991; Oliveira et al., 1992; Toro Santa Maria et al., 1993; Hofman et al., 1994; Abdel-Hafez and Al-Said, 1997; Pereira et al., 1999; Freire and Offord, 2003; Benezet et al., 2003). Antimicrobial activity of spices could be recognized as important factor for providing their inclusion in food conservation systems when pertinent measures are taken to assure their satisfactory microbiological quality. These measures must include action to control the moisture, good sanitary conditions in the processing, workers training, satisfactory transport conditions, proper storage, microbiological quality monitoring and actions of sanitary mark applied since harvest until insertion in foods.

**CONCLUSIONS**

Use of spices as microbial growth inhibitor in foods is often limited because of flavor considerations as effective antimicrobial dose may exceed the organoleptically accepted level (Pandit and Shelef 1994; Brull and Coote, 1999). Nonetheless, combinations of spices and other antimicrobial barriers could enhance the food shelf stability and microbial safety even in moderated levels. Due to this and due to the fact that spices are as GRAS, the antimicrobial properties of spices continue to be of interest (Pandit and Shelef, 1994). It is established that spices and their derivatives could be suitable alternatives for inclusion in food conservation systems and could act sometimes as main or adjuvant antimicrobial compounds. Before including spices and/or their derivatives in food conservation systems, some evaluations about microbiological quality, economic feasibility, antimicrobial effect for a long time and toxicity should be carried out.

**RESUMO**

Tem sido constante e crescente a busca por alternativos e eficientes compostos para conservação de total ou parcial substituição de aditivos antimicrobianos químicos tem sido crescente. As especiarias oferecem uma promissora alternativa para a segurança microbiana de alimentos. A atividade inibitória das especiarias e seus produtos derivados sobre o crescimento de bactérias, leveduras, fungos filamentosos e síntese de toxinas microbianas tem sido bem relatada, desta forma as especiarias poderiam ser utilizadas na conservação de alimentos como principais ou co-adjuvantes compostos antimicrobianos com vistas em assegurar a produção de alimentos microbiologicamente estáveis.

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