ELSEVIER

International Journal of Food Microbiology 43 (1998) 73–79

International Journal of Food Microbiology

Relationship between volatile components of citrus fruit essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*

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Received 31 December 1997; received in revised form 18 May 1998; accepted 8 June 1998

Abstract

This study examined the effect of volatile components of citrus fruit essential oils on *P. digitatum* and *P. italicum* growth. The hydrodistilled essential oils of orange (*Citrus sinensis* cvv. ''Washington navel'', ''Sanguinello'', ''Tarocco'', ''Moro'', ''Valencia late'', and ''Ovale''), bitter (sour) orange (*C. aurantium*), mandarin (*C. deliciosa* cv. ''Avana''), grapefruit (*C. paradisi* cvv. ''Marsh seedless'' and ''Red Blush''), citrange (*C. sinensis x Poncirus trifoliata* cvv. ''Carrizo'' and ''Troyer''), and lemon (*C. limon* cv. ''Femminello'', collected in three periods), were characterized by a combination of GC and GC/MS analyses. The antifungal efficacy of the oils was then examined at progressively reduced rates. Findings showed a positive correlation between monoterpenes other than limonene and sesquiterpene content of the oils and the pathogen fungi inhibition. The best results were shown by the citrange oils, whose chemical composition is reported for the first time, and lemon. Furthermore *P. digitatum* was found to be more sensitive to the inhibitory action of the oils.

Keywords: Citrus essential oils; Antimicrobial activity; Postharvest pathogens; *Penicillium digitatum*; *Penicillium italicum*; GC–MS; Statistical analysis

Many natural substances may play a fundamental role in the host plant/pathogen relationship: the essential oils produced by different plant genera are in many cases biologically active, endowed with antimicrobic, allelopathic, antioxidant and bio-regulatory properties (Caccioni and Guizzardi, 1994; Caccioni et al., 1995a,b; Deans, 1991; Elakovich, 1988; French, 1985; Vaughn and Spencer, 1991).

Citrus essential oils are present in fruit flavedo in great quantities. *Penicillium digitatum* (Pers.) Sacc. and *Penicillium italicum* Whem., the most harmful

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[1] Associated to the National Institute for the Chemistry of Biological Systems-C.N.R.

0168-1605/98/$19.00
PII: S0168-1605(98)00099-3

citrus fruit post-harvest pathogens, infect the fruit through micro-injuries produced in the flavedo during harvesting and processing. Flavedo lesions can involve the glands containing the essential oils, causing these oils to overflow. In fact Norman et al. (1967) and McCalley and Torres-Grifol (1992) have demonstrated that injured oranges release a much greater amount of terpene peel-oil constituents than healthy fruits. It may be supposed that *Penicillium* spp. conidia come into contact with the essential oils which could therefore play a role in the pathogenic process.

In previous studies was verified the biological activity of some components of orange and lemon oils; the most effective of them proved to be citral (Caccioni and Deans, 1993; Caccioni et al., 1995a,b).

In this study, we intended to examine the activity of volatile fractions of essential oils extracted from different citrus species and cultivars on the growth of *P. digitatum* and *P. italicum*.

1. Materials and methods

1.1. Plant material

Fruits of six orange cultivars (*Citrus sinensis* ''Washington navel'', ''Sanguinello'', ''Tarocco'', ''Moro'', ''Valencia late'' and ''Ovale''), mandarin (*C. deliciosa* ''Avana''), grapefruit (*C. paradisi* ''Marsh Seedless'' and ''Red blush''), bitter (sour) orange (*C. aurantium*) and citrange (*C. sinensis x P. trifoliata* ''Carrizo'' and ''Troyer'') were collected at the ripening stage. Fruits of lemon (*C. limon* cv ''Femminello'') were picked in three different periods (November, February and June). All the trees were cultivated in the experimental fields ''Palazzelli'' of the Citrus Experimental Institute, Lentini, Sicily.

1.2. Extraction of essential oils

Fresh rind tissue of each sample (flavedo and albedo, from 500 to 950 g) was subjected to steam distillation until there was no significant increase in the volume of the oil collected. The oils were dried over anhydrous sodium sulphate and stored under N_2 in sealed vials until required.

1.3. Analysis of essential oils

Analyses were performed on a Hewlett–Packard gas chromatograph model 5890 (Palo Alto, CA, USA), equipped with a flame ionisation detector (FID) and coupled with an electronic integrator. Analytical conditions: HP-1 dimethypolisiloxane capillary column (25 m $\times$ 0.2 mm I.D.), helium as carrier gas, injector and detector temperature 250 and 270°C, respectively. The oven temperature was held at 60°C for 6 min, then programmed from 60 to 250°C at 3°C/min.

GC/MS analyses were carried out on the same chromatograph equipped with a Hewlett–Packard MS computerised system, model 5971A, ionisation voltage 70 eV, electron multiplier 1700 V, ion source temperature 180°C, GC conditions as above.

Identification of components was based on GC retention times, computer matching with NBS library, comparison of the fragmentation patterns with those reported in the literature (Jennings and Shibamoto, 1980; Adams, 1995) and, whenever possible, co-injections with authentic samples. The reported values are the mean of three data readings taken from samples steam distilled at different times.

1.4. Strains of pathogens

Two strains of *Penicillium digitatum* and *P. italicum*, belonging to the Criof collection (University of Bologna), were used. The spores were obtained in vitro from monoconidial cultures after incubation (7 days at 20°C) on Potato Dextrose Agar (PDA) (Oxoid Ltd., Hampshire, UK) and subsequently suspended in sterile water. The concentration was adjusted at $5x10^5$ conidia ml^{-1} using an haemocytometer.

1.5. Antifungal test

Antifungal activity was studied by determining the dry weight of the pathogen mycelium after incubation in a liquid medium added to the oils, modifying the procedure already described by Deans (1991).

Flasks (50 ml) containing 10 ml of Saboraud Dextrose Broth (SDB) (Oxoid Ltd., Hampshire, UK) were inoculated with 20 μl of conidial suspension.

The oils were diluted in methanol (previously sterilized by filtration) and then added to each flask

to obtain concentrations ranging from 250 to 5000 ppm (v/v). The control flasks were added with the same doses of pure methanol (9 μl) used in treatments. The flasks were sealed with Parafilm™ and subjected to continuous agitation at 20°C for 5 days. Mycelium dry weight was obtained by filtration and oven-drying (65°C until constant weight) of the fungal cultures. Five flasks (replications) were used for each concentration tested. The inhibition index was calculated from the dry weight of the mycelium. The median effective doses (ED_{50}, ppm) were determined by log probit graphs (Bliss, 1934a,b; Finney, 1971). The relationship between the concentrations (%) of the various chemical classes in the oils and the antimicrobial efficacy (ED_{50}, ppm) were determined by linear regression. The significance were tested by analysis of variance ($P = 0.05$).

2. Results and discussion

Table 1 shows the volatile components of Citrus essential oils, whereas in Table 2 the components are grouped in classes for an easier comparison of the oils.

Concerning the *C. sinensis* species, the six cultivars analyzed here show a fairly similar composition, except for oxygenated hydrocarbons and sesquiterpenes, both mainly present in the ''Moro'' cv.

Concerning the sour orange (*C. aurantium*) and the mandarin (*C. deliciosa*, ''Avana'' *cv.*), the chemical composition of the essential oils is in accordance with data obtained from the literature (Tables 1 and 2) (Di Giacomo and Mincione, 1994).

The oils of the two cultivars of grapefruit (*C. paradisi*), ''Marsh Seedless'' and ''Red Blush'', show an almost superimposed chemical composition with a high content of aliphatic aldehydes (Table 2) and nootaktone (Table 1).

The lemon oil shows the highest amount of oxygenated monoterpenes. In particular, the oil of lemons collected in February showed the highest content of oxygenated compounds, being two geraniol–geranial and nerol–neral couples the main compounds. The ester and sesquiterpenes classes are present at the highest levels.

Finally, the oils from the fruits of the ''Troyer'' and ''Carrizo'' citranges (*C.sinensis x. Poncirus trifoliata*) have been, for the first time, analyzed (Tables 1 and 2). The peculiarity of these oils with respect to those of the other Citrus species is due to the high amount of sesquiterpenes. In both oils this class amounts to ca. 10%, whereas in all the other oils it never reaches 1%, being (*E*)-β-farnesene, α-elemene and β-sinensale the most important compounds (Table 1). A further differentiating aspect to the other Citrus oils is the presence of α-asarone. Concerning the oxygenated monoterpenes the ''Carrizo'' citrange shows twice the amount of that of ''Troyer''. Also significant is the content of aliphatic aldehydes (Table 2), exclusively represented by octanal. The content of aliphatic alcohols and esters is similar to that of the other citrus varieties.

Table 3 shows the antifungal activities of the fifteen citrus essential oils on *Penicillium digitatum* and *P. italicum*, expressed as ED_{50}. As can be seen from analysis of the data, the oils show extremely varied antifungal activity. The most active oils were found to be those of the two citranges ''Troyer'' and ''Carrizo'', while the activity of lemon oil from the February harvest was also high. Less effective, but still good, was the activity from grapefruit, mandarin, sour orange and lemon from the Summer harvest (June). Finally, with a partial exception in the case of the *cv.* ''Moro'', the antifungal activity of orange oils was found to be weaker, especially against *P. italicum*.

However *P. italicum* was found to be much more resistant to the antifungal activity of the oils: the ED_{50} generally being more than twice that found for *P. digitatum*. Only the oils of the citranges (''Carrizo'' and ''Troyer'') had an equivalent action on the two strains of fungus.

When the above data are considered together with the composition of the essential volatile oils (Tables 1 and 2) it would seen extremely difficult to correlate the fungitoxic activity to single compounds or classes of compounds. The various components of any oil may act sinergically while several compounds may have a stimulating action on fungal spore germination (French, 1985). Therefore, an holistic approach is necessary to explain the antimicrobial capabilities of an essential oil, whose performance could be the result of a certain quantitative balance of various components, where synergic and additive effects prevail over contrasting effects.

The analysis of variance for linear regression

Table 1
Chemical composition of Citrus essential oils[a]

Compound	A1	A2	A3	A4	A5	A6	AM	M1	P1	P2	L1	L2	L3	CZ	CY
α-Thujene	t	t	t	0.01	t	t	t	0.64	t	t	0.36	0.27	0.43	0.23	0.23
α-Pinene[b]	0.50	0.45	0.42	0.41	0.53	0.48	0.40	1.78	0.51	0.52	1.54	1.27	2.27	0.87	0.88
Camphene[b]	t	t	t	t	t	t	t	0.02	t	t	0.05	0.05	0.10	0.03	0.03
Sabinene[b]	0.27	0.54	0.13	0.83	0.43	0.34	0.08	0.17	0.54	0.54	1.52	0.93	19.52	0.51	0.56
β-Pinene[b]	0.04	0.04	t	0.06	0.04	0.03	0.33	1.41	0.07	0.07	9.42	8.34		2.16	1.37
Octanal[b]	0.29	0.46	0.34	0.53	0.37	0.09	0.08	0.06	0.58	0.34	0.06	0.06	0.07	1.40	0.99
Myrcene[b]	1.98	1.85	1.81	1.74	1.87	1.82	1.88	1.71	1.81	1.86	1.52	1.44	1.39	7.59	7.35
α-Phellandrene[b]	0.05	0.06	0.03	0.05	0.04	0.05	0.02	0.07	0.05	0.07	0.04	0.05	0.06	2.76	2.57
3-Carene[b]	–	0.22	0.05	0.13	0.03	0.22	0.01	t	–	–	t	t	t	t	t
α-Terpinene[b]	0.30	–	–	–	–	–	–	0.24	0.04	0.03	0.19	0.30	0.12	0.18	–
β-Phellandrene[b]	–	0.06	t	0.07	0.03	0.20	–	0.52	–	0.18	0.42	0.17	0.34	–	0.14
p-Cimene[b]	t	t	t	t	t	t	–	0.23	–	0.18				t	t
Limonene[b]	94.81	92.48	95.29	91.14	94.95	94.95	94.27	72.71	93.59	93.70	71.06	69.38	60.20	65.39	71.63
(*Z*)-β-Ocimene	t	t	t	t	t	t	t	t	t	–	0.02	0.04	–	0.05	0.03
(*E*)-β-Ocimene	0.02	0.05	0.01	0.05	0.03	0.01	0.23	0.02	0.29	0.21	0.07	0.07	0.15	1.78	1.12
γ-Terpinene[b]	0.02	0.09	0.04	0.18	0.06	0.04	0.02	17.17	0.07	0.08	8.06	8.44	9.45	0.20	0.10
Octanol[b]	0.07	0.21	0.18	0.24	0.12	0.03	0.33	0.03	0.14	0.14	0.01	0.04	0.06	0.20	0.13
cis-Linalol oxide	–	–	–	–	–	–	t	–	–	–	–	–	–	–	–
trans-Linalol oxide	–	–	–	–	–	–	0.05	–	0.23	0.21	–	–	–	–	0.06
Terpinolene[b]	0.07	0.01	0.02	0.06	0.03	0.05	0.02	0.82	0.02	0.02	0.39	0.47	0.52	0.10	0.05
Nonanal[b]	0.07	0.05	0.03	0.05	0.05	t	–	0.02	0.06	0.05	0.12	0.10	0.13	–	–
Linalol[b]	0.61	1.54	0.90	2.56	0.41	0.82	0.78	0.16	0.25	0.20	0.18	0.48	0.26	0.46	0.27
Citronellal[b]	0.06	0.03	0.02	0.04	0.03	0.02	–	0.02	–	–	0.08	0.07	0.04	0.04	0.03
iso-Pulegol	–	–	–	–	–	–	–	–	0.06	0.05	–	–	–	–	–
Nonanol[b]	–	–	–	–	–	–	–	–	t	t	–	–	–	–	–
Terpinene-4-ol[b]	0.08	0.24	0.06	0.31	0.15	0.10	0.06	0.27	0.17	0.18	0.34	0.67	0.60	0.55	0.23
α-Terpineol[b]	0.12	0.25	0.15	0.25	0.12	0.13	0.26	0.37	0.14	0.15	0.41	0.86	0.70	0.42	0.21
Decanal[b]	0.11	0.17	0.12	0.20	0.26	0.14	0.11	0.11	0.26	0.26	0.03	0.03	0.05	–	–
Carveol[b]	–	–	–	–	–	–	–	–	0.05	0.05	–	–	–	–	–
Octyl acetate[b]	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–
Nerol[b]	0.05	0.09	0.04	0.12	0.01	0.03	0.06	0.07	0.02	0.03	0.17	0.86	0.40	0.08	0.07
Neral[b]	0.14	0.12	0.04	0.18	0.07	0.08	0.03	t	0.06	0.05	0.90	0.85	0.38	–	–
Geraniol[b]	0.02	0.08	0.03	0.09	0.01	0.01	0.11	0.03	0.01	0.02	0.18	1.05	0.35	0.03	0.02
Perillaldehyde[b]	–	–	–	–	–	–	0.03	–	–	–	–	–	–	–	–
Geranial[b]	0.18	0.15	0.07	0.23	0.10	0.10	0.11	0.08	0.10	0.07	1.23	1.08	0.56	–	–
Decanol[b]	–	–	–	–	–	–	0.05	–	0.01	0.02	–	–	–	–	–
Thymol[b]	–	–	–	–	–	–	–	0.02	–	–	–	–	–	–	–
Citronellyl acetate	–	–	–	–	–	–	–	–	–	–	0.02	0.05	0.02	–	–
Terpinyl acetate	–	–	–	–	–	–	0.03	–	0.01	t	–	–	–	0.15	0.16
Neryl acetate	–	–	–	–	–	–	0.04	–	t	0.01	0.31	0.44	0.21	–	–
Geranyl acetate	–	–	–	–	–	–	–	–	0.05	0.06	0.23	0.45	0.45	–	–
Me-*N*-Me-anthranilate	–	–	–	–	–	–	–	0.46	–	–	–	–	–	–	–
α-Copaene[b]	–	–	–	–	–	–	–	–	0.04	0.04	–	–	–	0.03	0.02
α-Elemene	–	–	–	–	–	–	–	–	–	–	–	–	–	2.14	2.20
Dodecanal[b]	–	–	–	–	–	–	0.01	–	0.02	0.02	–	–	–	–	–
Decyl acetate	–	–	–	–	–	–	0.02	–	t	t	–	–	–	–	–
β-Bergamotene	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09	0.08
β-Caryophyllene[b]	0.01	0.04	t	0.02	0.03	t	0.07	0.06	0.18	0.17	0.19	0.15	0.13	0.52	1.60
trans-α-Bergamotene	–	–	–	–	–	–	–	–	–	–	0.17	0.28	0.23	0.15	0.15
α-Humulene[b]	–	–	–	–	–	–	–	0.02	0.02	0.02	0.01	0.03	0.02	0.34	0.37
2-Dodecenal[c]	–	–	–	–	–	–	–	–	0.03	0.02	–	–	–	–	–
(*E*)-β-Farnesene	–	–	–	–	–	–	–	–	–	–	–	–	–	2.60	2.12
β-Cubebene	–	–	–	–	–	–	0.05	–	–	–	–	–	–	0.06	0.30
β-Bisabolene	–	–	–	–	–	–	–	–	–	–	0.23	0.47	0.36	–	–
Aristolene	–	–	–	–	–	–	–	–	–	–	–	–	–	0.14	0.09

Table 1. Continued

Compound	A1	A2	A3	A4	A5	A6	AM	M1	P1	P2	L1	L2	L3	CZ	CY
Valencene[b]	t	0.02	t	0.03	0.01	t	–	–	–	–	–	–	–	0.31	0.44
(*E*,*E*)-α-Farnesene	–	–	–	–	–	–	–	0.05	–	–	–	–	–	0.37	0.18
γ-Cadinene	–	–	–	–	–	–	–	–	–	–	–	–	–	0.37	0.27
β-Elemene	–	–	–	–	–	–	0.01	–	–	–	–	–	–	0.57	0.59
(*E*)-Nerolidol[b]	–	–	–	–	–	–	0.07	–	–	–	–	–	–	0.09	0.05
α-Asarone	–	–	–	–	–	–	–	–	–	–	–	–	–	0.15	0.05
Cadinol[c]	–	–	–	–	–	–	–	–	–	–	–	–	–	0.18	0.10
β-Sinensal	–	0.03	t	0.05	–	t	–	–	–	–	–	–	–	2.00	1.30
α-Sinensal	–	0.02	t	0.05	–	t	–	0.16	–	–	–	–	–	–	–
Nootkatone	–	–	–	–	–	–	–	–	0.13	0.07	–	–	–	–	–

[a]Compounds are listed according to the elution order on HP-1 column, and values (area percent) represent averages of three determinations (t = trace < 0.01%). A1 = *C. sinensis* cv. Washington Navel; A2 = *C. sinensis* cv. Sanguinello; A3 = *C. sinensis* cv. Tarocco; A4 = *C. sinensis* cv. Moro; A5 = *C. sinensis* cv. Valencia Late; A6 = *C. sinensis* cv. Ovale; AM = *C. aurantium*; M1 = *C. deliciosa* cv. Avana; P1 = *C. Paradisi* cv. Marsh Seedless; P2 = *C. paradisi* cv. Red Blush; L1, L2, L3 = *C. limon* cv. Femminello (November, February, June); CZ = *C. sinensis x Poncirus trifoliata* – Carrizo Citrange; TY = *C. sinensis x Poncirus trifoliata* – Troyer Citrange.
[b]Co-injection with authentic sample.
[c]Correct isomer not identified.

Table 2
Components of Citrus essential oils grouped in classes and significant correlation with antifungal activity[a]

	A1	A2	A3	A4	A5	A6	AM	M1	P1	P2	L1	L2	L3	CZ	TY	Significance[b]	
																P. digitatum	*P. itaticum*
Minor Monoterpene Hydrocarbons	3.23	3.37	2.51	3.59	3.06	3.24	2.99	24.72	3.40	3.66	23.60	21.84	34.35	16.46	14.42	[a]	
Limonene	94.81	92.48	95.29	91.14	95.95	94.95	94.27	72.71	93.59	93.70	71.06	69.38	60.20	65.39	71.63		
Total Monoterpene Hydrocarbons	98.04	95.85	97.80	94.73	98.01	98.19	97.26	97.43	96.99	97.36	94.66	91.22	94.85	81.85	86.05		
Oxygenated Monoterpenes	1.26	2.50	1.31	3.90	0.90	1.29	1.49	1.02	1.09	1.01	3.49	5.92	3.29	1.67	0.89		
Total Monoterpenes other than Limonene	4.49	5.87	3.82	7.49	3.96	4.53	4.48	25.74	4.49	4.67	27.09	27.76	37.64	18.13	15.31	[a]	[a]
Sesquiterpenes	0.01	0.11	t	0.15	0.04	t	0.20	0.29	0.37	0.30	0.50	0.93	0.74	9.96	9.86	[a]	[a]
Aliphatic Aldehydes	0.47	0.68	0.49	0.78	0.68	0.23	0.20	0.19	0.94	0.69	0.24	0.19	0.25	1.40	0.99		
Aliphatic Alcohols	0.07	0.21	0.18	0.24	0.12	0.03	0.38	0.03	0.15	0.16	0.01	0.04	0.06	0.20	0.13		
Esters	–	–	–	–	–	–	0.28	0.46[c]	0.06	0.07	0.56	0.94	0.54	0.15	0.16		

[a]Values (area percent) represent averages of three determinations (t = trace < 0.01%). A1 = *C. sinensis* cv. Washington Navel; A2 = *C. sinensis* cv. Sanguinello; A3 = *C. sinensis* cv. Tarocco; A4 = *C. sinensis* cv. Moro; A5 = *C. sinensis* cv. Valencia Late; A6 = *C. sinensis* cv. Ovale; AM = *C. aurantium*; M1 = *C. deliciosa* cv. Avana; P1 = *C. paradisi* cv. Marsh Seedless; P2 = *C. paradisi* cv. Red Blush; L1, L2, L3 = *C. limon* cv. Femminello (November, February, June); CZ = *C. sinensis x Poncirus trifoliata* – Carizo Citrange; TY = *C. sinensis x Poncirus trifoliata* – Troyer Citrange.
[b]Analysis of variance (p 0.05) for linear regression between the concentrations (%) of the various chemical classes in the oils and the antimicrobial efficacy (ED_{50}).
[c]Methyl-*N*-methylanthranilate.

between the chemical composition of oils volatile fraction (Table 2) and the ED_{50} against *P. digitatum* and *P. italicum* (Table 3) showed significant correlation between the antimicrobial effect and the amount of minor monoterpenes hydrocarbons (monoterpenes hydrocarbons other than limonene), total monoterpenes other than limonene and sesquiterpenes. In particular, a positive correlation between the content of total monoterpenes other than limonene and antifungal activity has been verified for the oils, and the same holds true for sesquiterpenes. On the contrary, oxygenated monoterpenes do not show a

Table 3
Antifungal activity of citrus essential oils[a]

Citrus species	*Penicillium digitatum* ED_{50}	R squared	*Penicillium italicum* ED_{50}	R squared
Citrus sinensis				
A1 = Washington Navel	2180.2	0.906	5407.5	0.901
A2 = Sanguinello	1594.1	0.979	4277.4	1.000
A3 = Tarocco	1496.9	0.929	4470.6	0.960
A4 = Moro	1004.6	0.973	3147.2	1.000
A5 = Valencia Late	2245.6	0.896	4330.0	0.965
A6 = Ovale	2389.9	0.895	4436.3	0.967
Citrus aurantium				
AM = Sour orange	1015.4	0.863	1490.6	0.961
Citrus deliciosa				
M1 = Avana	713.3	0.900	1977.0	0.989
Citrus paradisi				
P1 = Marsh Seedless	910.3	0.979	1498.4	0.890
P2 = Red Blush	688.7	0.944	2361.7	0.974
Citrus limon				
L1 = Femminello (Dec.)	1056.4	0.991	2505.4	0.881
L2 = Femminello (Feb.)	574.1	0.987	1040.9	0.925
L3 = Femminello (Jun.)	569.1	0.968	1687.9	0.968
C. sinensis x Poncirus trifoliata				
CZ = Carrizo citrange	275.5	0.983	246.2	0.961
TY = Troyer citrange	311.8	0.965	251.2	0.968

[a]Median Effective Doses (ppm). Inhibitory concentration determined by log probit graphs. Methanol, at the used dose, is ineffective against the two fungi.

significant value of F in regression analysis. These results are, in our opinion, slightly misleading. Infact, even if a contribution of the monoterpene hydrocarbons in the antimicrobial action cannot be excluded a priori, we concluded that the oxygenated ones are more active, like previous studies showed (Caccioni and Guizzardi, 1994; Knobloch et al., 1989). Furthermore, carrying out a linear regression using only orange (6 *cvv*) and lemon (3 collections) oils, a highly significant correlation between the content of the oxygenated monoterpenes and antifungal activity has been obtained. In particular, among the orange *cvv*, the best results were given by oil from ''Moro'' *cv*, which has considerable oxygenated monoterpene content, as well as the highest amount of sesquiterpenes.

However, it is a known fact that citrus fruits have different levels of susceptibility to decay caused by *Penicillium* spp according to the *cv*., or, in the case of lemons, to the time of harvest (Pratella, 1979). As shown in this study, there is a substantial difference between the chemical composition and antifungal activities of the oils extracted from various types of citrus fruit. The activity on *Penicillium* spp is, in certain cases, extremely strong, as was seen in the case of citrange and lemon oils (Table 3). Essential oils might therefore represent a pre- formed barrier in situ, interfering greatly in host–pathogen relations. Recently, Ben-Yehoshua et al. (1992), (1995) and Rodov et al. (1995) postulated a significant relation between the presence of citral (mixture of two isomers geranial-neral) in the peel and the decay caused by *P. digitatum*. Citral itself is again indicated as the most active compound against *P. digitatum* and *P. italicum*, also in tests taking into account the antifungal capacity of the various components of citrus essential oils (Caccioni and Deans, 1993; Caccioni et al., 1995b).

Therefore, citrus essential oils could represent a pre-formed defence barrier, whose activity may be

integrated by lignin-like materials (Brown and Barmore, 1983) and phytoalexins (Ben-Yehoshua et al., 1992; Stange et al., 1993).

In conclusion, the increase in resistance of fruit to fungal infection, which can be achieved by breeding and genetic engineering (Esen and Soost, 1973; Starrantino, 1997), and the use of natural, low-toxicity substances is becoming more and more the up-to-date integrated control strategy to give a more acceptable and less environmentally damaging form of agriculture.

The antimicrobial abilities of essential oils, among which citrus oils, is also shown to be a particularly interesting field for applications within the food and cosmetics industries.

Acknowledgements

We thank Dr. P. Rapisarda (Istituto Sperimentale per l'Agrumicoltura, Acireale, CT) for the gift of authenticated plant material.

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