Artigo Original

Spices: alternative sources of antimicrobial compounds to use in food conservation

Especiarias: fontes alternativas de compostos antimicrobianos para utilização na conservação de alimentos

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RESUMO – *As especiarias têm sido reconhecidas como tendo propriedades antimicrobianas desde a antiguidade e atualmente elas têm surgido como alternativas para serem utilizadas em sistemas de conservação com o objetivo de produzir alimentos microbiologicamente estáveis. Estudos* in vitro *têm demonstrado a efetividade de especiarias e seus produtos derivados em inibir o crescimento de vários microrganismos deteriorantes e patogênicos de importância em alimentos e síntese de metabólitos microbianos tóxicos. Esta revisão tem o objetivo de abordar alguns aspectos que conduziram ao atual interesse em estudar a potencialidade antimicrobiana das especiarias, bem como enfatizar algumas pesquisas considerando a avaliação de sua atividade antimicrobiana sobre microrganismos de importância em alimentos.*

PALAVRAS-CHAVE – Especiarias; atividade antimicrobiana; conservação de alimentos.

SUMMARY – *Spices have been recognized as having antimicrobial properties since antiquity and nowadays they have risen as alternative to be used in food conservation systems in order to produce microbiologically stable foods.* In vitro *studies have shown the effectiveness of spices and by-products to inhibit various pathogenic and food-related spoiling microorganisms and synthesis of microbial toxic metabolites. This review aims to approach some aspects that led to the current interest in studying the antimicrobial potentiality of spices, as well as to emphasize some researches regarding the evaluation of their inhibitory action on food-related microorganisms.*

KEYWORDS – *Spices; antimicrobial activity; food conservation.*

INTRODUÇÃO

During the last 50 years, the food protection against spoiling and food-related pathogens has been reached by the use of various physical and chemical procedures (Benkeblia, 2004). Food conservation has become a complex challenger because there has been a continuous arising of new foods in the market requiring long and stable shelf-life and high protection against the microbial actions (Marino *et al*, 2001; Burt, 2004). Consumers have demanded more natural foods characterized for being free or having low levels of chemical additives and low impact on the environment characterizing the "green consumerism" (Burt, 2004). Moreover, it has been increasing the number of scientific reports about the isolation of microbial strains resistant to traditional antimicrobial methods applied by food industry (Daferera *et al*, 2005; Souza *et al*., 2005).

The increasing interest in replacing conventional food preservatives has impelled the research regarding the possible discovery of plant products having antimicrobial properties (Valero & Salmerón, 2003). Over 1340 plants are known as potential sources of antimicrobials compounds, but few of them have been scientifically studied (Velluti *et al*., 2003). Plants are characterized for possessing wide variation of volatile compounds being many designated as Generally Recognized as Safe-GRAS (Mansay, 2000; Newbern, 2000; Lanciotti *et al*., 2004). Being natural spices antimicrobial compounds appeal for consumers that question the safety of synthetic additives (Sagdiç, 2003).

Spices are rich in essential oils recognized for notable antimicrobial activity (Isamn, 2000; Prasad, 2000). The first report regarding the antimicrobial property of spices rose around 1880's when noted the antimicrobial effectiveness of mustard, clove, cinnamon and their essential oils (Boyle, 1955). Spices antimicrobial effectiveness depend on the kind of spice, its composition and concentration, kind and concentrations of the target microorganism, substrate composition, and processing and food storage conditions (Dorman & Deans, 2000; Marino *et al*., 2001). Many spices are recognized as having therapeutic properties (Table I) being used in folk medicine since antiquity (Isman, 2000; Mansaray, 2000; Srinivasan, 2005).

Among many spices primarily used for flavoring foods and at the same time have their antimicrobial potential recognized are garlic, onion, nutmeg, mustard, black-pepper, thyme, oregano, rosemary, mint, anis, basil, paprika, cassia, celery, dill, ginger, coriander, cilantro,

Recebido em 09/01/2006
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TABLE I
Medicinal properties of some spices

Spices	*Medicinal properties*
Asaofetida (*Ferula asafoetida* Linn.)	Anti-bacterial, anti-spasmodic, diuretic, expectorant, laxative, anti-asthmatic
Cumin (*Cuminum cyminum* Linn.)	Anti-spasmodic, carminative, digestive stimulant
Garlic (*Allium sativum* Linn.)	Anti-dyspeptic, anti-flatulent, useful for ear infection and ulcer treatment
Ginger (*Zingiber officinale* Linn.)	Useful for heart and blood diseases
Onion (*Allium cepa* Linn.)	Diuretic, emmenagogue, expectorant.
Back-pepper (*Piper nigrum* Linn.)	Anti-piretic
Red-pepper (*Capsicum annutum* Linn.)	Anti-inflammatory, analgesic, useful for indigestion treatment
Turmeric (*Curcuma longa* Linn.)	Anti-inflammatory, diuretic, laxative, useful for liver and blood diseases

cinnamon and cumin (Tassou *et al*., 2000; Sagdiç, 2003; Velluti *et al*., 2003; Baydar *et al*., 2004). Scientific assays regarding the antimicrobial properties of spices have been documented in recent years and the interest continues at the present (Cossetino et al., 1999; Dorman & Deans, 2000; Aligianis *et al*., 2001: Diaz *et al*., 2002; Lanciotti *et al*., 2004).

Antimicrobial activity of spice extracts

Antimicrobial activity of spice extracts has been noted on bacteria, moulds and pathogenic and/or spoiling yeasts, as well on the synthesis of toxic microbial metabolites. Sagdiç *et al*. (2002) studied the antimicrobial effectiveness of methanolic extracts from cumin, laurel, oregano, myrtle and thyme (1:1, 1:2, 1:5 and 1:10 v/v) on *E. coli* 0157:H7 (10^6CFU/mL). The researchers noted that excepting laurel extract, the other extracts until concentration 1:10 were able to inhibit the growth of *E. coli* 0157:H7 and deserved prominence the bactericidal effect showed by cumin, oregano, myrtle and thyme extract.

Konning *et al*. (2004) assaying the antimicrobial activity of methanolic extracts (3% v/v) of some medicinal plants from Ghana observed that pepper and ginger methanolic extract were able to inhibit the growth of Gram (+) (*Staphylococcus aureus* and *Bacillus subtillis*), Gram (-) bacteria (*E. coli* and *Pseudomonas aeuruginosa*) and fungi (*Candida albicans* and *Aspergillus niger*). Phytochemical screening showed presence of reducer sugars, glycosides and saponins in the ginger methanolic extract, while alkaloids, reducer sugars, tannins and saponins were found in the pepper one. Ngane *et al*. (2003) evaluating the antifungical activity of pepper ethanolic extract (1, 2 and 4mg/mL) found its effectiveness until 1mg/mL to inhibit the growth of *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Trichosporum rubrum*, *Scopulariopsis brevicaulis* and *Criptococcus neoformans*. Phytochemical analysis showed the presence of alkaloids, cumarinas and poliols in the assayed extract.

Chun *et al*. (2004) assayed the inhibitory activity of aqueous and methanolic extract from oregano (50, 100, 150 and 200µg/mL) on *Helicobacter pylori* and found high capacity of inhibition. Higher anti-*H. pilory* property showed by oregano methanolic extract was proposed to be related with its higher concentration of phenolic compounds when compared to the ethanolic extract. It was observed the presence of protocatequinic, cafeic, cumarinic and rosmarinic acid and quercetin in both extracts, although quantitative differences have been detected. Inhibitory activity of phenolic compounds on bacteria could take place by citossolic hyperacidity, breakage of electrons transport chain, cytoplasmic membrane disturbing, H^+-ATPase inhibition and channels inhibition (Shetty *et al*., 2004).

Sagdiç (2003) studied the inhibitory property of thyme and oregano hydrosols (10, 25, 50 and 75mL/100L) on *E. coli, S. aureus* and *Yersinia enterocolitica* (10^6-10^7CFU/mL). Hydrosols showed inhibitory activity on all assayed bacterial strains providing the appearance of large bacterial growth inhibition halos. Moreover, hydrosols at concentration 1:2 were able to cause kill of *S. aureus* in broth in maximum time of 4 days. Hydrosols, also called floral water or aromatic water, are byproducts from plant materials hidrodistillation and are characterized as complex mixtures of water soluble compounds and traces of essential oils.

Arora & Kaur (1999) assayed the inhibitory action of aqueous extracts from garlic, clove, black-pepper and chilli-pepper on various bacteria (*B. sphaericus, Enterobacter aerogens, E. coli, P. aeruginosa, S. aureus, Shigella flexneri*, *Salmonella typhi*) and yeasts (*C. albicans, C. apicola, C. acutus, C. catenulata, C. inconspicua, C. tropicalis, Rhodotorula rubra, Trignopsis variabilis*). Garlic extract was prominent in providing bactericidal and fungicidal effect in a short action time (2-5 hours). Inhibitory effectiveness of garlic aqueous extract on *Candida* species has been related to the oxidation of the tiol group (L-cisteyne glutatione-2-mercaptoethanol) causing enzymatic inactivation and yeast growth inhibition (Barone et al., 1977). Allicin is known as principal anti-*Candida* compound present in aqueous garlic extract.

Thyagaraja & Hosono (1996) studying the antifungal activity of methanolic extract from coriander, cumin, black-pepper, turmeric and asafoetida noted that asafoetida was active to inhibit *Rhizopus azigosporum, Mucor dimorphosphorus, Penicillium commune* and *Fusarium solani*. Trying to isolate the asafoetida antifungal compound was studied the antifungal action of the its aqueous (extracted with hot water) and lipidic (extracted with diethyl ether) fraction, being found that the active compound was present in the lipidic fraction due its capability to cause the mould mycelial growth inhibition.

Antimicrobial activity of spice essential oils

Essential oils (also named volatile oils) are aromatic oily liquids obtained from plant material as flowers, leaves, branches, stems, roots and fruits (Burt, 2004). Approximately 3000 essential oils are known, of which 300 are commercially important for flavor and fragrance market (Mourey & Canillac, 2002). Essential oils as antimicrobial agents present two principal characteristics:

i) their natural origin meaning more safety for consumers and environment; and

ii) there is low risk of rising microbial resistance to their action because essential oils are mixtures of several compounds that, apparently, present different antimicrobial action modes becoming more difficult the microbial adaptability (Daferera *et al*., 2003). Table II shows the major compounds of some spice essential oils known as having antibacterial properties.

Menon & Garg (2001) evaluating the inhibitory effect of clove essential oil (0.5 and 1.0% v/w) on *Listeria monocytogenes* in meat and cheese found that both assayed concentrations when stored at 7° and 30°C were able to cause statistically significant decreasing of the *L. monocytogenes* viable cells number in the assayed substrates. Hao *et al*. (1998a) and Hao *et al*. (1998b) reported inhibitory effect of eugenol, active compound of clove essential oil, on *L. monocytogenes* in beef and cooked poultry stored at 5° and 15°C.

Skandamis *et al.* (2002) assayed the effect of oregano essential oil on the survival/kill of *S. typhimurium* in beef stored at 5°C under different gaseous atmospheres and observed that the essential oil addition (0.85% v/w) presented significant reduction of *S. typhimurium* and spoiling micoflora. Antimicrobial activity of oregano essential oil has been related to its high concentration of phenolic compounds, which are able to dissolve into the microbial cytoplasmic membrane penetrating inside the microbial cell where they could interact with essential mechanisms for microbial metabolism (Marino *et al*., 2001). Generally, essential oils with prominent inhibitory property on food-borne pathogens present high percentage of phenolic compounds (Arora & Kaur, 1999).

Benkeblia (2004) found inhibitory property of onion and garlic essential oil until concentration 50μl/mL on *S. aureus, S. enteridis, F. oxysporum, P. cyclopium* and *A. niger*. The researchers reported that, instability of allicin, thyosulfates and related compounds, as well as their strong flavor and characteristic taste, unfortunately, could be limiting factor to apply garlic and onion essential oil as food additives.

Velluti *et al*. (2003) noted significant inhibitory effect of cinnamon, oregano and clove essential oil on the growth of *F. proliferatum* and *F. verticillioides* and synthesis of Fumonisin B_1 in maize grains with different water activities. Antifungal effectiveness was greater when the essential oils interacted with maize grains with higher water activities and it possibly happened due to water proportionate better penetration of essential oils into the grains intern parts.

Regarding the great number of different chemical compounds found in essential oils, their antimicrobial activity, possibly, could not be attributed to a specific mechanism. Otherwise, it is believed that their antimicrobial is developed by the attack on various targets in/on the microbial cell (Carlson *et al*., 2002). Cytoplasmic membrane coagulation, breakdown of protons motive force, breakdown of electron flux and active transport unbalance are some events responsible for promoting the antimicrobial property of essential oils (Sikkema, 1995; Carlson *et al*., 2002). These biological events have been believed for no occurring separately, as such as some of them are activated as consequence of other ones (Sikkema, 1995; Burt, 2004).

Inhibitory effect of spice essential oils against bacteria and fungi noted in laboratorial media has some times been different when assayed in solid foods (Skandamis & Nychas, 2000). Scientific reports have found that antimicrobial effectiveness of essential oils in *in vitro* antibacterial assays have just been noted in food matrix when the essential oil is present in higher concentrations (Smid & Gorris, 1999). The relation of *in vitro* effective concentration with that found in foods have been 2 folds in semi-skimmed milk (Karatzas *et al*., 2001), 10 folds in pig sausage (Pandit & Shelef, 1997), 50 folds in soup (Ultee & Smid, 2001) and 50-100 folds in cheese (Mendoza-Yepes *et al*., 1997). Higher nutrients availability in foods when compared to broth or synthetic agar could become some bacteria able to repair faster the cellular damage caused by essential oils compounds (Gili *et al*., 2002). Bacterial susceptibility to essential oils has been made stronger with food pH decrease, use of low store temperature and decreased oxygen availability in packages (Skandamys *et al*., 2000; Tsigarida *et al*., 2000).

Some few food preservatives having plant products as active compounds are commercially available. DMC Base Natural's is a food preservative produced by Domca S.A., Alhedín, Granda, Spain, and comprehend 50% of sage, citrus and rosemary essential oil and 50% of glycerol (Mendoza-Yepes *et al*., 1997). Protecta One and Protecta Two are herb extracts mixtures produced by Bavaria Corporation Apopka, Florida, USA, recognized as GRAS and ,although, the precise composition of these preservatives are not informed by producers, the extracts possibly have one or more essential oils dispersed in sodium chlorine and citrate chlorine solutions (Cutter, 2003).

TABLE II
Major compounds of some spices essential oils with antibacterial properties showed *in vitro* or in food models (data based on reports from literature) (Burt, 2004)

Spice common name	Scientific name	Major compounds	Approximate % composition*
Cilantro	*Coriandrum sativum* (immature leaves)	Linalol E-2-Decanal	26% 20%
Coriander	*Coriadrum sativum* (seeds)	Linalool	70%
		E-2-Decanal	–
Cinnamon	*Cinnamomum zeylanicum*	Trans-cinnamaldehydo	65%
Oregano	*Origanum vulgare*	Carvacrol	Traces-80%
		Thymol	Traces-64%
		γ-terpinene	2-52%
		p-Cymene	Traces-82%
Rosemary	*Rosmarinus officinalis*	α-pinene	2-25%
		Bornyl acetate	0-17%
		Canfor	2-14%
		1,8-cineole	3-89%
Sage	*Salvia officinalis*	Canfor	6-15%
		α-pinene	4-5%
		β-pinene	2-10%
		1,8-cineole	8-14%
		A-tujone	20-42%
Clove	*Syzigium aromaticum*	Eugenol	75-85¨%
		Eugenyl acetate	8-15%
Thyme	*Thymus vulgaris*	Thymol	10-64%
		Carvacrol	2-11%
		terpinene	2-31%
		p-cymene	10-56%

** P ercentage of total volatiles rounded up to the nearest whole number.*

CONCLUSIONS

Importance of spices as natural antimicrobials with renewed scientific interest in their rational application in food conservation can be noted by the increasing number of scientific reports in the last 90's. In a reality of search for natural antimicrobial compounds, the spices and by-products have risen up as interesting viable alternatives for the food industry to attend the consumers wish of getting foods free or with low levels of synthetic additives, but with the convenience of having long shelf-life and being innocuous for consumers. However, there has been a necessity of carrying out researchers emphasizing the spices antimicrobial action modes, interactions between spices and food components and synthetic additives, synergistic and antagonistic interactions between extracts or spices essential oils compounds, stability of spices by-products to food processing procedures (e.g. low pH, high temperatures, low temperatures) and toxicity of spices and by-products.

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