Available online at www.sciencedirect.com
ScienceDirect
Food Control 19 (2008) 1130–1138
FOOD CONTROL
www.elsevier.com/locate/foodcont

Antifungal activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils

M. Viuda-Martos, Y. Ruiz-Navajas, J. Fernández-López *, J. Pérez-Álvarez

Dpto. Tecnología Agroalimentaria, Escuela Politécnica Superior de Orihuela (Universidad Miguel Hernández), Ctra Beniel, km 3.2, E-03312 Orihuela (Alicante), Spain

Received 31 July 2007; received in revised form 10 December 2007; accepted 14 December 2007

Abstract

The objective of this work was to study the effect of the essential oils of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) on the growth of moulds commonly associated with food spoilage: *Aspergillus niger*, *Aspergillus flavus, Penicillium chrysogenum* and *Penicillium verrucosum*, using the agar dilution method. All the oils showed antifungal activity against all the moulds. Orange essential oil was the most effective against *A. niger*, mandarin essential oil was most effective at reducing the growth of *Aspergillus flavus* while grapefruit was the best inhibitor of the moulds *P. chrysogenum* and *P. verrucosum*. Citrus essential oils could be considered suitable alternatives to chemical additives for use in the food industry.

Keywords: Essential oils; Citrics; Moulds

1. Introduction

The growing awareness of consumers concerning the relation between food and health is revolutionising the food industry. New techniques such as high pressure, nanotechnology, irradiation, etc., are increasingly used to maximise the nutritional properties of foods, while new ingredients with functional properties contribute to improving health. The "elimination" of additives used in a wide variety of foods is demanded, while "natural" additives are seen as a benefit for both quality and safety.

In the face of this challenge, researchers are looking for new sources of ingredients and/or additives. One such source may be agro-food co-products. In the production of these new ingredients other, new co-products are generated, such as when fibre-rich citrus extracts are obtained (Lario et al., 2004). These extracts have been shown to have antimicrobial properties in several foodstuffs (Fernandez-Lopez, Zhi, Aleson-Carbonell, Pérez-Álvarez, & Kuri, 2005).

The use of chemical or synthetic agents with antimicrobial activity (as inhibitors, growth reducers or even inactivators) is one of the oldest techniques for controlling microorganism growth. The application of preservatives to foods is fundamental if their safety is to be maintained.

Natural antimicrobials, whether of microbial, animal or plant origin, which show bacteriostatic/fungistatic or bactericidal/fungicidal lengthen the useful life of foods and avoid, among other things, health-related problems, off-odors, unpleasant tastes, textural problems or changes in colour, which are basically caused by the enzymatic or metabolic systems of the principal microorganisms that lead to the alteration of foods (Feng & Zheng, 2007; López-Malo, Alzamora, & Guerrero, 2000).

Among natural antimicrobials are the essential oils (EOs) extracted from many plants and fruits, and many studies have described their antimicrobial effects (Angioni

* Corresponding author. Tel.: +34 966749734; fax: +34 966749677.
E-mail address: j.fernandez@umh.es (J. Fernández-López).

0956-7135/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2007.12.003

et al., 2003; Been, Akhila, & Abraham, 2007; Dehghan et al., 2007; Jo et al., 2004; Salgueiro et al., 2006; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2007). Essential oils and their main components possess a wide spectrum of biological activity, which may be of great importance in several fields, from food chemistry to pharmacology and pharmaceutics (Cristani, Mandalari, Sarpietro, Venuti, & Saija, 2007). The main advantage of essential oils is that they can be used in any foods and are considered generally recognized as safe (GRAS) (Kabara, 1991), as long as their maximum effects is attained with the minimum change in the organoleptic properties of the food. Such antimicrobial activity is due to the presence of bioactive substances such as flavonoids, terpenes, coumarines and carotenes (Tepe, Daferera, Sokmen, Sokmen, & Polissiou, 2005). The objective of this work was to study the effect of the essential oils of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) on the growth of moulds commonly associated with food spoilage including *Aspergillus niger*, *Aspergillus flavus, Penicillium chrysogenum* and *Penicillium verrucosum*.

2. Material and methods

2.1. Essential oils

The essential oil of orange (*C. sinensis* L.), ref. F70850L, was obtained by cold-pressing the peel. Its density at 20 °C was 0.85 g/mL, its refraction index at 20 °C was 1.47, while its boiling point was 49 °C. Lemon (*C. lemon* L.) ref. F70730L, mandarin (*C. reticulata* L.) ref. F70760L, and grapefruit (*C. paradisi* L.) ref. F71345L essential oils were obtained in the same way, while their corresponding densities and refraction indices (both at 20 °C) were: 0.85 g/mL and 1.48 (lemon), 0.85 g/mL and 1.47 (mandarin) and 0.85 g/mL and 1.47 (grapefruit). Their corresponding boiling points were 48, 49 and 52 °C. All the essential oils were purchased from Ravetllat Aromatics, (Barcelona, Spain).

2.2. Antimicrobial activity

2.2.1. Microbial strains

The essentials oils were individually tested against a panel of moulds, consisting of *P. chrysogenum* CECT 2784, *P. verrucosum* CECT 2906, *A. niger* CECT 2091 and *A. flavus* CECT 2685. All the above species were supplied by the Spanish Type Culture Collection (CECT) of the University of Valencia.

2.2.2. Agar dilution method

The food-pathogenic fungi were tested by the agar dilution method (Fraternale, Giamperi, & Ricci, 2003) with some modification, in the appropriate culture media (Potato Dextrose Agar, Oxoid, Basingstoke, Hampshire, England). The oils tested were added to the culture medium at a temperature of 40–45 °C, and then poured into Petri dishes (10 cm diameter). Concentrations 0.27%, 0.47%, 0.71% and 0.94% were tested for mandarin, lemon, orange and grapefruit essential oils. The moulds were inoculated, as soon as the medium had solidified. A disc (9 mm in diameter, Schlinder & Schuell, Dassel, Germany) of mycelial material, taken from the edge of five-day-old fungi cultures, was placed at the centre of each Petri dish. The Petri dish with the inoculum was then incubated at 25 °C. The efficacy of treatment was evaluated each day during nine days by measuring the diameter of the fungus colonised. The values were expressed in millimetres diameter/day. All tests were performed in triplicate.

2.3. Statistical analysis

Each parameter was tested in triplicate. Conventional statistical methods were used to calculated means and standard deviations. Statistical analysis (ANOVA) was applied to the data to determine differences ($p < 0.05$). To ascertain significant differences between the levels of the main factor, Tukey's test was applied between means (Afifi & Azen, 1979). Statistical data analysis was undertaken using the statistical package Statgraphics plus 2.0

3. Results and discussion

The essential oils of lemon, orange, mandarin and grapefruit at the concentrations assayed all showed the capacity to reduce or inhibit the growth of the moulds *P. chrysogenum, P. verrucosum, A. niger* and *A. flavus*. Tables 1–4 show the growth of the moulds *A. flavus, A. niger, P. chrysogenum* and *P. verrucosum,* respectively, during the nine days under study. Table 5 shows the% growth reductions obtained with the essentials oils at day 9.

In the case of *A. niger*, its growth was completely inhibited when a concentration of 0.94% of any of the EOs was used. Orange EO produced the greatest reduction in mycelium growth with this fungus at 0.27%, 0.47% and 0.71%, with percentage reductions of 29.5%, 36.4% and 48.1%, respectively. The second most effective EO with this mould was lemon EO, with reductions in mycelial growth of 21.6%, 26.0% and 40.0%, respectively, at the same concentrations.

The mandarin and grapefruit EOs caused the lowest percentage of mycelial reduction in *A. niger*, although the reduction obtained with the latter at 0.47% and 0.71% were close to those obtained at the same concentrations with lemon EO (14.7% and 31.9%, respectively), while at 0.27% the reduction provoked by this EO and mandarin EO (6.7% and 5.1%, respectively) were much below those obtained with orange and lemon EOs (29.5% and 21.6%, respectively).

In the case of *A. flavus,* with *A. niger*, total inhibition of growth was obtained with all the EOs at the highest concentration of 0.94%. In this case, though, the mandarin EO showed the highest inhibitions of mycelial growth with values of 55.5%, 62.8% and 64.8% at 0.27%, 0.47% and

Table 1
Antifungal activity of mandarin, lemon, orange and grapefruit essential oils using agar dilution method *upon Aspergillus flavus*

	Diameter (mean and SD $n = 3$) of mycelial growth (mm) including disc diameter of 9 mm									
	0 day	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days
Control	9.00 ± 0.00	10.2 ± 0.06	23.0 ± 0.07	37.4 ± 0.18	54.1 ± 0.03	66.2 ± 0.12	80.4 ± 0.13	87.3 ± 0.08	90.0 ± 0.00	90.0 ± 0.00
Mandarin 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	17.4 ± 0.03^{bL}	23.2 ± 0.16^{cL}	28.4 ± 0.17^{dL}	33.7 ± 0.18^{eL}	36.0 ± 0.11^{fL}	37.9 ± 0.16^{gL}	39.7 ± 0.17^{hL}	40.0 ± 0.11^{iL}
Mandarin 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	13.9 ± 0.06^{bA}	18.2 ± 0.11^{cA}	22.1 ± 0.09^{dA}	26.0 ± 0.10^{eA}	28.3 ± 0.06^{fA}	30.1 ± 0.17^{gA}	32.0 ± 0.19^{hA}	33.5 ± 0.18^{iA}
Mandarin 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	12.2 ± 0.08^{aW}	15.3 ± 0.09^{aW}	16.5 ± 0.17^{aW}	18.3 ± 0.07^{aW}	23.8 ± 0.18^{aW}	24.9 ± 0.08^{aW}	29.8 ± 0.11^{aW}	31.7 ± 0.11^{aW}
Mandarin 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.07^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}
Lemon 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	10.0 ± 0.07^{bM}	17.7 ± 0.07^{cM}	23.4 ± 0.08^{dM}	28.3 ± 0.07^{eM}	34.4 ± 0.07^{fM}	37.3 ± 0.10^{gM}	43.0 ± 0.08^{hM}	44.0 ± 0.07^{iM}
Lemon 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aB}	14.5 ± 0.08^{cB}	21.1 ± 0.13^{dB}	27.0 ± 0.12^{eB}	31.9 ± 0.17^{fB}	37.0 ± 0.10^{gB}	41.7 ± 0.07^{hB}	42.4 ± 0.06^{iB}
Lemon 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aX}	17.3 ± 0.16^{dX}	23.5 ± 0.16^{eX}	28.7 ± 0.14^{fX}	33.1 ± 0.08^{gX}	37.0 ± 0.10^{hX}	38.6 ± 0.10^{iX}
Lemon 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}
Orange 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	19.9 ± 0.15^{bN}	28.7 ± 0.07^{cN}	39.2 ± 0.18^{dN}	48.9 ± 0.07^{eN}	55.0 ± 0.10^{fN}	62.9 ± 0.17^{gN}	67.6 ± 0.13^{hN}	76.2 ± 0.12^{iN}
Orange 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	14.8 ± 0.08^{bC}	19.6 ± 0.11^{cC}	27.1 ± 0.07^{dC}	37.1 ± 0.06^{eC}	46.0 ± 0.11^{C}	52.0 ± 0.16^{gC}	60.6 ± 0.07^{hC}	67.3 ± 0.07^{iC}
Orange 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	12.5 ± 0.04^{bX}	16.2 ± 0.16^{cY}	23.1 ± 0.10^{dY}	31.2 ± 0.11^{eY}	37.8 ± 0.08^{fY}	44.7 ± 0.19^{gY}	52.5 ± 0.11^{hY}	61.2 ± 0.17^{iY}
Orange 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}
Grapefruit 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	19.1 ± 0.16^{bO}	25.2 ± 0.11^{1cO}	30.6 ± 0.06^{dO}	36.0 ± 0.07^{eO}	38.7 ± 0.12^{fO}	42.3 ± 0.07^{gO}	45.3 ± 0.06^{hO}	46.3 ± 0.18^{iO}
Grapefruit 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	17.0 ± 0.10^{bD}	22.7 ± 0.08^{cD}	26.4 ± 0.11^{dD}	30.3 ± 0.13^{eD}	32.5 ± 0.17^{fD}	37.0 ± 0.10^{gB}	40.2 ± 0.08^{hD}	41.0 ± 0.08^{iD}
Grapefruit 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	15.4 ± 0.08^{bY}	20.6 ± 0.10^{cZ}	24.3 ± 0.13^{dZ}	28.9 ± 0.09^{eZ}	32.0 ± 0.12^{fZ}	34.6 ± 0.14^{gZ}	36.9 ± 0.16^{hZ}	38.4 ± 0.09^{iZ}
Grapefruit 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}

Values followed by the same small letter within the same line are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (L–O) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (A–D) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (W–Z) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (R–V) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.

Table 2
Antifungal activity of mandarin, lemon, orange and grapefruit essential oils using agar dilution method upon *Aspergillus niger*

	Diameter (mean and SD $n = 3$) of mycelial growth (mm) including disc diameter of 9 mm									
	0 day	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days
Control	9.00 ± 0.00	12.7 ± 0.12	18.9 ± 0.04	29.5 ± 0.08	34.7 ± 0.16	44.1 ± 0.03	53.8 ± 0.14	64.5 ± 0.12	72.1 ± 0.04	80.9 ± 0.17
Mandarin 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	12.0 ± 0.04^{bL}	21.3 ± 0.18^{cL}	26.6 ± 0.16^{dL}	35.0 ± 0.21^{eL}	46.9 ± 0.09^{fL}	56.1 ± 0.01^{gL}	67.2 ± 0.19^{hL}	76.7 ± 0.13^{iL}
Mandarin 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	11.7 ± 0.16^{bA}	18.4 ± 0.04^{cA}	23.0 ± 0.11^{dA}	29.4 ± 0.12^{eA}	39.1 ± 0.16^{fA}	48.8 ± 0.10^{gA}	58.7 ± 0.10^{hA}	69.0 ± 0.05^{iA}
Mandarin 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	9.00 ± 0.11^{aW}	13.1 ± 0.11^{bW}	18.0 ± 0.13^{cW}	23.5 ± 0.04^{dW}	30.6 ± 0.11^{eW}	38.5 ± 0.12^{fW}	47.7 ± 0.13^{gW}	55.1 ± 0.10^{hW}
Mandarin 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}
Lemon 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	17.3 ± 0.20^{bM}	22.8 ± 0.16^{cM}	28.8 ± 0.02^{dM}	34.5 ± 0.06^{eM}	41.0 ± 0.18^{fM}	47.3 ± 0.07^{gM}	55.3 ± 0.18^{hM}	63.4 ± 0.04^{iM}
Lemon 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	12.2 ± 0.04^{bB}	19.0 ± 0.09^{cB}	25.4 ± 0.11^{dB}	30.0 ± 0.02^{eB}	39.1 ± 0.12^{fB}	44.6 ± 0.02^{gB}	53.0 ± 0.14^{hB}	59.8 ± 0.11^{iB}
Lemon 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	10.0 ± 0.11^{bX}	16.0 ± 0.04^{cX}	21.0 ± 0.20^{dX}	25.9 ± 0.10^{eX}	32.9 ± 0.04^{fX}	38.1 ± 0.09^{gX}	43.1 ± 0.21^{hX}	48.5 ± 0.08^{iX}
Lemon 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}
Orange 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	10.0 ± 0.03^{bN}	16.2 ± 0.03^{cN}	20.3 ± 0.17^{dN}	27.7 ± 0.03^{eN}	35.0 ± 0.17^{fN}	44.3 ± 0.15^{gN}	50.0 ± 0.18^{hN}	57.0 ± 0.05^{iN}
Orange 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aC}	13.0 ± 0.12^{cC}	18.2 ± 0.05^{dC}	24.6 ± 0.01^{eC}	29.1 ± 0.13^{fC}	37.0 ± 0.18^{gC}	43.6 ± 0.13^{hC}	51.5 ± 0.17^{iC}
Orange 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	9.00 ± 0.04^{aW}	9.0 ± 0.16^{aY}	9.9 ± 0.15^{bY}	14.6 ± 0.05^{cY}	21.7 ± 0.12^{dY}	27.2 ± 0.20^{eY}	35.1 ± 0.03^{fY}	42.0 ± 0.10^{gY}
Orange 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}
Grapefruit 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	15.4 ± 0.18^{bO}	24.0 ± 0.11^{cO}	32.0 ± 0.04^{dO}	42.6 ± 0.12^{eO}	51.7 ± 0.09^{fO}	60.9 ± 0.04^{gO}	70.2 ± 0.18^{hO}	75.4 ± 0.19^{iO}
Grapefruit 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	10.7 ± 0.03^{bD}	13.2 ± 0.10^{cC}	20.8 ± 0.12^{dD}	26.3 ± 0.08^{eD}	34.0 ± 0.19^{fD}	42.3 ± 0.17^{gD}	52.0 ± 0.12^{hD}	59.9 ± 0.10^{iB}
Grapefruit 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	9.00 ± 0.16^{aW}	11.2 ± 0.02^{bZ}	15.7 ± 0.09^{cZ}	22.7 ± 0.07^{dZ}	28.9 ± 0.10^{eZ}	37.4 ± 0.15^{fZ}	45.8 ± 0.05^{gZ}	52.6 ± 0.02^{hZ}
Grapefruit 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}

Values followed by the same small letter within the same line are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (L–O) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (A–D) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (W–Z) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (R–V) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.

Table 3
Antifungal activity of mandarin, lemon, orange and grapefruit essential oils using agar dilution method upon *Penicillium chrysogenum*

	Diameter (mean and SD $n = 3$) of mycelial growth (mm) including disc diameter of 9 mm									
	0 day	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days
Control	9.00 ± 0.00	11.4 ± 0.05	18.8 ± 0.10	20.0 ± 0.08	25.4 ± 0.03	33.7 ± 0.05	47.5 ± 0.02	56.3 ± 0.04	63.9 ± 0.04	78.8 ± 0.03
Mandarin 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	15.1 ± 0.08^{bL}	19.9 ± 0.10^{cL}	23.2 ± 0.04^{dL}	27.6 ± 0.05^{eL}	34.9 ± 0.05^{fL}	42.8 ± 0.04^{gL}	50.6 ± 0.02^{hL}	63.7 ± 0.09^{iL}
Mandarin 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	14.4 ± 0.15^{bA}	18.5 ± 0.12^{cA}	19.6 ± 0.02^{dA}	24.0 ± 0.03^{eA}	29.7 ± 0.03^{fA}	38.2 ± 0.03^{gA}	43.9 ± 0.01^{hA}	50.6 ± 0.10^{iA}
Mandarin 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	13.7 ± 0.11^{bW}	17.9 ± 0.11^{cW}	18.7 ± 0.02^{dW}	20.9 ± 0.04^{eW}	25.7 ± 0.03^{fW}	31.8 ± 0.03^{gW}	37.3 ± 0.04^{hW}	42.3 ± 0.07^{iW}
Mandarin 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}
Lemon 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	16.8 ± 0.10^{bM}	19.1 ± 0.12^{cM}	23.4 ± 0.08^{dM}	29.7 ± 0.09^{eM}	41.9 ± 0.06^{fM}	48.4 ± 0.08^{gM}	55.1 ± 0.07^{hM}	61.2 ± 0.08^{iM}
Lemon 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	13.9 ± 0.12^{bB}	15.2 ± 0.13^{cB}	16.8 ± 0.12^{dB}	25.8 ± 0.12^{eB}	32.4 ± 0.08^{fB}	38.0 ± 0.06^{gB}	41.5 ± 0.11^{hB}	48.5 ± 0.09^{iB}
Lemon 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	12.9 ± 0.09^{bX}	14.7 ± 0.11^{cX}	15.3 ± 0.09^{dX}	20.2 ± 0.10^{eX}	25.3 ± 0.07^{fX}	30.9 ± 0.10^{gX}	36.0 ± 0.06^{hX}	41.2 ± 0.11^{iX}
Lemon 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}
Orange 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	16.2 ± 0.11^{bN}	18.9 ± 0.14^{cM}	22.4 ± 0.11^{dN}	31.3 ± 0.13^{eN}	39.8 ± 0.11^{fN}	44.5 ± 0.03^{gN}	51.6 ± 0.05^{hN}	58.9 ± 0.08^{iN}
Orange 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	15.1 ± 0.10^{bC}	16.5 ± 0.10^{cC}	19.3 ± 0.10^{dC}	25.1 ± 0.10^{eC}	30.7 ± 0.10^{fC}	35.0 ± 0.05^{gC}	40.7 ± 0.02^{hC}	47.5 ± 0.11^{iC}
Orange 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	13.7 ± 0.13^{bW}	14.5 ± 0.08^{cX}	15.7 ± 0.03^{dY}	19.6 ± 0.08^{eY}	24.3 ± 0.10^{fY}	28.5 ± 0.04^{gY}	34.9 ± 0.10^{hY}	40.3 ± 0.09^{iY}
Orange 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}
Grapefruit 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	15.3 ± 0.10^{bL}	18.0 ± 0.09^{cO}	19.1 ± 0.07^{dO}	24.5 ± 0.10^{eO}	31.1 ± 0.10^{fO}	36.9 ± 0.06^{gO}	41.7 ± 0.07^{hO}	50.8 ± 0.09^{iO}
Grapefruit 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	14.7 ± 0.08^{bD}	16.9 ± 0.04^{cD}	18.0 ± 0.05^{dD}	21.2 ± 0.04^{eD}	28.0 ± 0.01^{fD}	33.1 ± 0.10^{gD}	39.1 ± 0.08^{hD}	46.3 ± 0.10^{iD}
Grapefruit 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	13.1 ± 0.07^{bZ}	13.2 ± 0.02^{cZ}	15.2 ± 0.11^{dX}	20.2 ± 0.11^{eX}	24.5 ± 0.03^{fZ}	28.3 ± 0.09^{gZ}	34.8 ± 0.13^{hY}	40.9 ± 0.07^{iZ}
Grapefruit 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}

Values followed by the same small letter within the same line are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (L–O) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (A–D) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (W–Z) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (R–V) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.

Table 4
Antifungal activity of mandarin, lemon, orange and grapefruit essential oils using agar dilution method upon *Penicillium verrucosum*

	Diameter (mean and SD $n = 3$) of mycelial growth (mm) including disc diameter of 9 mm									
	0 day	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days
Control	9.00 ± 0.00	9.31 ± 0.02	18.0 ± 0.04	20.6 ± 0.03	24.4 ± 0.09	35.9 ± 0.08	49.5 ± 0.10	53.2 ± 0.11	65.8 ± 0.08	80.6 ± 0.13
Mandarin 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	14.9 ± 0.03^{bL}	19.5 ± 0.04^{cL}	21.4 ± 0.06^{dL}	26.8 ± 0.10^{eL}	33.9 ± 0.06^{fL}	42.1 ± 0.09^{gL}	52.5 ± 0.11^{hL}	66.8 ± 0.03^{iL}
Mandarin 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	14.1 ± 0.07^{bA}	17.5 ± 0.03^{cA}	19.7 ± 0.07^{dA}	24.1 ± 0.09^{eA}	29.8 ± 0.08^{fA}	38.2 ± 0.07^{gA}	46.0 ± 0.08^{hA}	55.5 ± 0.08^{iA}
Mandarin 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	13.5 ± 0.04^{bW}	16.9 ± 0.08^{cW}	18.8 ± 0.06^{dW}	21.0 ± 0.07^{eW}	25.8 ± 0.08^{fW}	31.9 ± 0.07^{gW}	40.9 ± 0.06^{hW}	46.3 ± 0.11^{iW}
Mandarin 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}
Lemon 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	16.8 ± 0.02^{bM}	19.0 ± 0.07^{cM}	22.4 ± 0.09^{dM}	29.7 ± 0.10^{eM}	41.9 ± 0.08^{fM}	48.4 ± 0.10^{gM}	54.9 ± 0.07^{hM}	64.1 ± 0.15^{iM}
Lemon 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	14.1 ± 0.02^{bA}	15.1 ± 0.06^{cB}	16.7 ± 0.07^{dB}	25.7 ± 0.08^{eB}	32.4 ± 0.09^{fB}	38.0 ± 0.06^{gB}	40.2 ± 0.08^{hB}	50.0 ± 0.11^{iB}
Lemon 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	12.0 ± 0.05^{bX}	14.7 ± 0.08^{cX}	15.3 ± 0.07^{dX}	20.2 ± 0.07^{eX}	25.3 ± 0.07^{fX}	30.8 ± 0.07^{gX}	34.7 ± 0.06^{hX}	42.6 ± 0.17^{iX}
Lemon 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}
Orange 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	15.8 ± 0.07^{bN}	18.0 ± 0.09^{cN}	19.1 ± 0.06^{dN}	24.5 ± 0.08^{eN}	31.0 ± 0.06^{fN}	38.9 ± 0.10^{gN}	42.6 ± 0.08^{hN}	52.8 ± 0.09^{iN}
Orange 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	14.6 ± 0.02^{bB}	17.0 ± 0.07^{cC}	18.1 ± 0.07^{dC}	21.2 ± 0.09^{eC}	28.1 ± 0.11^{fC}	36.2 ± 0.11^{gC}	40.3 ± 0.09^{hB}	45.6 ± 0.16^{iC}
Orange 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	11.9 ± 0.11^{bX}	16.3 ± 0.08^{cY}	17.3 ± 0.08^{dY}	20.3 ± 0.07^{eX}	24.6 ± 0.10^{fY}	31.3 ± 0.10^{gY}	35.6 ± 0.10^{hY}	41.7 ± 0.05^{iY}
Orange 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}
Grapefruit 0.27%	9.00 ± 0.00^{aL}	9.00 ± 0.00^{aL}	16.2 ± 0.05^{bO}	17.8 ± 0.11^{cN}	23.6 ± 0.07^{dO}	27.4 ± 0.06^{eO}	39.9 ± 0.07^{fO}	44.6 ± 0.08^{gO}	52.1 ± 0.10^{hO}	59.6 ± 0.07^{iO}
Grapefruit 0.47%	9.00 ± 0.00^{aA}	9.00 ± 0.00^{aA}	15.6 ± 0.08^{bC}	16.7 ± 0.11^{cD}	19.4 ± 0.08^{dD}	25.3 ± 0.08^{eD}	30.8 ± 0.08^{fD}	35.1 ± 0.09^{gD}	42.8 ± 0.11^{hC}	48.5 ± 0.16^{iD}
Grapefruit 0.71%	9.00 ± 0.00^{aW}	9.00 ± 0.00^{aW}	12.1 ± 0.09^{bX}	14.6 ± 0.04^{cX}	15.8 ± 0.10^{dZ}	19.7 ± 0.09^{eZ}	24.5 ± 0.10^{fY}	28.6 ± 0.07^{gZ}	35.8 ± 0.09^{hY}	42.2 ± 0.10^{iZ}
Grapefruit 0.94%	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}	9.00 ± 0.00^{aR}

Values followed by the same small letter within the same line are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (L–O) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (A–D) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (W–Z) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (R–V) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.

Table 5
Reduction percentage values of orange, lemon, mandarin and grapefruit essential oils upon the growth of *Aspergillus flavus, Aspergillus niger, Penicillium verrucosum* and *Penicillium chrysogenum*

	% Growth reduction (mean and SD $n = 3$)				
	Concentration (%)	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium verrucosum*	*Penicillium chrysogenum*
Orange	0.27	29.5 ± 0.04^{aA}	15.4 ± 0.03^{bA}	26.1 ± 0.6^{cA}	25.3 ± 0.02^{dA}
	0.47	36.4 ± 0.12^{aF}	25.2 ± 0.08^{bF}	39.9 ± 0.7^{cF}	39.7 ± 0.08^{cF}
	0.71	48.1 ± 0.13^{aJ}	32.0 ± 0.01^{bJ}	47.7 ± 0.06^{cJ}	48.8 ± 0.10^{dJ}
	0.94	100.0 ± 0.003^{aR}	100.0 ± 0.00^{aR}	100.0 ± 0.00^{aR}	100.0 ± 0.00^{aR}
Lemon	0.27	21.6 ± 0.05^{aB}	51.1 ± 0.14^{bB}	20.5 ± 0.01^{cB}	22.3 ± 0.07^{dB}
	0.47	26.0 ± 0.09^{aG}	52.9 ± 0.11^{bG}	38.0 ± 0.02^{cG}	38.4 ± 0.06^{dG}
	0.71	40.0 ± 0.12^{aK}	57.2 ± 0.03^{bK}	47.2 ± 0.16^{cK}	47.7 ± 0.03^{dK}
	0.94	100.0 ± 0.00^{aR}	100.0 ± 0.00^{aR}	100.0 ± 0.00^{aR}	100.0 ± 0.00^{aR}
Mandarin	0.27	5.1 ± 0.11^{aC}	55.5 ± 0.02^{bC}	17.2 ± 0.07^{cC}	19.2 ± 0.09^{dC}
	0.47	14.7 ± 0.08^{aH}	62.8 ± 0.07^{bH}	31.2 ± 0.11^{cH}	35.7 ± 0.10^{dH}
	0.71	31.9 ± 0.06^{aL}	64.8 ± 0.08^{bL}	42.6 ± 0.12^{cL}	46.3 ± 0.04^{dL}
	0.94	100.0 ± 0.00^{aR}	100.0 ± 0.00^{aR}	100.0 ± 0.00^{aR}	100.0 ± 0.00^{aR}
Grapefruit	0.27	6.7 ± 0.03^{aD}	48.6 ± 0.10^{bD}	34.5 ± 0.09^{cD}	35.5 ± 0.18^{dD}
	0.47	25.7 ± 0.09^{aI}	54.4 ± 0.12^{bI}	43.5 ± 0.17^{cI}	41.2 ± 0.08^{dI}
	0.71	35.0 ± 0.11^{aM}	57.4 ± 0.06^{bM}	48.3 ± 0.12^{cM}	48.1 ± 0.07^{dM}
	0.94	100.0 ± 0.00^{aR}	100.0 ± 0.00^{aR}	100.0 ± 0.00^{aR}	100.0 ± 0.00^{aR}

Values followed by the same small letter within the same line are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (A–D) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (F–I) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (J–M) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.
Values followed by the same letter (R–U) within the same column are not significantly different ($p > 0.05$) according to Tukey's multiple range test.

0.71%, respectively. This EO was followed in order of effectiveness by lemon and grapefruit EOs, while orange EO obtained values approximately half of those obtained with the most effective (mandarin) 15.4%, 25.2% and 32.0%, respectively for the same concentrations.

In so far as *P. verrucosum* is concerned, grapefruit EO produced the best reduction figures, with values of 34.5%, 43.5% and 48.3% at concentrations of 0.27%, 0.47% and 0.71%, respectively. This was followed by lemon EO with slightly lower reductions of 20.4%, 38.0% and 47.2% at the same concentrations. Orange and mandarin showed the lowest reductions, although it must be pointed out that at 0.94% all four EOs produced the total inhibition of this mould, along with *A. niger* and *A. flavus*.

Grapefruit was also the most effective EO in reducing the growth of *P. chrysogenum* (reduction percentages of 35.5%, 41.2% and 48.1%), which is similar to the reductions obtained with *P. verrucosum*. As with this mould, too, lemon was the next best EO, showing values similar to those obtained with grapefruit when used at concentrations of 0.47% and 0.71% (38.4% and 47.7%, respectively). As mentioned above all four EOs produced total inhibition at 0.94%.

Few studied have examined the antifungal activity of citrus essential oils (Caccioni, Guizzardi, Biondi, Renda, & Ruberto, 1998; Sharma, & Tripathi, 2006 a,b; Shukla, Shahi, & Dixit, 2000). Citrus essential oils are a complex mixture of volatile compounds that show, among other properties, antifungal activity by reducing or totally inhibiting fungal growth in a dose-response manner (Sharma & Tripathi, 2006b). This activity may be produced by a single major compound or by the synergistic or antagonistic effect of various compounds (Deba, Xuan, Yasuda, & Tawata, 2007). Several authors have attributed the antifungal capacity of citrus essential oils to the presence of components such as el D-limonene, linalool or citral Alma et al., 2004; Bezic, Skocibusic, & Dunkic, 2005; Rasooli, Moosavi, Rezaee, & Jaimand, 2002; Rodov, Ben-Yoshua, Fang, Kim, & Ashkenazi, 1995; Sonboli, Babakhani, & Mehrabian, 2006; Tepe et al., 2006) which are present in differing concentrations in citric EOs (Vekiari et al., 2002; Veriotti & Sacks, 2001). Other author attributed this function to the phenolic compounds: the amphipathicity of these compounds can explain their interactions with biomembrane and thus the antimicrobial activity (Veldhuizen, Tjeerdsma-van Bokhoven, Zweijtzer, Burt, & Haagsman, 2006). In fact, the hydrophilic part of the molecule interacts with the polar part of the membrane, while the hydrophobic benzene ring and the aliphatic side chains are buried in the hydrophobic inner part of the bacterial membrane (Cristani et al., 2007). Furthermore, the involvement of the hydroxyl group in the formation of hydrogen bonds and the acidity of these phenolic compounds may have other possible explanations (Cristani et al., 2007).

Possible action mechanisms by which mycelial growth may be reduced or totally inhibited have been proposed. It is commonly accepted that it is the toxic effects of the EO components on the functionality and structure of the cell membrane that is responsible for the aforesaid activity (Knobloch, Pauli, Iberl, Wigand, & Weis, 1989; Sikkema, de Bont, & Poolman, 1995). For example, Uribe, Ramirez, and Peña (1985) relate low EO concentrations with changes in the cell structure, which would inhibit respiration and alter the permeability of the microbe cell membrane, while high concentrations would provoke severe damage to the membrane and the loss of homeostasis, leading to cell death (Carson, Me, & Riley, 2002). Conner and Beuchat (1984) suggested that the antimicrobial activity is produced by interactions provoked by EOs in the enzymatic systems related with energy production and in the synthesis of structural components of the microbial cells. Omidbeygi, Barzegar, Hamidi, and Nafhdibadi (2007), on the other hand, suggests that components of the essential oils cross the cell membrane, interacting with the enzymes and proteins of the membrane, so producing a flux of protons towards the cell exterior which induces changes in the cells and, ultimately, their death. Cristani et al. (2007) reported that the antimicrobial activity is related to ability of terpenes to affect not only permeability but also other functions of cell membranes, these compounds might cross the cell membranes, thus penetrating into the interior of the cell and interacting with critical intracellular sites. Daferera, Ziogas, and Polissiou (2000) reported that the fungitoxic activity of EOs may have been due to formation of hydrogen bonds between the hydroxyl group of oil phenolics and active sites of target enzymes

Lucini, Zunino, López, and Zygadlo (2006) indicated that mycelial growth inhibition is caused by the monoterpenes present in essential oils. These components would increase the concentration of lipidic peroxides such as hydroxyl, alkoxyl and alkoperoxyl radicals and so bring about cell death. For Sharma and Tripathi (2006b), the EOs would act on the hyphae of the mycelium, provoking exit of components from the cytoplasm, the loss of rigidity and integrity of the hypha cell wall, resulting in its collapse and death of the mycelium.

4. Conclusions

The essential oils of orange, lemon, mandarin and grapefruit show antifungal activity against the fungi *A. niger*, *A. flavus, P. chrysogenum* and *P. verrucosum*. Orange EO is the most effective against *A. niger*, while mandarin is the best inhibitor of *A. flavus.* In the case of *P. chrysogenum* and *P. verrucosum*, grapefruit EO is the most effective growth reducer.

It seems, then, that citrus EOs could be considered suitable alternatives to chemical additives for use in the food industry, attending to the needs for safety and satisfying the demand of consumers for natural components.

Acknowledgments

The financial support provided by the Consellerias de Cultura, Educación y Deporte, Agricultura Pesca y Alimentación (Generalitat Valenciana) through Project GV04B-679 and from Tecnología y Nutrición de la Dieta Mediterránea Master's Course is gratefully acknowledged.

References

Afifi, A. A., & Azen, S. P. (1979). *Statistical analysis. A computer arieted approach* (2nd ed.). London: Academic Press Inc.

Alma, M. H., Nitz, S., Kollmannsberger, H., Digrak, M., Efe, F. T., & Yilmaz, N. (2004). Chemical composition and antimicrobial activity of the essential oils from the gum of Turkish Pistachio (*Pistacia vera* L.). *Journal of Agricultural and Food Chemistry, 52*(12), 3911–3914.

Angioni, A., Barra, A., Arlorio, M., Coisson, J. D., Russo, M. T., Pirisi, F. M., et al. (2003). Chemical composition, plant genetic differences, and antifungal activity of the essential oil of *Helichrysum italicum* G. Don ssp. microphyllum (Willd) Nym. *Journal of Agricultural and Food Chemistry, 51*(4), 1030–1034.

Been, J., Akhila, R., & Abraham, E. (2007). Antimicrobial activity and chemical composition of essential oil from *Hedychium coronarium*. *Phytotherapy Research, 21*(5), 439–443.

Bezic, N., Skocibusic, M., & Dunkic, V. (2005). Phytochemical composition and antimicrobial activity of *Satureja montana* L. and *Satureja cuneifolia* Ten. essential oils. *Acta Botanica Croatica, 64*(2), 313–322.

Caccioni, D. R. L., Guizzardi, M., Biondi, D. M., Renda, A., & Ruberto, G. (1998). Relationship between volatile components of citrus fruit essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*. *International Journal of Food Microbiology, 43*(1/2), 73–79.

Carson, C. F., Me, B. J., & Riley, T. V. (2002). Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrobial Agents and Chemotherapy, 46*, 1914–1920.

Conner, D. E., & Beuchat, L. R. (1984). Effects of essential oils from plants on growth of food spoilage yeasts. *Journal of Food Science, 49*, 429–434.

Cristani, M., d'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M. G., Micieli, D., & et al. (2007). Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. *Journal of Agricultural and Food Chemistry*, available on line.

Daferera, D. J., Ziogas, B. N., & Polissiou, M. G. (2000). GC–MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on Penicillium digitatum. *Journal Agricultural and Food Chemistry, 48*, 2576–2581.

Deba, F., Xuan, T. D., Yasuda, M., & Tawata, S. (2007). Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. Var. Radiata. *Food Control*. Available on line.

Dehghan, G., Solaimanian, R., Shahverdi, A. R., Amin, G., Abdollahi, M., & Shafiee, A. (2007). Chemical composition and antimicrobial activity of essential oil of *Ferula szowitsiana* D.C. *Flavour and Fragrance Journal, 22*(3), 224–227.

Feng, W., & Zheng, X. (2007). Essential oils to control *Alternaria alternata in vitro* and *in vivo*. *Food Control, 18*, 1126–1130.

Fernandez-Lopez, J., Zhi, N., Aleson-Carbonell, L., Pérez-Álvarez, J. A., & Kuri, V. (2005). Antioxidant and antibacterial activities of natural extracts: application on cooked meat balls. *Meat Science, 69*, 371–380.

Fraternale, D., Giamperi, L., & Ricci, D. (2003). Chemical composition and antifungal activity of essential oil obtained from in vitro plants of *Thymus mastichina* L. *Journal of Essential Oil Research, 15*, 278–281.

Jo, C., Park, B. J., Chung, S. H., Kim, C. B., Cha, B. S., & Byun, M. W. (2004). Antibacterial and antifungal activity of citrus (*Citrus unshiu*) essential oil extracted from peel by-products. *Food Science & Biotechnology, 13*, 384–386.

Kabara, J. J. (1991). Phenols and chelators. In N. J. Russell & G. W. Gould (Eds.), *Food preservatives* (pp. 200–214). Glasgow: Blackie.

Knobloch, K., Pauli, A., Iberl, B., Wigand, H., & Weis, N. (1989). Antibacterial and antifungal properties of essential oil components. *Journal of Essential Oil Research, 1*, 119–128.

Lario, Y., Sendra, E., García-Pérez, J., Fuentes, C., Sayas-Barberá, E., Fernández-López, J., et al. (2004). Preparation of high dietary fiber powder from lemon juiced by-products. *Innovative Food Science & Emerging Technologies, 5*, 113–117.

López-Malo, A., Alzamora, S. M., & Guerrero, S. (2000). Natural antimicrobials from plants. In M. Stella, S. M. Alzamora, M. S. Tapia, & A. Lopez-Malo (Eds.), *Minimally processed fruits and vegetables, fundamental aspects and applications* (pp. 237–363). Gaithersburg: Aspen Publishers.

Lucini, E. I., Zunino, M. P., López, M. L., & Zygadlo, J. A. (2006). Effect of monoterpenes on lipid composition and sclerotial development of *Sclerotium cepivorum* Berk. *Journal of Phytopathology, 154*, 441–446.

Omidbeygi, M., Barzegar, M., Hamidi, Z., & Nafhdibadi, H. (2007). Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. *Food Control, 18*(12), 1518–1523.

Rasooli, I., Moosavi, M. L., Rezaee, M. B., & Jaimand, K. (2002). Susceptibility of microorganisms to *Myrtus communis* L. essential oil and its chemical composition. *Journal of Agricultural Science and Technology, 4*(3/4), 127–133.

Rodov, V., Ben-Yoshua, S., Fang, D. Q., Kim, J. J., & Ashkenazi, R. (1995). Preformed antifungal compounds of lemon fruit: Citral and its relation to disease resistance. *Journal of Agricultural and Food Chemistry, 43*, 1057–1061.

Salgueiro, L. R., Pinto, E., Goncalves, M. J., Costa, I., Palmeira, A., Cavaleiro, C., et al. (2006). Antifungal activity of the essential oil of Thymus capitellatus against *Candida*, *Aspergillus* and *dermatophyte* strains. *Flavour and Fragrance Journal, 21*(5), 749–753.

Sharma, N., & Tripathi, A. (2006a). Fungitoxicity of the essential oil of Citrus sinensis on post-harvest pathogen. *World Journal of Microbiology & Biotechnology, 22*, 587–593.

Sharma, N., & Tripathi, A. (2006b). Effects of *Citrus sinensis* (L.) Osbeck epecarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microbiological Research*, Available on line.

Shukla, A. C., Shahi, S. K., & Dixit, A. (2000). Epicarp of *Citrus sinensis*: A potential source of natural pesticides. *Indian Phytopathology, 53*, 468–471.

Sikkema, J., de Bont, J. A. M., & Poolman, B. (1995). Mechanisms of membrane toxicity of hydrocarbons. *Microbiological Reviews, 59*, 201–222.

Sonboli, A., Babakhani, B., & Mehrabian, A. R. (2006). Antimicrobial activity of six constituents of essential oil from Salvia. *Zeitschrift fur Naturforschung Section C, Biosciences, 61*(3/4), 160–164.

Tepe, B., Daferera, D., Sokmen, A., Sokmen, M., & Polissiou, M. (2005). Antimicrobial and antioxidant activities of the essential oils and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chemistry, 90*, 333–340.

Tepe, B., Akpulat, H. A., Sokmen, M., Daferera, D., Yumrutas, O., Aydin, E., et al. (2006). Screening of the antioxidative and antimicrobial properties of the essential oils of Pimpinella anisetum and Pimpinella flabellifolia from Turkey. *Food Chemistry, 97*(4), 719–724.

Uribe, S., Ramirez, J., & Peña, A. (1985). Effects of beta-pinene on yeast membrane functions. *Journal of Bacteriology, 161*, 1195–1200.

Vekiari, S. A., Protopapadakis, E. E., Papadopoulou, P., Papanicolaou, D., Panou, C., & Vamvakias, M. (2002). Composition and seasonal variation of the essential oil from leaves and peel of a Cretan lemon variety. *Journal of Agricultural and Food Chemistry, 50*(1), 147–153.

Veldhuizen, E. J., Tjeerdsma-van Bokhoven, J. L., Zweijtzer, C., Burt, S. A., & Haagsman, H. P. (2006). Structural requirements for the antimicrobial activity of carvacrol. *Journal of Agricultural and Food Chemistry, 54*, 1874–1879.

Veriotti, T., & Sacks, R. (2001). High-speed GC and GC/time-of-flight MS of lemon and lime oil samples. *Analytica Chemistry, 73*(18), 4395–4402.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez-Álvarez, J. A. (2007). Antifungal activities of thyme, clove and oregano essential oils. *Journal of Food Safety, 27*, 91–101.